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Official Title:	Nonmyeloablative peripheral blood mobilized hematopoietic precursor cell transplantation for sickle cell disease and β-thalassemia in individuals with higher risk of transplant failure
NCT number:	NCT02105766
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CLINICAL RESEARCH PROTOCOL

Protocol# 14-H-0077
Drug: Pentostatin / Cyclophosphamide / Alemtuzumab
IND: Exempt

Date: 13 FEB 2024

Title: Nonmyeloablative peripheral blood mobilized hematopoietic precursor cell transplantation for sickle cell disease and β-thalassemia in individuals with higher risk of transplant failure

Other underlying words: Peripheral blood stem cells, graft-versus-host disease, sirolimus (Rapamune®), low dose irradiation, alemtuzumab (Campath®), cyclophosphamide (Cytoxan®), pentostatin (Nipent®)

Principal Investigator: Matthew Hsieh MD, CMTB, NHLBI

Subjects of study:	**Number**	**Sex**	**Age range**
Patients:	81	either	≥4 years
Donors	81	either	10-80 years

Project involves ionizing radiation?	Yes
Off site project?	No
Multi-Institutional project?	No
DSMB	Yes

Referral Contact:
NIH Office of Patient Recruitment 1-800-411-1222 (TTY 1-866-411-1010)

PRECIS

Our ongoing nonmyeloablative allogeneic peripheral blood stem cell (PBSC) transplant protocol (03-H-0170) for patients with severe sickle cell disease (SCD) and β-thalassemia from HLA-matched family donors has excellent results thus far. Our long term leukocyte engraftment rate is 85-90% with the same disease-free survival. None of the engrafted patients had acute sickle-related events, significant toxicity associated with the conditioning regimen, or any evidence of graft versus host disease (GVHD).

While these results rival the transplant outcomes from low risk transplant patients with β-thalassemia, there are areas for improvement. The first is the 10-15% graft rejection rate, where a majority of these individuals were male donor and female recipient pairs. Another limitation is the significant delay in donor red cell engraftment in one recipient who had pre-existing allo-antibody to donor red cells from previous transfusions. Also we have excluded another group of individuals with preformed antibodies, recipients having major ABO incompatibility to the donors.

To overcome these limitations (and reduce the transplant failure rate) in this new protocol, we will continue our nonmyeloablative approach in the patients with SCD and β-thalassemia with HLA-matched family donors, but using an increased intensity regimen in a subset considered at high risk for transplant failure. This modified regimen consists of pentostatin and oral cyclophosphamide, which we hypothesize will reduce both the T cells that mediate leukocyte rejection and the B/plasma cells that produce anti-donor erythrocyte antibodies. The main transplant backbone will remain as alemtuzumab, low dose total body irradiation of 300 cGy, and sirolimus; the transplant graft will remain as unmanipulated G-CSF mobilized, T-cell replete, PBSC product for hematopoietic and lymphoid reconstitution.

The primary endpoint of this study is the percentage/number of patients who have sustained donor type hemoglobin at 1 year post transplant for male donors – female recipients. The primary endpoint for those with pre-existing antibodies is the presence of donor red cells with reticulocytes ≥30 k/uL at 2 years post-transplant. Other endpoints include the toxicity of the pentostatin-cyclophosphamide regimen, the degree of donor-host chimerism necessary for long-term graft survival and disease amelioration, incidence of acute and chronic GVHD, incidence of graft rejection, transplant-related morbidity, as well as disease-free and overall survival. Since SCD and β-thalassemia are non-malignant disorders of red cells, severe GVHD, lack of donor erythrocyte (prolonged donor red cell aplasia), or graft rejection is collectively considered transplant failure.

TABLE OF CONTENTS

SEE ALSO: BONE MARROW TRANSPLANT CONSORTIUM GUIDELINES
Marrow stem cell transplant section
Hematology branch, NHLBI
http://intranet.cc.nih.gov/bmt/

1.0 OBJECTIVES

Using a nonmyeloablative preparative regimen followed by HLA-matched, related donor allogeneic granulocyte colony-stimulating factor (filgrastim, G-CSF) mobilized peripheral blood stem cell transplant in (PBSCT) in patients with sickle cell disease (SCD) and β-thalassemia at increased risk for transplant failure, we hope to:

1.1 determine if the addition of pentostatin and cyclophosphamide improves engraftment rate in male donor-female recipient pairs (cohort 1) and allows clinically meaningful donor erythropoiesis in those with pre-existing antibody (cohort 2)

1.2 determine the regimen failure rate, defined as graft rejection, severe GVHD (acute GVHD grade 3 or higher or extensive chronic GVHD), or prolonged donor red cell aplasia (>2 years post-HSCT)

1.3 examine the level of chimerism required to maintain both graft survival as well as hematologic normalcy using a regimen containing pentostatin, cyclophosphamide, alemtuzumab, and low total body irradiation.

1.4 evaluate the safety, efficacy, and toxicity of the pentostatin and oral cyclophosphamide regimen, including disease-free survival, transplant-related mortality, overall survival, and compare this new regimen to 03-H-0170

1.5 evaluate the longitudinal effects of this transplant on neuropsychological functioning and quality of life (see Addendum A), and other end organ function

2.0 BACKGROUND

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only available cure for patients with sickle cell disease (SCD) and β-thalassemia, but has been infrequently pursued due to its associated complications. The majority of patients who are otherwise eligible for bone marrow transplant (BMT) do not have a suitable donor. In addition, the unacceptable risk of death from conventional BMT renders many patients, especially those with nonmalignant disorders, ineligible for what may otherwise be curative therapy. Recently however, in both malignant and non-malignant disorders, it has been shown that these high intensity regimens are not necessary for engraftment and survival, and many centers are currently exploring nonmyeloablative conditioning regimens in order to reduce the toxicity associated with this treatment modality. While successful engraftment has been reported in the majority of patients conditioned with reduced intensity regimens, these regimens still carry significant toxicity and have not significantly reduced the risk of GVHD. For patients with congenital severe anemias, the replacement of abnormal or absent erythroid cells with normal donor-derived erythroid cells is required for disease amelioration. These disorders constitute an ideal situation for a nonmyeloablative conditioning regimen as only a proportion of normal cells will need to engraft given the survival and proliferative advantage of the donor-derived erythroid cells as compared to the host cells. Further, as a nonmyeloablative regimen will allow autologous recovery with a low risk of adverse consequences to the recipient if the graft should fail, graft failure is preferable to the development of severe GVHD. As such, we propose the development of an immunosuppressive but nonmyeloablative transplant regimen consisting of pentostatin and cyclophosphamide before our backbone of alemtuzumab, low dose radiation, and sirolimus, in patients with either SCD or β -thalassemia.

Sickle Cell Disease

SCD is a well described genetic disorder associated with significant morbidity and mortality. It affects one of every 600 African-Americans in the United States alone [1]. The disease is characterized by recurrent vaso-occlusive crises as a consequence of abnormal hemoglobin polymerization in areas of low oxygen tension. As a result, patients develop functional asplenism leading to a high risk of infections from encapsulated organisms, recurrent pain crises, acute chest syndrome, pulmonary hypertension, kidney failure, and neurologic events, as well as sudden death as the most serious consequences of this disease.[2] More recently, sickle hepatopathy and iron overload have been discovered to increase mortality in patients with SCD, as patients with ferritin $\geq$1000 ug/L or direct bilirubin >0.4mg/dL led to

significantly decreased survival as compared to patients with ferritin <1000ug/L and direct bilirubin <0.4 mg/dL (Jordan Feld and Theo Heller, manuscript in preparation).

The medical costs of this disease are enormous, with estimates of $40,000 per patient per year (year 2000 figures) for chronic transfusion therapy and chelation alone, but do not include the impact on quality of life of those with the disease[3]. An overlapping symptom complex also occurs in patients with the double heterozygous forms of SCD, sickle-C, and sickle β-thal[0] disease, and in fact, these patients cannot always be differentiated clinically but only by means of a laboratory test. While transfusions can prevent further neurologic events in patients at risk, iron overload is common, resulting in significant end-organ toxicity. The most common form of treatment in SCD had been erythrocyte transfusions, and more recently hydroxyurea.[4-6] Hydroxyurea results in a significant reduction in the number of painful crises per year and a decreased frequency of acute chest syndrome,[4] and has become the treatment of choice for many individuals with SCD. Unfortunately, hydroxyurea is not curative, and does not appear to reverse established end-organ damage.

Several important interventions have led to an improvement in the overall life expectancy of patients with SCD, most notable among these are the use of pneumococcal vaccines and the prophylactic use of penicillin during childhood. Hydroxyurea has also been suggested to improve survival in patients with SCD.[7] However, life expectancy remains significantly shortened compared to the national average with that of an affected male being 47 years versus the national average of 72.[2] There are no factors to predict better survival among patients, further complicating the decision to proceed with higher risk treatments, especially during childhood when such treatments may be better tolerated. In one study of 3,764 patients, 18 percent of the patients died with overt organ failure, and early mortality was highest among patients with symptomatic disease. Importantly however, another 33 percent who appeared to be clinically free of organ failure died during an acute sickle crisis.[2]

The only established cure for patients with SCD remains allogeneic BMT; however, the procedure has only been applied to highly selected children.[8-10] In adults, the higher burden of accumulated end-organ damage would be expected to result in higher transplant associated morbidity and mortality, beyond that reported in children, including seizures and intracranial hemorrhage. As a result, this method has traditionally only been offered to those patients less than the age of 16 with either end-organ damage or symptomatic disease due to their demonstrated higher early mortality rate.

We currently have an ongoing protocol for adult patients with severe congenital anemias and a 6/6 human leukocyte antigen (HLA)-matched sibling donor using a conditioning regimen consisting of alemtuzumab, 300 cGy total body irradiation (TBI), and sirolimus for GVHD prophylaxis. Our results to date show a leukocyte engraftment rate a disease-free survival of 85%. These data are excellent, and are very close to the results achieved with myeloablative conditioning in children. However, with additional experience and sufficient accrual to date, we have experienced some limitations with this current approach (See section 3.0).

Thalassemia

Thalassemia is the most common genetic disorder worldwide,[11] and is a result of either defective or absent synthesis of one or more of the globin chain subunits of the hemoglobin tetramer. Inadequate accumulation of the globin subunits results in ineffective erythropoiesis. There is marked heterogeneity ranging from profound anemia resulting in death in utero to a relatively benign anemia. Thalassemias are designated by the globin chain, α or β, whose synthesis is affected, and by major or minor, denoting homozygosity or heterozygosity. β-thalassemia major, or Cooley's anemia, is a clinically severe anemia caused by the inheritance of two β -thalassemia alleles. As a result, circulating red blood cells are small and distorted, containing very small amounts of hemoglobin. Further, the accumulation of free α-globin molecules in erythrocytes results in ineffective erythropoiesis.

In patients with β-thalassemia major, the resulting anemia is so severe that most patients require lifelong red blood cell transfusions. This chronic transfusion therapy necessitates the use of iron chelation to prevent the long-term consequences of iron overload. Other treatments such as butyrate and hydroxyurea have been explored, but these have had only limited success, are not well tolerated (especially butyrate), and most importantly, are not curative.

Prior to the regular use of deferoxamine for iron chelation, only 25% of patients survived to the age of 25 years. For patients born after 1967, for whom such chelation has been available, one study estimated that the probability of being

alive at 20 years of age remains low at 67%,[12] and only 40% among 20 year old patients will be free of complications. The most common cause of death related to iron overload is heart disease, followed by infection and liver disease.[13]

Even with chelation, red cell transfusion incurs other risks including transmission of blood borne pathogens such as HIV and hepatitis C, as well as the risk of transfusion reactions. Alloimmunization makes access to blood products more difficult, and even more expensive to continue. Deferoxamine, although necessary to prevent iron overload, is itself not benign. Side effects from iron chelation include visual and auditory neurotoxicity, and allergic reactions, including anaphylaxis.[14-17] Moreover, the therapy is not well tolerated, impact on quality of life, and often results in poor compliance, especially in adolescents. There are oral alternatives now, deferasirox and deferiprone, with equivalent iron unloading, more tolerable side effect profile, and improved compliance.

The only available cure for β-thalassemia major is bone marrow transplantation; however, myeloablative BMT has many potential associated risks. Risk groups have been identified based upon three variables: hepatomegaly >2 cm, portal fibrosis of any degree, and inadequate compliance with chelation therapy, with Class I, II, and III having none, 1-2, or all three of the variables, respectively. Bone marrow transplantation is most successful in Class I children who undergo transplantation early, with a DFS of 90-93% and TRM of 3-4%.[18] For higher risk patients, the survival and DFS decrease to 86 and 82% and drops further to 62 and 51% in the highest risk category. Results in adults (>16 years of age) are similar at 65 and 62% disease free and overall survival, respectively.[19] These studies have been performed using HLA-matched sibling donors. Although there are newer iron chelators available, cost/access to medication and compliance continues to be the main issues in suboptimal chelation. In the present day, the morbidity associated with iron overload remains, and extends into transplant related outcomes.

As adults have a higher rejection rate and also a higher incidence of GVHD, better conditioning regimens are needed. For high risk children (Class II or III), improvements have been seen when using lower dose conditioning, resulting in less morbidity and mortality, suggesting the rational to apply nonmyeloablative methods to adults.[20, 21] The use of Pesaro classification are still being used currently.

3.0 SCIENTIFIC AND CLINICAL JUSTIFICATION

Unlike patients who undergo allogeneic peripheral blood stem cell (PBSC) transplantation for malignant indications, patients with non-malignant disorders such as SCD and thalassemia do not require full and/or rapid donor engraftment to cure their disease.[9, 22-24] While it is generally accepted that GVHD is less severe in patients conditioned with low intensity preparative regimens,[25] graft rejection is preferable to debilitating GVHD in the setting of severe congenital anemias.

Host T and B lymphocytes remaining at the time of allogeneic HSCT can mediate a host-versus-graft response (rejection) that results in failure to engraft. In our current study (03-H-0170) of allogeneic HSCT using matched sibling donors with the platform of host conditioning with alemtuzumab followed by 300 cGy of TBI, the overall graft rejection rate is 10-15% (success rate of 85-90%); 0% in sex-matched pairs, 14% in female-into-male recipient, and 50% in male-into-female transplant combination (P=0.0164 Fisher's exact test, male-to-female compared to all other combinations together). Higher graft rejection rate seen in male-into-female pairs has been observed in murine[26] and human transplant settings.[27, 28] Thus with our low intensity approach, antigens on the Y chromosome and perhaps other minor HLA antigens may represent important immunologic barrier to overcome.

Another limitation of the current study is pre-existing red cell antibody to donor red cells. One patient who had anti-JKa antibody pre-transplant; this antibody persisted post-transplant, caused red cell aplasia for up to 1.5 years post transplant, and rendered him transfusion dependent during this time. There were two other patients who were reticulocytopenic and required red cell support for a similar duration post-transplant. Additionally, we have been excluding individuals with major ABO incompatibility or patients demonstrating strong titer red cell antibody to donor red cells. Thus in these settings, additional pre-transplant interventions are justified to reduce the risk of transplant failures. In this protocol, we hypothesize that adding immune-depleting and immune-suppressive chemotherapy regimen prior to the alemtuzumab/TBI therapy will reduce the risk of graft rejection. The chemotherapy regimen that we will evaluate consists of pentostatin plus daily, dose-adjusted, oral cyclophosphamide ("PC regimen").

Pentostatin is an FDA-approved medication that is primarily used for the treatment of hairy cell leukemia[29] and chronic lymphocytic leukemia.[30, 31] Pentostatin, which has a unique mechanism of action that involves inhibition of the enzyme, adenosine deaminase (ADA), that is deficient in a large proportion of patients with severe combined immune deficiency (SCID), also mediates significant immune suppression and immune depletion.[32] The immune modulating effects of pentostatin, which comprise a significant side effect in cancer therapy, can be harnessed in the transplantation setting to prevent graft rejection. An initial study by Pavletic et al demonstrated the lymphodepleting effects and safety of pentostatin (a total of 12 mg/m^2 over three days) when administered in sequence with nonmyeloablative doses of TBI (200 cGy) in the context of allogeneic HSCT.[33] In another study, pentostatin (a total of 8 mg/m^2 over two days) was safely administered in sequence with 600 cGy of TBI prior to allogeneic HSCT.[34] As such, pentostatin can represent an effective component to host conditioning regimens for the purpose of preventing graft rejection. However, to date, pentostatin has been used less frequently than other immune modulating conditioning, which has primarily involved the purine analogue fludarabine.

In murine transplantation models, we set out to compare the immune depleting and immune suppressive effects of pentostatin relative to fludarabine[35]. In these studies we found that pentostatin was advantageous relative to fludarabine because at a given level of host myeloid cell depletion, there was a significantly greater magnitude of host B and T cell depletion. In addition, host T cells that remained after pentostatin-based conditioning were significantly more immune supppresed relative to host T cells remaining after fludarabine-based conditioning. Importantly, in these studies, we found that optimal host immune modulation was achieved when intermittently dosed pentostatin was administered in combination with daily dosing of cyclophosphamide. These results indicated that, pentostatin, which can have a biological half-life (in terms of inhibiting the ADA enzyme) of up to one week, is best administered in combination with low-dose therapy with DNA alkylators to achieve maximal immune depletion while minimizing myeloid cell depletion. Finally, in these experiments, we found that host recipients of pentostatin/cyclophosphamide (PC) conditioning were significantly less likely to reject a fully MHC-disparate hematopoietic cell transplant relative to recipients of fludarabine/cyclophosphamide conditioning.

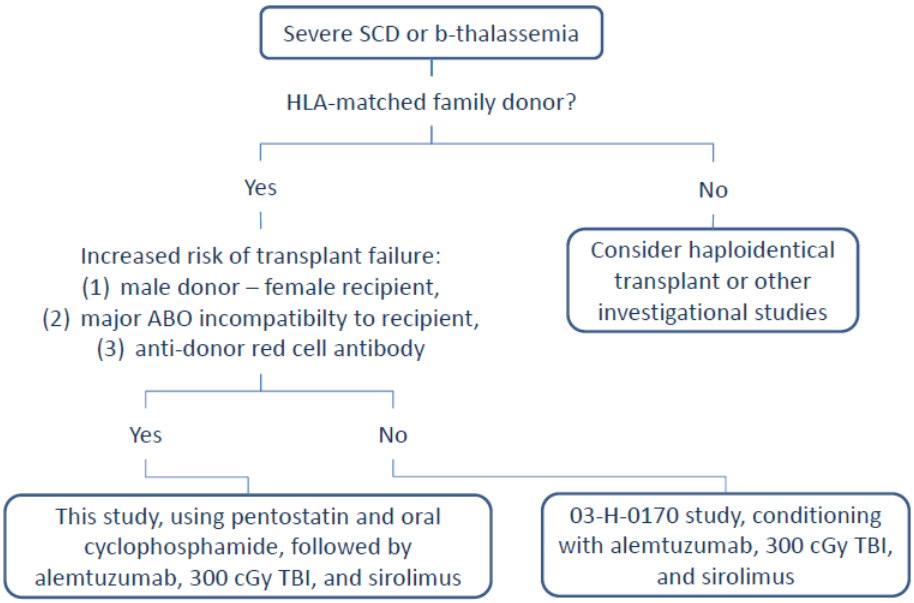
Based on these murine results, we initiated a pilot clinical trial of PC conditioning in the setting of HLA-matched sibling allogeneic HCT at the NIH Clinical Center (NCI Protocol 08-C-0088; Low Intensity Allogeneic Hematopoietic Stem Cell Transplantation Therapy of Metastatic Renal Cell Carcinoma Using Early and Multiple Donor Lymphocyte Infusions Consisting of Sirolimus-Generated Donor Th2 Cells). In an attempt to maximize host immune depletion with relative sparing of host myeloid cells, we designed a 21-day pre-transplant host conditioning regimen consisting of a total of 12 mg/m^2 of pentostatin administered in equal doses on days 1, 8, and 15 of the 21-day regimen; and, in this context, patients received a flat, daily dose of cyclophosphamide at the relatively modest dose of 200 mg per day, each day of the 21-day regimen. However, to ensure sparing of host myeloid cells, we also designed a dose adjustment scheme whereby daily doses of cyclophosphamide would be held in the event that an ANC value were to be reduced. Using this regimen, in 12 consecutive patients, we achieved the protocol-defined target level of immune depletion (end of regimen ALC value of < 200) without a single case of significant neutropenia (no case of grade 3 toxicity). And, each of the 12 patients achieved prompt alloengraftment without any case of graft rejection. These results indicate that the PC regimen can be safely administered and is effective when used alone for the prevention of graft rejection.

In other murine models, we next evaluated the ability of the PC conditioning to abrogate host immune responses against foreign protein, namely the anti-cancer immunotoxin SS1P.[36] Immunogenicity of foreign protein therapies is a significant clinical obstacle, as many patients are limited to only a single course of protein therapy due to neutralizing antibody formation; such antibody formation is induced by B cells, with significant cooperativity from host T cells. As such, we hypothesized that the PC regimen might be particularly effective for preventing host immunogenicity to SS1P. Indeed, in these murine studies, we found that the PC regimen safely modulated host immunity and allowed for up to six cycles of immunotoxin therapy without induction of neutralizing antibody formation. Based on these results, we initiated a clinical translation of SS1P therapy preceded by host immune modulation with the PC regimen (NCI Protocol 11-C-0160; A Pilot Study of Pentostatin Plus Cyclophosphamide Immune Depletion to Decrease Immunogenicity of SS1P in Patients with Mesothelioma). To improve the feasibility of the regimen, this protocol is evaluating a somewhat truncated version of the PC regimen, with pentostatin currently

being administered at a dose of 4 mg/m^2 (on days 1, 5, 9, and 13) with daily, dose-adjusted cyclophosphamide being administered on days 1 through 14 of the regimen. Initial results from the first 10 patients treated on this protocol indicate that the PC regimen indeed is safe and is effective in preventing or delaying host immune responses against the foreign protein therapy. In combination, the results of our studies in the allogeneic and autologous setting indicate that the dose-adjusted PC regimen can be safely administered and yield effective immune modulation.

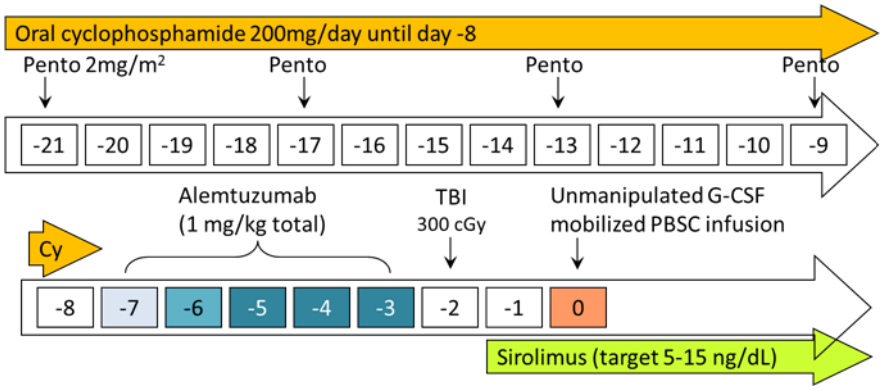
In sum, these results suggest that adding PC regimen may represent an effective modality to reduce graft rejection or antibody production against donor red cells in the setting of allogeneic HSCT in the population with SCD and β-thalassemia. The combined regimen already contain 3 lymphodepleting agents (pentostatin, cyclophosphamide, and alemtuzumab) 8-12mg of dexamethasone is used as premedication for each pentostatin dosing, and TBI. The addition of ritixumab pre-transplant for patients with pre-existing antibody to donor red cells may be necessary, especially in those with severe hemolysis from allo- (transfusion related) or auto-antibodies. We may have these patients undergo plasma exchange (section 7.2) if they have major ABO mismatch to their donors.

We hypothesize that the duration of neutropenia (ANC<500) with the PC and transplant regimen should be similar to the currently protocol, average of 16-21 days. An important objective of the current protocol will evaluate the safety and efficacy of the PC regimen when administered over a 14-day interval. To maximize safety of administration, the PC therapy is administered separately and followed by the alemtuzumab and TBI. And to further ensure safety of this regimen in a SCD or β-thalassemia patient that may have increased end-organ dysfunction relative to the cancer patient population, the dose of pentostatin will be reduced to a level of 2 mg/m^2/day, which has been shown to be effective in the lymphoid neoplasia setting.[31] We also intend to transplant male donors – female recipients first and measure appropriate antibody titers pre- and post-HSCT (see section 11.1). Knowing the duration of neutropenia and the degree of antibody reduction with this combined regimen will help us decide if any anti-plasma cell therapy, e.g. bortezomib, lenolidomide, and/or others, given pre- or post-HSCT, would be necessary.



4.0 STUDY DESIGN

Pentostatin will be given on days -21, -17, -13, -9 and oral cyclophosphamide from days -21 to -8, with the intention to be administered in the outpatient setting. Alemtuzumab will follow on days –7 to –3, and 300 cGy TBI on day –2. Sirolimus will start at a loading dose for three doses on day -1 and adjusted to maintain trough levels between 5-15 ng/mL. The PBSC graft targeted to deliver ≥10 x 10^6 CD34$^+$ cells/kg (minimum ≥5 x 10^6) will be infused on day 0.



The proposed number of subjects will include both cohorts. The first cohort of patients will be male donor – female recipients to see if this new regimen will yield higher rate of durable donor leukocyte chimerism. We will also measure anti-A, anti-B, and/or other red cell antibody titers from this initial cohort to determine the feasibility of transplanting patients with pre-existing antibodies (major ABO mismatch or other anti-donor red cell antibody). The information gathered from this cohort of patients will help to determine the degree of antibody titer reduction in those with pre-existing antibodies and determine if anti-plasma cell therapy is needed.

The second cohort of patients will be patients with pre-existing antibodies (major ABO mismatch or other anti-donor red cell antibody). The primary endpoint for this group will be different than the first cohort (section 11.1 and 11.2). It is very likely that red cell aplasia (6 months to 2 years post-transplant) and prolonged duration of red cell transfusion are expected in this second group, thus a later time point to determine treatment success is justified. Once we begin to enroll in the second cohort, enrollment in the first cohort may occur concurrently.

5.0 ELIGIBILITY ASSESSMENT AND PATIENT SELECTION

The target subject population is a subset of patients who have severe disease and HLA matched family donors, and who are at increased risk for transplant failure, defined as male donors and female recipients, major ABO incompatibility to the recipient (Appendix B), or presence of antibody in the recipients to donor red cells from alloimmunization. The remainder of the inclusion and exclusion criteria is very similar to the other HLA matched transplant study (03-H-0170).

5.1 **Inclusion criteria- recipients** (must fulfill one disease category in 5.1.1 and all of 5.1.2)

5.1.1 **Disease specific**

5.1.1.1 Patients with severe sickle cell disease (not limited to Hb SS, SC, or Sβ-thal) at high risk for disease-related morbidity or mortality, defined by having severe end-organ damage (A, B, C, D, or E) or potentially modifiable complication(s) not ameliorated by hydroxyurea or sickle specific therapy (F):

A. Stroke defined as a clinically significant neurologic event that is accompanied by an infarct on cerebral MRI or cerebral arteriopathy requiring chronic transfusion therapy; OR
B. Sickle cell-related renal insufficiency defined by a creatinine level ≥ 1.5 times the upper limit of normal and kidney biopsy consistent with sickle cell nephropathy OR nephrotic syndrome OR creatinine clearance less than < 50mL/min OR requiring peritoneal or hemodialysis[37-39]; OR
C. Tricuspid regurgitant jet velocity (TRV) of ≥ 2.5 m/s[40, 41] at baseline; OR
D. Recurrent priapism defined as at least 2 episodes of an erection lasting >4 hours involving the corpora cavernosa and corpus spongiosa[42]; OR
E. Sickle hepatopathy defined as EITHER ferritin >1000mcg/L OR direct bilirubin >0.4 mg/dL at baseline

Any one of the below complications:

F.

Complication	Eligible for hydroxyurea	Eligible for HSCT
Vaso-occlusive crises	At least 3 hospital admissions in the last year [4]	More than 1 hospital admission per year while on therapeutic dose of hydroxyurea or sickle cell therapy[2]
Acute chest syndrome	2 prior ACS	any ACS while on hydroxyurea [43]
Osteonecrosis of 2 or more joints	And significantly affecting their quality of life by Karnofsky score 50-60 (Appendix B)	And on hydroxyurea where total hemoglobin increases <1 g/dL or fetal hemoglobin increases <2.5 times the baseline level
Red cell alloimmunization	Transfusion dependent	Total hemoglobin increases <1 g/dL while on hydroxyurea

5.1.1.2 Patients with beta-thalassemia who have grade 2 or 3 iron overload, determined by the presence of 2 or more of the following:
- portal fibrosis by liver biopsy
- inadequate chelation history (defined as failure to maintain adequate compliance with chelation with deferoxamine initiated within 18 months of the first transfusion and administered subcutaneously for 8-10 hours at least 5 days each week)
- hepatomegaly of greater than 2cm below the costochondral margin

5.1.2 **Non-disease specific:**

5.1.2.1 Age ≥4 years

5.1.2.2 6/6 HLA matched family donor available

5.1.2.3 Ability to comprehend and willing to sign an informed consent

5.1.2.4 Negative β-HCG, when applicable

5.2 **Exclusion criteria –recipient** (any of the following would exclude the subject from participating)

5.2.1 ECOG performance status of 3 or more (See Appendix A)

5.2.2 Evidence of uncontrolled bacterial, viral, or fungal infections (currently taking medication and progression of clinical symptoms) within one month prior to starting the conditioning regimen. Patients with fever or suspected minor infection should await resolution of symptoms before starting the conditioning regimen.

5.2.3 Major anticipated illness or organ failure incompatible with survival from PBSC transplant

5.2.4 Pregnant or lactating

5.3 **Inclusion criteria –donor**

5.3.1 6/6 HLA matched family donor deemed suitable and eligible, and willing to donate, per clinical evaluations who are additionally willing to donate blood for research. Matched related donors will be evaluated in accordance with existing Standard NIH Policies and Procedures for determination of eligibility and suitability for clinical donation. Note that participation in this study is offered to all matched related donors, but is not required for a donor to make a stem cell donation, so it is possible that not all related donors will enroll onto this study.

5.4 **Exclusion criteria- donor**

5.4.1 None

6.0 CLINICAL EVALUATION OF THE PATIENT

Bone marrow aspirates and biopsies will be read by a pathologist. Samples will be ordered and tracked through the CRIS screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

6.1 Pre-Study Evaluation

	Pre-transplant Screening X= required O=Optional
Procedure: Consent Forms	
14-H-0077 Treatment consent	X
Procedure: History & Physical	
Medical history (to determine baseline symptoms)	X
Physical examination	X
ECOG or Karnofsky Performance Status, Lansky (pediatrics only)	X
Vital signs, Weight	X
Height	X
Procedure: Blood for clinical laboratory tests	
Local lab: Hematology	
CBC w/diff	X
Reticulocyte Count	X
PT/PTT	X
D-dimer	X
Hemoglobin Electrophoresis	X
Genotyping Analysis of Hemoglobin A, F, SS, SC or Sβ-Thalassemia patients and donors only	O
RBC Phenotype	X
STR profile, recipient	X
WBC chimerism (post-transplant)	
Type and antibody screen (anti-red cell antibodies)	X
Isohemagglutinin titer	X
Direct Antiglobulin Screen	X
CMV/EBV PCR; Quantitative, Blood -On the first day of Pentostatin	O
Local lab: HLA typing	
HLA – A, B, C and DR, DQ (low resolution)	X
HLA Confirmatory A,B,C, DRB1	X
HLA Antibody Screen- Class 1 A, B, C	X
HLA Antibody Screen - Class 2 DRB1, DQB1	X
KIR Genotype	X

Sequence Based HLA-C	X
Local lab: Chemistry	
Acute care panel	X
Mineral panel	X
Hepatic panel	X
Creatine Kinase	X
Lactate Dehydrogenase	X
Protein, total	X
Uric Acid	X
Lipid panel	X
Lipoprotein Profile	O
Apolipoprotein Panel	O
Iron and Transferrin (include ferritin)	X
Folate	O
Vitamin B12	O
Troponin I	X
B-HCG serum pregnancy (females only)	X
Pre-albumin	O
Local lab: Endocrine testing	
Thyroid Stimulating Hormone	X
T3/Triiodothyronine (total)	X
FT4/ Thyroxine, Free	X
Growth hormone	X
Insulin-like growth factor 1	X
Adrenocorticotropic hormone	X
Morning cortisol	X
Fasting insulin	X
Fasting glucose	X
Hemoglobin A1C (for Thalassemia patients)	X
Serum fructosamine (for SCD patients)	X
25 hydroxy Vitamin D	X
Follicle Stimulating Hormone (males and females)	X
Luteinizing Hormone (males and females)	X
Testosterone, Total and Free (males only)	X
Anti-mullerian hormone (females only)	X
Prolactin (females only)	X
Estradiol (females only)	X

Progesterone (males and females)	X
Local lab: Blood for DTM viral markers	
HBs Ag, Screening	X
Anti-HCV Antibody	X
Anti-HTLV-I/II Antibody	
Anti-HBc Antibody	
Anti-HIV-1/2 Antibody	
West Nile Virus	
HIV-1/HCV/HBV NAT	X
T. cruzi Antibody	X
~~RPR~~ RPR/ Syphilis Total Antibody, Serum	X
Anti-Cytomegalovirus IgG, IgM Antibody	X
Anti-EBV Antibody Panel	X
Anti-HAV Antibody, Total	X
Anti-HAV IgM	X
Anti-HBc IgM	X
Anti-HBs Antibody	X
Anti-HSV Type 1/2 Antibody, IgG	X
Anti-Toxoplasma IgG	X
Anti-Toxoplasma IgM (Mayo)	X
Anti-Varicella-Zoster Virus IgG	X
Local lab: Blood for immunological studies	
Lymphocyte Phenotyping TBNK	X
Immunoglobulins, Quantitative	X
Pro-BNP/ Pro Brain Natriuretic Peptide	X
Haptoglobin	X
Local lab: Evaluate sickle nephropathy	
Blood	
Cystatin C	X
CRP, High Sensitivity, Comprehensive	X
Osmolality, serum	X
Random urine	
Urinalysis	X
Phosphorus, Inorganic, Urine	X
Uric acid	X
Protein /Creatinine Ratio	X
Albumin/Creatinine Ratio	X

Osmolality, urine	X
24 hour urine	
Creatinine	X
Protein	X
Albumin	X
Phosphorus, Inorganic, Timed Urine	X
Uric acid	X
Creatinine clearance	X
Electrophoresis, Timed Urine	X
Blood for storage: Research samples	
Pre transplant research labs (One 10ml EDTA and one 4mL SST)	X
CD34 Sample to be drawn when collecting autologous PBCS for backup: -Draw Pre Plerixafor and approximately 4hrs post Plerixafor (at the start of apheresis) labs. The timing of these blood draw may be changed per PI discretion.	X X
Procedures/ Consults:	
Collection of Autologous PBSC for Backup	O
Bone Marrow Harvest for Backup	O
ACTH stimulation (CRIS order: Cortisol Serial 01-08)	X
Oral glucose tolerance test (OGTT)	X
Echocardiogram	X
EKG	X
Pulmonary function tests	X
6-min walk	X
24 hour Holter monitor	X
Chest x-ray (CRIS order: DX Chest-Pa & Lat)	X
Bone marrow aspirate and biopsy	X (cytogenetics, next gen sequencing – mayo test NGSHM)
Liver MRI	X
Cardiac MRI	X
Bone dexa scan	X
Reproductive health assessment	X
Sperm / testicular tissue or oocyte banking, if requested	X
Brain MRI/MRA	X
Quality of life tests: Neuropsychology/PROMIS-57	X
Dental consult	X
Ophthalmology consult	X

Social work consult	X
Pre-transplant education class	X
Venous assessment	X
Exchange or simple transfusion	X
Transfusion Medicine Consult	X
Abdominal ultrasound	X
Miscellaneous protocol requirements:	
Plasma exchange (major ABO mismatch recipient as needed)	O
Discontinue: Iron chelation 2 days before starting PC regimen	X
Discontinue: Hydroxyurea or other SCD therapy 1-3 days prior to starting PC regimen	X

6.2 Day 0- Day 100

X=Required O=Optional	**Inpatient**	**Day 30 (+/-7 days)**	**Day 60 (+/-7 days)**	**Day 100 (+/-7 days)**
Procedure: History & Physical				
Medical history (to determine baseline symptoms)	Per Inpatient team	X	X	X
Physical examination	Per Inpatient team	X	X	X
ECOG or Karnofsky Performance Status, Lansky (pediatrics only)				X
Vital signs, Weight	X	X	X	X
Procedure: Blood for clinical laboratory tests				
Local lab: Hematology				
CBC w/diff	X	X	X	X
Reticulocyte Count	X twice weekly			
PT/PTT	X twice weekly	X	X	X
Hemoglobin electrophoresis		X	X	X
RBC Phenotype				O
WBC chimerism		X	X	X
Type and antibody screen (anti-red cell antibodies)	X weekly	X	X	X
Isohemagglutinin titer				X
CMV/EBV PCR; Quantitative, Blood	X weekly	X	X	X
Local lab: Chemistry				
Acute care panel	X	X	X	X
Mineral panel	X	X	X	X
Hepatic panel	X	X	X	X

Creatine Kinase		X	X	X
Lactate Dehydrogenase		X	X	X
Uric Acid		X	X	X
Lipid panel	X every 2 weeks	X	X	X
Iron and Transferrin (include Ferritin)				X
B-HCG serum pregnancy (females only)				O
Pre-albumin	X twice weekly			
Sirolimus level	X 2-3 per week	X	X	X
Local lab: Evaluate sickle nephropathy				
Random urine				
Urinalysis		X	X	X
Protein Creatinine Ratio, Urine		X	X	X
Albumin/Creatinine Ratio, Urine		X	X	X
Blood for storage: Research samples				
Post-Transplant Research labs (One 6ml EDTA and one 4mL SST)		X	X	X
Campath research levels (One 4mL SST)- ***Day 7, 14, 21, 28/30 post- transplant (+/-2 days***)	XXXX*	X		
Procedures/ consults:				
Echocardiogram				X
Pulmonary function tests				X
6-min walk				X
Bone marrow aspirate +/- biopsy				O
Quality of life tests: Neuropsychology/PROMIS-57				X (Monitoring Test Battery)

**denotes multiple procedures*

6.3 Beyond Day 100

It is our intention to have patients return for all these follow-up visits. However, some patients, especially international ones, may have financial, visa related, or other difficulties that limit their visits. In those situations, we will work with their local physicians to get some of the testing done and/or mail kits for blood testing and provide vaccinations if needed.

X=required; O=optional	**M6 (+/- 1month)**	**M12 (+/-1 month)**	**M18 (+/-1 month)**	**M24 (+/-1 month)**	**M36 (+/-6 month)**	**M48 (+/-6 month)**	**M60 (+/-6 month)**
Procedure: History & Physical							
Medical history (to determine baseline symptoms)	X	X	O	X	X	X	X
Physical examination	X	X	O	X	X	X	X
ECOG or Karnofsky Performance Status, Lansky	O	X	O	X	X	X	X

X=required; O=optional	**M6 (+/- 1month)**	**M12 (+/-1 month)**	**M18 (+/-1 month)**	**M24 (+/-1 month)**	**M36 (+/-6 month)**	**M48 (+/-6 month)**	**M60 (+/-6 month)**
(pediatrics only)							
Vital signs, Weight	X	X	O	X	X	X	X
height	O	X	O	X	X	X	X
Procedure: Blood for clinical laboratory tests							
Local lab: Hematology							
CBC w/diff	X	X	O	X	X	X	X
Reticulocyte Count	X	X	O	X	X	X	X
PT/PTT	X	X	O	X	X	X	X
D-dimer	X	X	O	X	X	X	X
Hemoglobin electrophoresis	X	X	O	X	X	X	X
RBC Phenotype	O	O	O	O	O	O	O
WBC chimerism	X	X	O	X	X	X	X
Type and antibody screen (anti-red cell antibodies)	X	X	O	X	O	O	O
Isohemagglutinin titer	X	X	O	X	O	O	O
CMV/EBV PCR	X	X	O	X	O	O	O
Local lab: Chemistry							
Acute care panel	X	X	O	X	X	X	X
Mineral panel	X	X	O	X	X	X	X
Hepatic panel	X	X	O	X	X	X	X
Lactate Dehydrogenase	X	X	O	X	X	X	X
Lipid panel	X	X	O	X	X	X	X
Iron and Transferrin (include Ferritin)	X	X	O	X	X	X	X
B-HCG serum pregnancy (females only)	O	O	O	O	O	O	O
Sirolimus level	X	X	O	O	O	O	O
Local lab: Endocrine testing							
Thyroid Stimulating Hormone	O	X	O	X	X	X	X
T3/Triiodothyronine (total)	O	X	O	X	X	X	X
FT4/ Thyroxine, Free X	O	X	O	X	X	X	X
Serum fructosamine	O	X	O	X	X	X	X
25 hydroxy Vitamin D	O	X	O	X	X	X	X
Follicle Stimulating Hormone (males and females)	O	X	O	X	X	X	X
Luteinizing Hormone (males and females)	O	X	O	X	X	X	X

X=required; O=optional	**M6 (+/- 1month)**	**M12 (+/-1 month)**	**M18 (+/-1 month)**	**M24 (+/-1 month)**	**M36 (+/-6 month)**	**M48 (+/-6 month)**	**M60 (+/-6 month)**
Testosterone, Total and Free (males only)	O	X	O	X	X	X	X
Anti-mullerian hormone (females only)	O	X	O	X	X	X	X
Estradiol (females only)	O	X	O	X	X	X	X
Progesterone (females only)	O	X	O	X	X	X	X
Local lab: Blood for immunological studies							
Lymphocyte TBNK	O	X	O	X	O	O	O
Immunoglobulins, quantitative	O	X	O	X	O	O	O
Haptoglobin	O	X	O	X	O	O	O
Local lab: Evaluate sickle nephropathy							
Blood							
Cystatin C	O	X	O	X	O	O	O
CRP, High Sensitivity, Comprehensive	O	X	O	X	O	O	O
Random urine							
Urinalysis	O	X	O	X	O	O	O
Protein/Creatinine ratio	O	X	O	X	O	O	O
Albumin/Creatinine ratio	O	X	O	X	O	O	O
Blood for storage: Research samples							
Post-Transplant Research Labs (One 6ml EDTA)	X	X	O	X	X	X	X
Procedures/ consults:							
Echocardiogram	O	X	O	X	O	O (one betw yr 4 and 5)	O
Pulmonary function tests	O	X	O	X	O	O (one betw yr 4 and 5)	O
6-min walk	O	X	O	X	O	O (one betw yr 4 and 5)	O
Brain MRI/MRA	O	X	O	X	O	O (one betw yr 4 and 5)	O
Bone dexa scan	O	X	O	X	O	O (one betw yr 4 and 5)	O
Bone marrow aspirate +/- biopsy	O	O	O	O	O	O	O
Liver MRI	O	O	O	O	O	O	O
Cardiac MRI	O	O	O	O	O	O	O
Reproductive health assessment	O	X	O	X	O	O	O
Quality of life tests: Neuropsychology PROMIS-57	O	X	O	X	O	O	O

After 5 years follow-up visits are not mandatory, but yearly communication with the patient and the referring physician may continue.

7.0 TREATMENT PLAN

7.1 Exchange Transfusion (Appendix C)

Prior to the PC regimen, those patients with SCD who are not routinely (exchange) transfused may undergo an exchange transfusion per Department of Transfusion Medicine (DTM) procedure for a goal HbS $\leq$30% just prior to receiving the preparative regimen in order to decrease the likelihood of neurologic and other sickling events that may be precipitated by the transplant procedure. Exchange transfusion may be omitted, and rituximab may be used instead, in selected individuals with prior severe transfusion or auto-antibody related hemolysis.

7.2 Plasma Exchange

For patients with major ABO mismatch, plasma exchange may be performed prior to the PC regimen as this procedure is effective in removing these antibodies, which are more of the IgM type. For patients with antibodies to minor antigens on donor red cells, which are typically IgG type and less efficiently removed by plasma exchange, we will discuss with DTM and consider plasma exchange.

7.3 Collection of Autologous Peripheral Blood Stem Cells (PBSC) (Appendix C)

Collection of autologous PBSC for backup in case of graft rejection and delayed autologous recovery may be performed before the start of the preparative regimen in patients with thalassemia. Use of G-CSF as a mobilizing agent has yielded unreliable results in this setting. However, plerixafor has been effectively used as a sole agent to mobilize stem cells in patients with severe β-thalassemia, including those with and without prior splenectomy[44]. The target stem cell dose will be $\geq$ 2 x 10^6 CD34 cells/kg, and the minimum dose for proceeding to transplant will be $\geq$ 1 x 10^6 CD34 cells/kg. If use of plerixafor does not result in collection of a sufficient number of CD34 cells, G-CSF may be added to plerixafor in a subsequent synergistic attempt to improve CD34 yield. Both agents have also been used to safely mobilize a patient with thalassemia[44].

Collection of back up hematopoietic stem cells in individuals with SCD poses risks that outweigh the potential benefits. G-CSF has been associated with extensive morbidity (including vaso-occlusive crises, acute chest syndrome, and multi-organ failure) and mortality in patients with SCD[45]. Plerixafor is currently being studied in patients with SCD, so the risks are unknown. Bone marrow harvest is also being studied in patients with SCD undergoing gene therapy studies. The risk of general anesthesia may be significant in patients with severe disease, and fluid shifts associated with removal of bone marrow may lead to significant morbidity. Therefore, the risks of autologous cell collection may outweigh the benefits in patients with SCD, and hence autologous cells will not routinely be collected in that patient population. There may be clinical situations where autologous cells may be collected, e.g. a small or young donor with a very large recipient, less than target number of stem cells collected from the donor, a recipient having HLA-antibodies, or other concern that may hinder engraftment.

7.4 Peripheral blood progenitor cell collection (Appendix C)

Donors will sign NHLBI standard of care protocol for HSC mobilization and collection. This section further clarifies the target HSC dose and type of HSC graft needed for this study. The target collection number for progenitor cells is $\geq$10 x 10^6 CD34+ cells/kg.

For adult donors, this product will be collected in advance and cryopreserved. The volume processed per apheresis procedure will be determined by DTM medical staff on the day of apheresis, based on peak CD34 cell mobilization response to filgrastim (G-CSF) and the CD34 cell dose needed, based on kilogram weight of the recipient. This will range from 15 to 35 liters processed per day for 2 to 3 days, not to exceed a total of 75 liters over 3 days. A day 3 apheresis procedure will only be performed if the minimum dose of 5 million per kg is not met after the first two day collection. The minimum dose for proceeding to transplant will be $\geq$ 5 x 10^6 CD34 cells/kg. In order to meet the minimum dose, the donor may undergo a second mobilization a minimum of 2 weeks later. If after two such attempts,

an inadequate cell number has been collected, the patient and donor will be withdrawn from the protocol, unless another donor is available. Red cells in the graft may be removed in major ABO incompatible grafts and minor ABO incompatible grafts may have plasma removed per Transfusion Medicine protocol. Additional mobilization agent, e.g. plerixafor, may be used with GCSF as per standard of care/Bone Marrow Transplant Consortium Standard Operating Procedures.

For adult donors who undergo G-CSF mobilization and PBSC collection outside of the NIH Clinical Center, the volume of each apheresis to be processed and the days of apheresis will be determined by the institutional practice of that outside facility. The goal is to target ≥5 x 10^6 CD34 cells/kg. Also, refer to sections 8.1.15 and 8.2.

Pediatric donor cell collections will be done as per routine standard of care/Bone Marrow Transplant (BMT) Consortium Standard Operating Procedures. PBMC will be cryopreserved at the NIH Department of Transfusion Medicine until use.

7.5 Preparative regimen

All drugs will be given intravenously if possible based on the dosing formulation. All other concomitant medications or special procedures noted in the protocol shall follow standard NHLBI transplant protocols/procedures. Iron chelation should be discontinued about 2 days before initiating the pentostatin and cyclophosphamide (PC) regimen. Hydroxyurea should be discontinued 1-3 days prior to first dose of PC regimen to avoid overlapping myelosuppression.

The 14-day PC regimen will be administered just proximal to the alemtuzumab and the TBI with the intent to be administered as an outpatient. As such, pentostatin will be administered at a dose of 2 mg/m^2 on days -21, -17, -13, and -9. A total pentostatin dose of 8 mg/m^2 will be utilized, which is equal or somewhat reduced dose relative to previous transplantation studies. On the day of each pentostatin infusion, 8-12 mg of dexamethasone (IV or PO) and 8mg IV or 16-24 mg PO of ondansetron will be administered 30-60 minutes prior to, and 1 L of 0.9% saline will be infused over 60-120 minutes prior to pentostatin infusion. Pentostatin, diluted in 500 mL of 0.9% sodium chloride, will then be infused IV over 30-60 minutes. Oral dexamethasone of 4mg may be administered on days -20, -19; -16, -15; -12, -11; -8 and -7; oral ondansetron, 8mg twice daily, may be administered throughout the 14-day PC regimen. Changes in the anti-emetics, hydration, or administration of pentostatin are allowed at the discretion of the PI or designee.

Although pentostatin is >90% renally excreted, there is no established consensus on how to dose in those with impaired renal functions. Page 2 of pentostatin package insert reports that after a dose of 4mg/m^2, the terminal half-life was measured to be 6 hours in patients with creatinine clearance (CrCl) >60 mL/min vs 18 hours in those with CrCl <50 (http://hemonc.org/docs/packageinsert/pentostatin.pdf). Lathia and colleagues[46] reduced pentostatin dosing based on CrCl and found the drug exposure and toxicity was comparable in all groups (CrCl >60 mL/min – 4 mg/m^2, 41-60 mL/min – 3 mg/m^2, 21-40 – 2mg/m^2, ≤20 mL/min – 1 mg/m^2). Thus serum creatinine or cystatin C levels will be obtained prior to each dose of pentostatin, and since our starting dose is already at 2mg/m^2, dose adjustment for renal dysfunction is as follows:

- 2 mg/m^2 of pentostatin if CrCl ≥ 60 mL/min/1.73m^2, 1.5 mg/m^2 of pentostatin if CrCl 40-59 mL/min/1.73m^2
- 1.0 mg/m^2 of pentostatin if CrCl 20-39 mL/min/1.73m^2, 0.5 mg/m^2 of pentostatin if CrCl <20 mL/min/1.73m^2

Estimate of creatinine clearance is obtained by 24-hour urine, creatinine or cystatin C.

$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times [0.85 \; if \; Female]}{72 \times \text{Serum Creatinine (in mg/dL)}}$$

Cyclophosphamide will be administered at a daily dose of 200 mg per day on days -21 through -8 as per the schema detailed below. In children <16 years of age, 3mg/kg of cyclophosphamide, rounded to the nearest 25mg increment

(not to exceed 200mg), will be used. Cyclophosphamide will be either reduced or omitted depending on whether the lymphocyte depletion target has been achieved and whether there is any myeloid cell toxicity. The stated goal of the PC regimen is to enter the interval of alemtuzumab therapy with substantial immune depletion (absolute lymphocyte count [ALC] value < 100) and without grade 3 neutrophil toxicity (absolute neutrophil count [ANC] value <1000).

Cyclophosphamide dose adjustment based on ALC and ANC values						
Day of cycle[1]		ALC value at time of evaluation		ANC value at time of evaluation[2]		Cyclophosphamide dose[3]
-21		Any		> 1000		200
-17		≥ 400		> 1000		200
		200-399		500-999		100
		< 200		< 500		0
-13		≥ 200		> 1000		200
		101-199		500-999		100
		< 100		< 500		0
-9		≥ 100		> 1000		200
		50-99		500-999		100
		< 50		< 500		0

[1] Pentostatin will not be dose-adjusted based on ALC/ANC values.
[2] For ANC values <1000, decrease in cyclophosphamide dosing only – patients will not receive G-CSF.
[3] Cyclophosphamide dose indicated will be continued daily until the next ALC/ANC measurements (performed on days -17, -13, and -9). For young children, dose will be 3mg/kg, rounded to the nearest 25mg increment.

Days –7 to –3 Alemtuzumab (Campath®) IV given in an escalating dose over a total of 5 days Diphenhydramine doses and/or route of administration can be adjusted as per inpatient or research team
Day –7: Diphenhydramine 1mg/kg (maximum 50mg) IV and acetaminophen 10-15mg/kg (maximum 650mg) PO, then followed 30 minutes later by alemtuzumab 0.03mg/kg in 100mL normal saline infused over 2 hours
Day –6: Diphenhydramine 1mg/kg (maximum 50mg) IV and acetaminophen 10-15mg/kg (maximum 650mg) PO, then followed 30 minutes later by alemtuzumab 0.1mg/kg in 100mL normal saline infused over 2 hours
Day -5 to Day -3: Diphenhydramine 1mg/kg (maximum 50mg) IV and acetaminophen 10-15mg/kg (maximum 650mg) PO, then followed 30 minutes later by alemtuzumab 0.3 mg/kg in 100mL normal saline infused over 2 hours. For obese patients, alemtuzumab may be capped at 30mg per dose
Day -2: Total body irradiation, dose of 300 cGy, delivered as per the Department of Radiology standard of practice
Day –1: Sirolimus (Rapamune®) will be started at 5mg PO q4h x three doses at day -1, and continued at 5mg PO daily to target trough levels of 5-15 ng/mL. For children <16 years of age, 1mg/m2 PO q4h (not to exceed 5mg per dose) x three doses at day -1, and continued 1mg/m2 PO daily will be administered.
Day 0: Infusion of unmanipulated G-CSF - mobilized peripheral blood stem cells

7.6 TBI

Energy: All patients should be treated with a linear accelerator using energies higher than 4MV.

Timing: It is anticipated that TBI will be delivered on day -2.

Technique: TBI will be delivered with lateral fields using extended SAD values of 600cm. Tissue compensators will be used as appropriate for all patients. Gonadal shielding will be used in male patients.

Dose/Fractionation: Patients will receive TBI to a total dose of 300 cGy in a single fraction.

Dose Modifications: Occasionally, the total dose/technique of TBI may require modifications due to patient factors (unexpected or severe (grade 4-5) adverse events, serious medical illnesses not conducive to stable patient transfer, patient refusal, etc) or treatment factors (linear accelerator machine offline, etc.) Modifications to the radiation treatment will be at the discretion of the treating radiation oncologist in collaboration with the Principal Investigator.

7.7 GVHD prophylaxis

Sirolimus (Rapamune®) will be started on day -1 with goal trough levels of 5-15ng/ml. The dose may be reduced in the presence of hepatic or renal insufficiency. The sirolimus will be given for a minimum of one year; however, the total duration of administration will be determined by the presence or absence of GVHD and the level of donor /recipient chimerism.

If at least one year post-transplant the recipient displays >50% lymphoid donor chimerism, the sirolimus dose can be tapered. Donor/recipient chimerism levels will be checked periodically during and after the taper. If lymphoid and/or myeloid donor chimerism levels decrease by $\geq$ 20%, the sirolimus dose may be increased back to the most recent dose at which donor chimerism was maintained; other immunosuppression may be used as clinically indicated.

Subjects will be advised not to take medication with grapefruit juice and not to take St. John's wort while on sirolimus. Subjects must also be advised about limiting exposure to sunlight and UV light due to an increased risk of skin cancer. Women of childbearing potential will be informed of the potential risks during pregnancy and that they should use effective contraception prior to initiation of drug.

7.8 Central venous line placement

A central venous catheter will be placed by a surgeon, interventional radiologist, or vascular access device specialist prior to transplantation.

7.9 Infection Prophylaxis

Penicillin VK 250 mg PO BID (or equivalent) from day 0 until pneumococcal vaccinations are completed post-transplant. Prophylaxis of pneumocystis will begin when neutrophil engraftment is established, which may be later than day 30. Because of possible liver dysfunction, nystatin or micafungin may be used first line, but other anti-fungal agents may be substituted for prophylaxis. Prophylaxis and treatment of infections will otherwise be administered according to BMT consortium guidelines.

Patients will be offered influenza vaccination, as seasonally indicated, when they are at least 6 months post-transplant per CDC HSCT guidelines.

7.10 Fever Regimen: see BMT Consortium guidelines

7.11 Bleeding prophylaxis and blood product support (See Appendix C)

When possible, platelet counts will be maintained at or higher than 50,000/ul for patients with SCD (which is higher than usually maintained for non-sickle cell patient transplants) throughout the transplant to diminish the risk of intracranial bleeding. Peri-transplant target hemoglobin will be kept above 9 g/dL in patients with SCD. Otherwise, packed red blood cell and platelet transfusions will be given according to BMT consortium guidelines.

7.12 Contraindication

Filgrastim (G-CSF) is a relative contraindication for all patients with sickling disorders and will only be given with the consent of the principal investigator, lead associate investigator, or the attending protocol investigator.

7.13 **Cardiac MRI**

Cardiac MRI may be performed at baseline and 1 year post-transplant to assess factors such as cardiac structure, cardiac volumes, left ventricular ejection fraction, iron deposition, myocardial fibrosis, and extracellular volume fraction with gadolinium enhanced methods where indicated. We may evaluate the effect of transplant on those factors.

8.0 MANAGEMENT OF PATIENT COMPLICATIONS

The major complications are viral reactivation, acute and chronic GVHD, and relapse of the original disease. Patients with these complications will be treated along the following lines:

8.1 **Viral reactivation:** see BMT consortium guidelines

8.2 **Acute GVHD:** Sirolimus may be continued and treated according BMT consortium guidelines

8.3 **Chronic GVHD:** Sirolimus may be continued and treated according BMT consortium guidelines

8.4 **Graft rejection and falling chimerism (poor engraftment):** This transplant protocol uses a nonmyeloablative preparative regimen. Therefore, autologous recovery (relapse of disease) is anticipated in patients who fail to engraft. For falling chimerism in the myeloid and/or CD3 compartment, another course of alemtuzumab as in the conditioning or unselected stem cell product ('boost') from the original donor may be used after preconditioning- with Busulfan IV 3.2 mg/kg on days -5, -4, and -3, and Campath IV 0.03mg/kg on day -7, 0.1mg/kg on day -6, and 0.2mg/kg on days -5 and 4, followed by sirolimus or equivalent immunosuppressant as in protocols 14-H-0111 and 15-H-0098. These medications are widely used at the Clinical Center and other transplant centers, and their side effects are well known. Autologous rescue collection before stem cell boost is likely not needed, but may be considered if one or more risk factors for pancytopenia post infusion are present (e.g. age >35, low stem cell number of the boost, or low blood counts before at the start of the pre-conditioning regimen).

For patients who had initial donor engraftment and subsequent graft rejection (donor myeloid and CD3 chimerism of 0%), there is currently no standard approach; watch and wait until resurgence of sickle related complications (pain crisis, acute chest syndrome, etc), restart hydroxyurea or sickle specific therapy, or another stem cell transplant are all options one can consider. We will discuss these options, and the patient can be offered to participate in other applicable clinical trials, or be referred back to their primary physician depending on what is considered to be in the best interest of the patient.

8.5 **Post-transplant hemolysis**: IVIg, corticosteroids, eculizumab, rituximab, or other therapies may be used to treat the hemolysis as per published literature or practice guidelines.

9.0 RESEARCH STUDIES

The amount of blood that may be drawn from adult recipients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period.

For pediatric recipients, no more than 5 mL/kg may be drawn for research purposes in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period.

Up to 50 mL of donor (adult or pediatric) blood may be collected prior to cell donation (and prior to GCSF mobilization) for research. Once a standard leukapheresis product from a donor has been found to meet target cell dose, 1mL of additional cells from the collection can be used for research under this protocol.

Storage: Research specimens will be stored under the supervision of the principal investigator. Samples will not be labeled with the patient's name. Samples will be assigned a unique code known only to the principal investigator, which will serve as a link to the patient's clinical information collected as part of this research protocol. No samples will be provided to investigators outside the branch without IRB notification and an executed MTA. Therefore confidentiality is protected.

Tracking: Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Samples will not be sent outside NIH without IRB notification and an executed MTA.

End of study procedures: Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

Loss or destruction of samples: Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

Data management: The principal investigator will be responsible for overseeing entry of data into an in-house password protected electronic system or locked research file system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contract data manager will assist with the data management efforts.

All human subjects' personally identifiable information (PII), eligibility, and consent verification will be recorded. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant, e.g., study-specific identifying number (SSPIN or other unique code, or minimum PII required for subject identification.

Data will be abstracted from Clinical Center progress notes as well as from progress notes forwarded from home physicians. Laboratory data from NIH will be imported electronically from CRIS into an in-house database. Laboratory values from referring home physicians will be entered into the system.

End of study procedures: Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value.

Loss or destruction of data: Should we become aware that a major breech in the plan to protect patient confidentiality and trail data has occurred, the IRB will be notified. Data will not be sent outside NIH without IRB notification and an executed MTA or CTA.

9.1 Chimerism studies

We will use PCR analysis of microsatellites to identify the contribution of the donor marrow to post transplant hematopoiesis and to detect donor lymphocytes according to the schedule listed in Section 6.0.

9.2 Bone Marrow Samples – exploratory

Up to 32 mL of bone marrow aspirate and biopsy will be collected for research studies at the pre-transplant evaluation. Bone marrow aspirate and/or biopsy may be collected at day 100 post-transplant, and/or when sustained donor erythroid chimerism is attained or if clinically indicated. These may help elucidate the contribution of the progenitor cells to the circulating component.

9.3 Transthoracic Echocardiography – exploratory

Transthoracic echocardiography will be performed to assess TR jet velocity at study onset Echoes may be performed yearly post transplantation, or more frequent as clinically indicated.

9.4 Immune reconstitution – exploratory

Lymphocyte subpopulations (percentage and absolute number of CD3, CD4, CD8, CD16, CD56, and CD19 cells) and immunoglobin levels (IgG, IgA, and IgM) will be qualified
according to Section 6.0.

9.5 Assessment of cytokines and lymphocyte function – exploratory

Donor serum and lymphocytes will be collected pre-transplant. Patient serum and lymphocytes will be collected pre-transplant and post-transplant. Serum tumor necrosis factor, interferon-gamma, and interleukin-17 may be quantified. Levels may continue to be followed based on the results.

Patient effector T cell subsets may be measured at each time point by intracellular cytokine staining for interferon gamma, interleukin-4, interleukin-17, and FoxP3. Biochemical analysis of mTOR signaling complexes in patient T cells will be measured by Western blot for phospho p70 S6 kinase and phospho AKT in response to anti CD3 plus anti CD28 stimulation in vitro.

CMV pp65 specific T cell responses may be measured in cases where either donor or recipient is CMV positive. These will be assessed by intracellular cytokine staining for interferon-gamma and FoxP3 in response to pooled overlapping pp65 peptides.

In cases where patient/donor pairs have received influenza vaccine due to the seasonal timing of the transplant, we may measure influenza specific T cell responses by intracellular cytokine staining for interferon-gamma, interleukin-4, interleukin-17, and FoxP3 in response to influenza peptides.

In cases where GVHD or graft rejection is observed, we may assess donor/host allo-reactivity by CFSE dilution assay in the presence and absence of sirolimus in vitro. We may also measure mTOR signaling in patient T cells by Western blot for phospho p70 S6 kinase and phospho AKT in response to anti CD3 plus anti CD28 stimulation in the presence or absence of sirolimus in vitro.

All of these research tests may be run at the laboratory of Dr. Jonathan Powell at Johns Hopkins Hospital. Samples will be assigned a unique code known only to the principal and associate investigators. Samples will not be sent outside NIH without an executed material transfer agreement or collaborative research agreement.

9.6 **Neuropsychologic testing**: see Addendum A – exploratory

9.7 **Quality of life testing:** see Addendum A – exploratory

9.8 **Other potential studies:** as described on the IRB list of approved Laboratory Research Studies

10.0 BIOSTATISTICAL CONSIDERATIONS

10.1 Study Endpoints and Sample Size

Cohort 1: male donors – female recipients, the primary endpoint is treatment success at **1 year** post-transplant, defined as sustained donor type hemoglobin on hemoglobin electrophoresis for patients with SCD and transfusion independence for patients with β-thalassemia. We intend to enroll 4-6 patients in this cohort before enrollment in the next cohort. Once the enrollment in cohort 2 has begun, simultaneous enrollment to both cohorts can occur.

Cohort 2: patients with pre-existing antibody to donor red cells (either major ABO mismatch or other anti-donor red cell antibody), the primary endpoint is presence of donor red cells, detected by hemoglobin electrophoresis or donor type red cell antigen, and reticulocyte count ≥30 k/uL at **2 years** post-transplant. It is very likely that red cell aplasia (6 months to 2 years post-transplant historically observed at NIH in non-myeloablative transplants) and prolonged duration of red cell transfusion are expected in this second group, thus a later time point to determine treatment success is justified.

This study will start with a sample size of 81 recipients, which will include both cohorts of subjects. We anticipate a 2:1accrual pattern (male donor/female recipients: pre-existing antibody recipients).

The sample size estimation for cohort 1 is based on a one-sided 0.05 hypothesis test with null hypothesis the true success rate is <= L, where we examine different sample sizes associated with different values of L, We plan to enroll 56 subjects in this cohort. Table below shows the number of patients needed to achieve a lower one-sided 95% confidence bound of success rate ranging from 0.50 to 0.70 under different actual success rate. Based on our previous study 03-H-0170 (success rate of 85%), we target the overall success range of 75%-95%. For example, if

the true success rate is 85% then 24 subjects are required to have 80% power for the lower 95% confidence interval to exceed 0.60, and 56 subjects are needed to have 70% power for the lower confidence interval to exceed 0.70.

Sample size table based on one-sided significance level of 0.05 and 80% power for cohort 1

	True success rate				
Lower 1-sided 95% Confidence bound	0.75	0.8	0.85	0.9	0.95
>0.50	27	19	13	10	8
>0.55	40	26	18	13	9
>0.6	68	38	24	16	11
>0.65	142	63	35	22	14
>0.7	520	129	56	31	18

The results from protocol 03-H-0170 suggest that the male-into-female transplant regime has a failure rate of at least 50%. Consequently, there is interest in demonstrating the success rate of the regimen tested here is greater than 50%. In this cohort, we propose that our primary analysis formally test the null hypothesis that the success rate is ≤ 50% with a one-sided test and alpha level of 0.05. For a final sample size of N=56 and a true success rate of 75% then there is 98% power to reject this null hypothesis; if the true rate is 70% then power falls to 91%.

The sample size estimation for cohort 2 is calculated based on a wide range of possible success rate since we do not have data available to estimate the actual rate. We plan to enroll 25 subjects in this cohort. For example, if the actual success rate is 50%, then the sample size would be sufficient to give a lower 95% confidence bound of ≥25% with 80% power.

Sample size table based on one-sided significance level of 0.05 and 80% power for cohort 2

	True success rate						
Lower 1-sided 95% Confidence bound	0.2	0.3	0.4	0.5	0.6	0.7	0.8
>0.15	361	49	20	11	7	5	4
>0.25		502	63	25	13	8	6
>0.35			592	71	27	14	9
>0.45				634	74	27	14
>0.55					625	71	26
>0.65						567	63
>0.75							460

Secondary endpoints

1. $CD34^+$ cell dose, $CD3^+$ cell dose
2. Degrees of donor-recipient lymphoid and myeloid chimerism by microsatellite PCR analysis; erythroid chimerism by CBC, reticulocyte count, hemoglobin electrophoresis, red cell phenotype for donor antigen, or if necessary by flow analysis. Peripheral blood or bone marrow samples will be used for chimerism on days +30, +60 and +100, and periodically thereafter. More frequent monitoring may be required.
3. Incidence of acute and chronic GVHD, graft rejection, or red cell aplasia >2 years after transplant. These together count toward the combined endpoint for regimen failure (section 11.2).
4. Transplant-related mortality, disease-free survival and overall survival
5. Neutrophil recovery (days to neutrophil count of 0.5 x 10^9/L)
6. Platelet recovery (days to platelet count of 50 x 10^9, days to transfusion independence)
7. Red cell recovery (days to recovery of reticulocyte count ≥30 k/uL, detection of donor red cells, transfusion independence)

Exploratory endpoints

1. Anti-A, anti-B, and/or other red cell antibody titer pre- and post-HSCT
2. Immune reconstitution, cytokines levels, and lymphocyte function post-transplant
3. Quality of life and neuropsychologic function post-transplant (Addendum A)
4. Effect of transplant on neurologic, cardio-pulmonary, renal, liver, and gonadal organ function

10.2 Stopping Rules

Because this regimen is expected to have very low mortality in patients with non-life threatening conditions, the study will be stopped and the design re-evaluated after any death. We will also monitor for graft rejection (at 1 year post-transplant), severe GVHD (acute grade III-IV and chronic extensive at any time point post-transplant), or prolonged red cell aplasia (at >2 years post-transplant), which we would collectively consider as regimen failure.

We will stop the study if posterior probability that the regimen failure rate is greater than 30% exceeds 0.90. Stopping means that serious consideration will be given to modifying or terminating the protocol. Stopping boundaries are given in the table below. Beta prior distributions are used for these calculations with parameters alpha, beta = 2.1, 4.9 for regimen failure.

Note that one need not evaluate all the number of patients in the first column in order to stop. For example, if 4 of the first 4 patients fail to engraft, we will have crossed the boundary given in the first row. For 25 patients, stopping rule would be met if 12 patients failed. The monitoring plan was evaluated by simulation. If the true regimen failure rate is .3, we will stop about 20% of the time. True regimen failure rates of .2, .4, and .6 entail stopping about 2%, 72% and >99% of the time respectively. This is acceptable performance for a stopping rule.

Table: Stopping rule for cohort 1 (male donor-female recipient)

# of patients with at primary endpoint determination	Regimen Failures
5	4
10	6
15	8
20	10
25	12
30	13
35	15
40	17
45	18
50	20
55	22
60	24

Table: Stopping rule's operating characteristics for cohort 1

Probability of regimen failure = p	0.20	0.30	0.40	0.60
Proportion of Stopped Studies	1.7%	20.2%	71.6%	>99%
Average number of subjects	59.2	52.8	35.0	11.2

Average number of failures	11.8	15.8	14.0	6.7

Similar stopping rules with its operating characteristics can be calculated for cohort 2 (see tables below). For this cohort, the study will be stopped if posterior probability that the regimen failure rate is greater than 30% exceeds 0.90.

Table: Stopping rule for cohort 2 (antibody group)

# of patients with at primary endpoint determination	Regimen Failures
5	4
10	6
15	8
20	10
25	12

Table: Stopping rule's operating characteristics for cohort 2

Probability of regimen failure = p	0.20	0.30	0.40	0.60
Proportion of Stopped Studies	1.4%	11.2%	38.6%	94.5%
Average number of subjects	24.8	23.6	20.3	10.8
Average number of failures	5.0	7.1	8.1	6.5

As of July 2022, 4 patients (see table below) have developed acute leukemia or myelodysplasia. The timing when these events occurred post-HSCT, type of leukemia, and predisposing factors are very different. The causes of these events are uncertain, but may include any of the following: severe sickle cell disease, inflammatory marrow from frequent vaso-occlusive crises, iron overload with increased reactive oxygen species adding additional marrow inflammation/injury, prior hydroxyurea, chemotherapy in the transplant process, radiation, immunosuppression, viral infection or reactivation, and other yet unknown. Based on the review of literature (as of March 2021), the rate of leukemia (including acute, chronic, and myelodysplasia) is estimated <10% (Brunson et al, Blood 2017; Seminog et al, JRSM 2016; Li et al Mod Path 2019; Eapan et al Lancet Haem 2019). Thus, if another patient develops acute leukemia, chronic leukemia, or myelodysplasia, the protocol would stop accrual and discussion with DSMB and IRB would follow to determine the next steps for the protocol. This criterion was met based on data reported to DSMB in January 2022, which recommended cohort 1 to stop accrual, cohort 2 to continue enrollment, and report each additional patient who develop leukemia or myelodysplasia.

Patient	**Heme malignancy/MDS**	**Donor chimerism at heme malignancy finding**	**Comments**	**Inclusion in DSMB report**
7 (cohort 1)	Ph+ ALL, 3 yrs after transplant	CD3 36%, CD14/15 89% 2.5 yrs post HCT, dropped to CD3 25%, CD14/15 30% at ALL diagnosis,	recovered to CD3 20% and CD14/15 100% after chemo	2020

26 (cohort 2)	Complex cytogenetics, treatment related AML, 3 months after transplant	CD3 10% CD14/15 16%		Included in the initial 2022 DSMB report
9 (cohort 2)	Inv 3(q21,26), 20q deletion, 4 yrs after transplant	CD3 58% CD14/15 97%. These numbers have been same and stable throughout post-HCT		2022
16 (cohort 2)	7q deletion in marrow 3.5 yrs after transplant	0%, patient had primary graft failure with autologous recovery		2022

10.3 Interim Analysis

We propose an interim efficacy analysis of the primary null hypothesis that the success probability is ≤ 0.50 after N=28 outcomes have been evaluated. An O'Brien-Fleming spending function will be used to calculate the p-value threshold used for this interim analysis. For conducting the interim analysis with N = 28 (corresponding to information time = 0.50) the nominal p-value threshold is approximately 0.0056. The flexibility of the spending function allows for interim analyses to be conducted at other times, or for more than one interim analysis. However, as this is an unblinded trial it is preferable to have an a priori specified time for analysis. If the trial is not stopped early the p-value threshold used at the end of the study will be adjusted to reflect any interim analysis that were conducted.

Also, given the strong interest in secondary outcomes and obtaining greater precision regarding the success probability, the investigators may continue to enroll to the N=56 ceiling even if a significant finding is discovered at the interim analysis. In this way, the discovery of a significant finding at the interim analysis would support the early disclosure of a promising therapy but still allow for additional learning opportunities and treatment for others in this cohort.

No interim analysis is planned for the second cohort of 25 participants due to the small size of the group.

10.4 Off study criteria

10.4.1 *Withdrawal by the patient from the transplant procedure*

Patients and their donors will be given ample opportunity to withdraw from the study prior to admission for transplant. Thereafter, the nature of the procedure does not permit safe withdrawal from the protocol. The patient and donor have the right at any time to elect not to participate in the research aspects of the protocol (donation of blood and bone marrow for non-routine tests).

10.4.2 *Withdrawal by the physician from experimental protocol*

Patients with disease relapse may be taken off protocol but will continue to be monitored by our institution for a minimum of 6 months post-transplant for possible infectious complications related to the conditioning regimen. The patient will then be consented to the Hematology Branch evaluation and treatment protocol (94-H-0010) for alternative treatments or referred back to his/her referring physician depending on what is considered to be in the best interest of the patient.

11.0 DATA SAFETY AND MONITORING

11.1 Safety Monitoring

Principal Investigator: The safety of interventions and treatments associated with this protocol will be under continuous review by the investigative team. Accrual, efficacy and safety data will be monitored by the PI.

IRB. Prior to implementation of this study, the protocol and the proposed patient consent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to the 45 CFR 46 code of federal regulations. This committee will also approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual, follow up of subjects, or data analysis continues.

DSMB: The NHLBI Data safety and Monitoring Board will review the protocol at regular intervals. A progress report will be forwarded to the DSMB at these times and their recommendations will be expeditiously implemented. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

11.2 Grading of adverse events

The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTCAE version 4.0. A copy of the criteria can be down-loaded from the CTEP home page at **http://ctep.cancer.gov/reporting/ctc.html**.

Grading of adverse events

Grade	Category	Description
1	Mild	Mild; asymptomatic; clinical or diagnostic observations only; intervention not indicated
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
4	Life threatening	Life-threatening consequences; urgent intervention indicated
5	Death	Death related to AE

Attribution of adverse events

Relationship	**Attribution**	**Description**
Unrelated to investigational agent/intervention1	Unrelated	The AE *is clearly **NOT** related* to the intervention
	Unlikely	The AE is doubtfully related to the intervention
Related to investigational agent/intervention[1]	Possibly	The AE may be related to the intervention
	Probably	The AE ***is likely related*** to the intervention
	Definitely	The AE ***is clearly related*** to the intervention

1NOTE: AEs listed as 'possibly, probably, or definitely' related to the investigational agent/intervention are considered to have a suspected 'reasonable causal relationship' to the investigational agent/intervention (ICH E2A).

11.3 NIH IRB and CD reporting

Expedited Reporting

Events requiring expedited reporting will be submitted to the IRB per Policy 801 "Reporting Research Events".

Reports to the IRB at the time of Continuing Review:

The PI or designee will refer to HRPP Policy 801 "Reporting Research Events" to determine IRB reporting requirements.

Reports to the CD:

The PI or designee will refer to NHLBI DIR guidelines to determine CD reporting requirements and timelines.

Grade 3 or higher adverse event recording will start at the time of conditioning regimen, will be followed until satisfactory resolution, through final study visit, and reported to the IRB at the time of continuing review, unless otherwise noted below.

The research team will be responsible for assessing AEs. Information on AEs will be solicited from subjects through questions from study personnel and/or information volunteered by the subject.

12.0 HUMAN SUBJECT PROTECTIONS

12.1 Rationale for subject selection

All patients with confirmed thalassemia or SCD, as defined in section 5.1, will be considered for the protocol. Gender, ethnic background, and/or race will not be taken into consideration.

Strategies for patient recruitment: Hematologists and internists throughout the country will be informed of the protocol by letter. Information about the protocol will be posted on Clinicaltrials.gov, Clinical Center studies, and the NHLBI Patient recruitment websites. The protocol will also be listed in the physician's data query (PDQ).

12.2 Participation of children

12.2.1 *As stem cell transplant recipients*

As the risk of this new regimen is better known and the protocol with alemtuzumab and 300 cGy TBI (03-H-0170) being successfully adopted in children with little toxicity, we will begin to include children from age 4 and older.

12.2.2 *As participants in laboratory research studies*

Pediatric participants may participate in those laboratory studies that the IRB finds involves no greater than minimal risk to children provided that adequate provisions are made for soliciting the assent of the children and the permission of their parents or guardians (see section 13.6).

12.3 Hazards and discomforts- recipient

12.3.1 *Related to the transplant*

The mortality from conventional BMT may be as high as 40%. While transplant-related morbidity and mortality is low in our current study (03-H-0170), the HSCT procedure nevertheless carries significant risk. It is therefore only appropriate to carry out this experimental procedure in the context of debilitating conditions and with full informed consent from the patient, donor, and immediate family. The specific hazards of this study using a nonmyeloablative preparative regimen and high PBSC content graft are graft rejection, graft-versus-host disease, disease relapse, and infectious complications. Major discomforts are those of nausea, anorexia, diarrhea, fever, malaise, and intolerance of the isolation period. It is estimated that approximately 10% or more of transplant patients experience depression, which may worsen after transplant. Additionally, the transplant process may worsen pre-existing medical condition(s).

Side effects of those drugs novel to nonmyeloablative transplantation are described in detail below:

12.3.2 *Related to Alemtuzumab (Campath®)*

Boxed Warning

Hematologic Toxicity: Serious and, in rare instances fatal, pancytopenia/marrow hypoplasia, autoimmune idiopathic thrombocytopenia, and autoimmune hemolytic anemia have occurred in patients receiving Campath therapy. **Single doses of Campath greater than 30 mg or cumulative doses greater than 90 mg per week should not be administered because these doses are associated with a higher incidence of pancytopenia.**

Infusion Reactions: Campath can result in serious, and in some instances fatal, infusion reactions. Patients should be carefully monitored during infusions and Campath discontinued if indicated. **Gradual escalation to the recommended maintenance dose is required at the initiation of therapy and after interruption of therapy for 7 or more days.**

Infections, Opportunistic Infections: Serious, sometimes fatal bacterial, viral, fungal, and protozoan infections have been reported in patients receiving Campath therapy. Prophylaxis directed against *Pneumocystis carinii* pneumonia (PCP) and herpes virus infections has been shown to decrease, but not eliminate, the occurrence of these infections.

The safety and efficacy of alemtuzumab were evaluated in a multicenter, open-label, non-comparative study in 93 patients with refractory B-cell chronic lymphocytic leukemia (B-CLL) who had been previously treated with alkylating agents and had failed treatment with fludarabine, and side effects are detailed below. Previous treatment with alkylating agents and fludarabine may have contributed to both the range and severity of the side effects observed.

Infusion-related: adverse events resulted in discontinuation of alemtuzumab therapy in 6% of the patients. The most commonly reported infusion-related adverse events include rigors in 89% of patients, drug-related fever in 83%, nausea in 47%, vomiting in 33%, and hypotension in 15%. Other frequently reported infusion-related events include rash in 30% of patients, fatigue in 22%, urticaria in 22%, dyspnea in 17%, pruritus in 14%, headache in 13%, and diarrhea in 13%. Acute infusion-related events were most common during the first week of therapy. Antihistamines, acetaminophen, antiemetics, meperidine, and corticosteroids, as well as incremental dose escalation were used to prevent or ameliorate infusion-related events.

Infections: In the earlier studies all patients were required to receive anti-herpes and anti-PCP prophylaxis. Forty (43%) of 93 patients experienced 59 infections (one or more infections per patient) during treatment or within 6 months of the last dose. Of these, 34 (37%) patients experienced 42 infections that were of Grade 3 or 4 severity; 11 (18%) were fatal. Fifty-five percent of the Grade 3 or 4 infections occurred during treatment or within 30 days of the last dose. In addition, one or more episodes of febrile neutropenia (ANC 500 cells/μL) were reported in 10% of patients. The following types of infections were reported: Grade 3 or 4 sepsis in 12% of patients with one fatality, Grade 3 or 4 pneumonia in 15% with five fatalities, and opportunistic infections in 17% with four fatalities. Candida infections were reported in 5% of patients; CMV infections in 8% (4% of Grade 3 or 4 severity); Aspergillosis in 2% with fatal Aspergillosis in 1%; fatal Mucormycosis in 2%; fatal Cryptococcal pneumonia in 1%; *Listeria monocytogenes* meningitis in 1%; disseminated Herpes zoster in 1%; Grade 3 Herpes simplex in 2%; and *Torulopsis* pneumonia in 1%. PCP pneumonia occurred in one (1%) patient who discontinued PCP prophylaxis. In one of the earlier studies where anti-herpes and anti-PCP prophylaxis was optional, 37 (66%) patients had 47 infections while or after receiving Campath therapy.

Immunosuppression/Opportunistic Infections: Alemtuzumab induces profound lymphopenia. Anti-infective prophylaxis is recommended upon initiation of therapy and for a minimum of 2 months following the last dose of Alemtuzumab or until $CD4^+$ counts are 200 cells/μL. The median time to recovery of $CD4^+$ counts to 200/μL was 2 months, however, full recovery (to baseline) of $CD4^+$ and $CD8^+$ counts may take more than 12 months. Because of

the potential for transfusion-associated GVHD in severely lymphopenic patients, irradiation of any blood products is recommended.

Hematologic:

- Pancytopenia/Marrow Hypoplasia: Alemtuzumab therapy was permanently discontinued in six (6%) patients due to pancytopenia/marrow hypoplasia. Two (2%) cases of pancytopenia/ marrow hypoplasia were fatal.
- Anemia: Forty-four (47%) patients had one or more episodes of new onset NCI-CTC Grade 3 or 4 anemia. Sixty-two (67%) patients required RBC transfusions. In addition, erythropoietin use was reported in nineteen (20%) patients. Autoimmune hemolytic anemia secondary to Alemtuzumab therapy was reported in 1% of patients. Positive Coombs test without hemolysis was reported in 2%.
- Neutropenia: Sixty-five (70%) patients had one or more episodes of NCI-CTC Grade 3 or 4 neutropenia. Median duration of Grade 3 or 4 neutropenia was 28 days (range: 2 – 165 days).
- Thrombocytopenia: Forty-eight (52%) patients had one or more episodes of new onset Grade 3 or 4 thrombocytopenia. Median duration of thrombocytopenia was 21 days (range: 2 – 165 days). Thirty-five (38%) patients required platelet transfusions for management of thrombocytopenia. Autoimmune thrombocytopenia was reported in 2% of patients with one fatal case of Alemtuzumab -related autoimmune thrombocytopenia.
- Lymphopenia: The median $CD4^+$ count at 4 weeks after initiation of Alemtuzumab therapy was 2 (two)/μL, at 2 months after discontinuation of Alemtuzumab therapy, 207/μL, and 6 months after discontinuation, 470/μL. The pattern of change in median $CD8^+$ lymphocyte counts was similar to that of $CD4^+$ cells. In some patients treated with Alemtuzumab, $CD4^+$ and $CD8^+$ lymphocyte counts had not returned to baseline levels at longer than 1-year post therapy.

Cardiac: The following were reported in at least one patient treated on studies where Campath-1H was used as a single agent: cardiac failure, cyanosis, atrial fibrillation, cardiac arrest, ventricular arrhythmia, ventricular tachycardia, angina pectoris, coronary artery disorder, myocardial infarction, and pericarditis. Some of these cardiac abnormalities may be irreversible. For this reason, we will monitor subjects with an echocardiogram, a 24 hour holter monitor and serum troponin levels before treatment begins. After the last dose of Campath-1H, and at the 3 month follow up visit these tests may be obtained as clinically indicated. We will also closely monitor subjects for cardiac symptoms and ask them to immediately report any cardiac symptoms (palpitations, irregular pulse, difficulty in breathing, dizziness, swelling in the ankles, chest discomfort or pain).

12.3.3 *Related to Sirolimus:* Those reported with ≥ 3% and <20% incidence in patients on Sirolimus treatment are listed here. Refer to the package insert for additional side effects.

- Body as a Whole: abdomen enlarged, abscess, ascites, cellulitis, chills, face edema, flu syndrome, generalized edema, hernia, Herpes zoster infection, lymphocele, malaise, pelvic pain, peritonitis, sepsis;
- Cardiovascular System: atrial fibrillation, congestive heart failure, hemorrhage, hypervolemia, hypotension, palpitation, peripheral vascular disorder, postural hypotension, syncope, tachycardia, thrombophlebitis, thrombosis, vasodilatation;
- Digestive System: anorexia, dysphagia, eructation, esophagitis, flatulence, gastritis, gastroenteritis, gingivitis, gum hyperplasia, ileus, liver function tests abnormal, mouth ulceration, oral moniliasis, stomatitis;
- Endocrine System: Cushing's syndrome, diabetes mellitus, glycosuria, hypercholesterolemia, hyperlipidemia;
- Hematologic and Lymphatic System: ecchymosis, leukocytosis, lymphadenopathy, polycythemia, thrombotic thrombocytopenic purpura / hemolytic-uremic syndrome;
- Metabolic and Nutritional: acidosis, alkaline phosphatase increased, BUN increased, creatine phosphokinase increased, dehydration, healing abnormal, hypercalcemia, hyperglycemia, hyperphosphatemia, hypocalcemia, hypoglycemia, hypomagnesemia, hyponatremia, lactic dehydrogenase increased, SGOT increased, SGPT increased, weight loss;
- Musculoskeletal System: arthrosis, bone necrosis, leg cramps, myalgia, osteoporosis, tetany, muscle injury
- Nervous System: anxiety, confusion, depression, dizziness, emotional lability, hypertonia, hyperesthesia, hypotonia, insomnia, neuropathy, paresthesia, somnolence;

- Respiratory System: dyspnea, changes in PFTs, asthma, atelectasis, bronchitis, cough increased, epistaxis, hypoxia, lung edema, pleural effusion, pneumonia, rhinitis, sinusitis, diffuse alveolar hemorrhage;
- Skin and Appendages: fungal dermatitis, hirsutism, pruritus, skin hypertrophy, skin ulcer, sweating;
- Special Senses: abnormal vision, cataract, conjunctivitis, deafness, ear pain, otitis media, tinnitus;
- Urogenital System: albuminuria, or proteinuria, bladder pain, dysuria, hematuria, hydronephrosis, impotence, kidney pain, kidney tubular necrosis, nocturia, oliguria, pyelonephritis, pyuria, scrotal edema, testis disorder, toxic nephropathy, urinary frequency, urinary incontinence, urinary retention, kidney injury.

Less frequently occurring adverse events included: mycobacterial infections, Epstein-Barr virus infections, BK virus-associated nephropathy, skin cancer, lymphoma, pericardial effusion, posterior reversible encephalopathy syndrome (PRES), and pancreatitis.

12.3.4 *Related to Pentostatin*

Pentostatin is cleared by a renal mechanism (90%). As such, the pentostatin dose must be reduced for renal insufficiency. The primary toxicity is related to opportunistic infection due to T cell depletion or low blood counts. Common (>10%) side effects include fever, headaches, fatigue, nausea, vomiting, diarrhea, rash, pain, myelosuppression, and respiratory symptoms. Occasional (1-10%) side effects include changes in liver function tests, electrolytes, kidney function tests, blood pressures, heart rhythms; also chills, sweating, anxiety, confusion, dizziness, dry or itchy skin. At higher doses, CNS toxicity may include seizures, coma, and death. Interstitial pulmonary toxicity or edema has also been described.

12.3.5 *Cyclophosphamide*

Most commonly (>10%), patients may develop anorexia, nausea, vomiting, diarrhea, mucositis, myelosuppression, gonadal dysfunction, alopecia, and immunosuppression. Occasionally (1-10%), they may develop hemorrhagic cystitis, nasal stuffiness with rapid administration, flushing, rash, kidney tubular necrosis (which usually resolves with drug discontinuation) or SIADH. Rarely (<1%), patients may experience transient blurred vision, cardiac toxicity with arrhythmias, hyperpigmentation, impaired wound healing, myocardial necrosis, hepatotoxicity, weakness, hemorrhagic colitis, nail changes, bladder fibrosis, pulmonary fibrosis, and secondary malignancies.

12.3.6 *Related to Hydroxyurea*

Hydroxyurea most commonly leads to myelosuppression, and blood counts will be frequently monitored during hydroxyurea administration. Less commonly, hydroxyurea can cause alopecia, dermatomyositis-like skin changes, hyperpigmentation, nail discoloration/atrophy, skin ulcers, drowsiness, anorexia, constipation, diarrhea, nausea, vomiting, and elevated hepatic enzymes. Rarely, hydroxyurea can cause edema, chills, fever, dizziness, disorientation, hallucinations, headache, malaise, seizure, facial/peripheral erythema, skin atrophy, hyperuricemia, pancreatitis, peripheral neuropathy, weakness, increased creatinine, pulmonary fibrosis, and acute diffuse pulmonary infiltrates. Secondary leukemias have been described after prolonged use in patients predisposed to developing leukemia.

12.3.7 *Related to Radiation*

Side effects of radiation have been well described.[47] The most common include nausea and mucositis. There also exists a risk of hypothyroidism, cataracts, interstitial pneumonitis, nephropathy, and an unspecified long term risk of developing secondary malignancies.[48] Importantly, the majority of the non-neoplastic effects were sub clinical and/or reversible. [49] There is also a risk of sterility following TBI. Recovery of gonadal function has been reported to be 10-14% with a pregnancy incidence of <3% following TBI[50, 51]. However, these results are reported in patients who have history of hematologic malignancies, and therefore have also received prior chemotherapy. Further, the dose of TBI that they received was higher, at least 1000cGy. The incidence of sterility has not been reported in patients with nonmalignant hematologic diseases who have received lower doses of TBI. However, the risk of sterility exists, but is presumed to be lower than previously reported results. In a further attempt to decrease the risk of sterility, testicular shielding will be applied. We will discuss the option of gamete storage with subjects of child-bearing age. Studies attempting to evaluate the risk induced by radiation alone suggest that there is a higher rate of solid tumors after radiation based regimens. Curtis et al. reported on 19,229 patients and found a cumulative incidence rate of 2.2% at 10 years, and 6.7% at 15 years, with higher doses of TBI associated with a

higher risk of solid cancers.[52] However, the more important risk factor appears to be related to the level of immunosuppression, as GVHD was also strongly linked to an increased risk of solid tumor development. In fact, some studies have shown no increased risk with radiation therapy,[53, 54] but the highest risk factor was felt to be the presence of chronic GVHD and long term treatment with cyclosporine.[55, 56] Therefore the actual risk cannot be quantified for the low dose of 300cGy to be used in this trial; however the risk is presumed to be lower. The radiation exposure from bone density scans are very low, even lower than chest x-rays, thus in comparison to the 300 rads of TBI, the additional risk from bone density scans are negligible.

12.3.8 *Related to Antimicrobials in general*

Allergic reactions, renal impairment (gentamicin, vancomycin, amphotericin, acyclovir), "red man" syndrome (vancomycin), hepatic damage (acyclovir, rifampicin)

12.3.9 *Related to bone marrow aspirate and biopsy:*

No major risks are involved with bone marrow aspirate and biopsy. However, there is a small risk of infection, pain, bleeding, and hematoma formation at the site of the aspiration with the procedure.

12.3.10 *Related to blood draws*

No major risks are involved with blood draws. Minor complications including bleeding, pain, and hematoma formation at the site of blood draws; vasovagal reactions, thrombus formation, or infection may rarely occur.

12.3.11 *Related to cardiac monitoring*

EKG: An electrocardiogram (EKG) is a test that measures the electrical activity of the heartbeat. With each beat, an electrical impulse (or "wave") travels through the heart. This wave causes the muscle to squeeze and pump blood from the heart. A technician will put patches (electrodes) on the chest, arms and legs. The electrodes are soft and don't cause any discomfort when they're put on or taken off by the technician. The machine only records the EKG. It doesn't send electricity into the body. There's no pain or risk associated with having an electrocardiogram

Transthoracic ECHO: The ECHO uses sound waves to visualize and evaluate the function of the heart. There are no associated risks

Holter Monitor: The Holter involves wearing a monitor for 24 hours during which time the electrical activity of the heart is recorded. There are no associated risks other than the inconvenience of wearing the apparatus.

12.3.12 *Related to central line placement*

A catheter may be placed in a large vein of the neck, chest, or arm using local anesthetic. Patients will sign a separate consent for the line placement procedure. Only trained experienced staff will place the line in order to minimize these procedure-related risks

The risks from the procedure are low; they include bleeding, bruising, or infection at the site of insertion. Very rarely (less than 1% of the time), the line placement may nick a vein causing one lung to collapse during line insertion. If the lung collapses, a tube may have to be inserted into the chest and remain in place until the lung re-expands. Because of this risk, patients will have a chest x-ray following the procedure to make sure the line is in the correct place and that the lung is not collapsed. Once placed, the line will remain in place until drug administration is complete.

12.3.13 *Related to brain magnetic resonance imaging*

There is a very small risk that the patient will experience an allergic reaction, and these are usually easily controlled with medications. Anaphylactic reactions can rarely occur. Patients with severe kidney disease requiring dialysis who undergo MRI with intravenous contrast can develop a rare but severe disease called nephrogenic systemic fibrosis that leads to hardening of skin, joints, internal organs, and eyes. Patients with severe kidney disease will not receive intravenous contrast. The majority of patients will not require sedation. However, all patients that do require sedation will be offered monitored anesthesia care through the Department of Anesthesia and Surgical Services (DASS), Clinical Center, NIH, and a separate consent will be completed as needed.

Gadolinium-based contrast agents (GBCAs): Gadolinium is an FDA approved medication used to improve MRI images. Gadolinium contrast is an injected medication used to improve MRI images. Most patients experience a metallic taste when gadolinium contrast is injected. Some (2%) report mild symptoms such as headache, nausea or vomiting, or a rash near the injection site. Rarely (<0.1%) patients experience severe symptoms such as wheezing, shortness of breath, and low blood pressure as part of an allergic (anaphylactoid) reaction that may require emergency medical treatment.

In a few cases per million, usually in patients with severe kidney disease, gadolinium contrast can cause a rare, debilitating or even fatal, skin disease called Nephrogenic Systemic Fibrosis (NSF) that cause thickening of the skin and other organs. Since physicians became aware of the disease, began screening patients at risk of kidney disease, and switched to safer ("macrocyclic") forms of gadolinium contrast, new reports of NSF are much more rare.

There are also reports of gadolinium retained in the brain, bone, and skin. It is not known whether this is important to health. We use "macrocyclic" forms of gadolinium contrast, such as gadolinium contrast, such as gadobutrol, that are thought to reduce this risk.

12.3.14 ***Related to neuropsychological testing***

Depending on the patient's age, neuropsychological testing will take up to 1.5 to 2 hours to perform. During this time, the patient may experience frustration, fatigue, or distress. If these symptoms occur, the patient will be allowed to take a break. He or she will not have to answer every question, and testing will be discontinued if the patient asks. The clinical social worker and/or study investigators will be available if the patient would like to discuss his/her concerns.

12.3.15 ***Related to Busulfan (Busulfex®)***

The most common adverse events (>10%) expected with busulfan are as follows:
Cardiovascular: Fast heart rate, swelling in your legs, chest pain, high blood pressure, low blood pressure
Central nervous system: Insomnia, headache, fever, chills, dizziness, anxiety, depression, confusion
Dermatologic: Rash, hair loss, itching
Endocrine and Metabolic: Electrolyte imbalance, high blood sugar
Gastrointestinal: Nausea, vomiting, diarrhea, inflammation of the digestive tract, poor appetite, abdominal pain
Hematologic: decrease in blood cell production
Hepatic: changes in liver enzymes
Local: Site injection reaction
Musculoskeletal: Back pain, weakness, joint pain, muscle pain
Renal: Kidney damage
Respiratory: Cough, runny nose, shortness of breath, pneumonia, sore throat

Less common (1-10%) include abnormal heart rhythm, heart failure, hallucination, delirium, seizures, brain hemorrhage, severe rash, blockage of the intestine, inflammation of the pancreas, blood in the urine or lungs, asthma, and liver failure.

12.4 **Hazards and discomforts- donor**

12.4.1 *Related to blood draw*

No major risks are involved with blood draws. Minor complications including bleeding, pain, and hematoma formation at the site of blood draws, vasovagal reactions or infections may rarely occur.

12.4.2 *Related to neuropsychological testing (Donors and Recipients)*

Depending on the patient's age, neuropsychological testing will take up to 1.5 to 2 hours to perform. During this time, the patient may experience frustration, fatigue, or distress. If these symptoms occur, the patient will be allowed to take a break. He or she will not have to answer every question, and testing will be discontinued if the

patient asks. The clinical social worker and/or study investigators will be available if the patient would like to discuss his/her concerns.

12.5 **Risks in relation to benefit**

12.5.1 *For adult transplant subjects*

Clinically the approach is ethically acceptable because we are targeting a patient group with a debilitating and often lethal hematological disease, incurable with conventional treatments other than allogeneic BMT. The protocol aims to decrease the risk of transplant rejection and related mortality, thus making more patients candidates for potentially curative therapy.

12.5.2 *For adult donors*

The risks of the study procedures for adult donors under this protocol are minimal. By being in this study, donors will receive medical evaluation of their health. While there are no direct benefits of neuropsychological testing, psych test results may reveal personal strengths and weaknesses. Adult donor participation in this study may also help inform future studies and contribute to new ways of making transplant procedures safer and more effective. The clinical team will communicate all risks to the subjects at the time of consenting.

12.5.3 *For pediatric transplant subjects (ages 4-18)*

The inclusion of children satisfies the criteria set forth in 45 Code of Federal Regulations 46, Subpart D as follows:

(a) *the risk is justified by the anticipated benefit to the subjects*: We are offering pediatric subjects with a probably lethal hematological disease, incurable with conventional treatments other than allogeneic BMT, an alternative to symptomatic therapy.

(b) *the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches.* The protocol aims to decrease the risk of transplant-related mortality, thus making more patients candidates for potentially curative therapy; and

(c) adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians, as set forth in 46.408.

12.5.4 *For pediatric participants involved in laboratory research studies*

The inclusion of children satisfies the criteria set forth in 45 Code of Federal Regulations 46, Subpart D: 46.404 as follows:

(a) *The research does not involve greater than minimal risk*. Blood specimens for research are obtained concurrently with clinically indicated sampling. Therefore, there is no risk associated with sample collection for research because research will only be performed on material obtained during standard clinical intervention.

(b) Only those laboratory tests approved by the IRB and involving not greater than minimal risk will be conducted. Research will not include genetic testing. Therefore, there is no genetic testing-associated risk.

(c) Adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians, as set forth in 46.408.

12.6 **Informed consent**

Informed consent will be conducted following OHSRP Policy 301- Informed Consent.

An IRB-approved consent form will be provided to the subject electronically or by hard copy for review prior to consenting. The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved platforms).The investigational nature and objectives of this trial, the procedures, and their attendant risks and discomforts and potential benefits will be carefully explained to the subject in a private setting. The subject will be given as much time as they need to review the document and to consult with their family, friends, and personal health care providers. In addition, a study team member will be available to answer any questions.

A signed and dated informed consent document will be obtained by any investigator authorized to consent prior to entry onto the study. Consent may be obtained with required signatures on the hard copy of the consent or on the electronic document.

When a document that is in electronic format is used for obtaining consent, this study may use the iMed platform which is 21 CFR, Part 11 compliant, to obtain the required signatures.

During the consent process, participants and investigators may view the same approved consent document simultaneously when participant is being consented in person at the Clinical Center or both may view individual copies of the approved consent document on screens in their respective locations remotely. Signatures may be obtained either by both directly signing on the device that the consenting investigator is using (when in person) or through iMed Mobile Signature Capture (remotely) which allows texting or emailing a link to the participant. That link allows the participant to review the consent, then proceed to sign on the device they are using.

Whether hard copy or electronic consent, both the investigator and the participant will sign the document with a hand signature using a pen, finger, stylus, or mouse.

When done remotely, if the participant prefers to sign a hard copy, they may be instructed to sign and date the consent document during the discussion and mail, secure email or fax the signed document to the consenting investigator.

Whether in person or remotely, the privacy of the participant will be maintained.

Finally, the fully signed informed consent document will be stored in the electronic medical record, and the subject will receive a copy of the signed informed consent document.

If the patient is a minor, the parent who signs the consent for the minor must be a legally recognized parent or guardian. Where deemed appropriate by the clinician, and the child's parent or guardian, the child will also be included in all discussions about the trial and a minor's assent will be obtained. Assent will only be used in children who can understand it. In cases where parents share joint legal custody in making medical decisions of their child (e.g. by a custody agreement or court order) both parents must give their parental permissions regardless of level of risk of the research. Exceptions may be made if one parent is deceased, becomes incompetent or is not reasonably available (e.g. in prison). The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent.

Consent for Minors when they reach the age of majority:

When a pediatric subject reaches age 18, continued participation will require consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. Should sample or data analysis continue following completion of active participation and the subject has reached 18 years of age, we will attempt to contact the subject using the last known contact information to obtain consent for continued use of data or samples collected during their prior visit. Given the length of time that may have transpired for some of the subjects since their last visit for this study, we request waiver of informed consent for those individuals who after good faith efforts to contact them, we are unable to contact or who were taken off study.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d), each of which must be addressed in relation to the protocol:

(1) The research involves no more than minimal risk to the subjects.

a. Analysis of samples and data from this study involves no additional risks to subjects.

(2) The waiver or alteration will not adversely affect the rights and welfare of the subjects.

a. This is an FDA-regulated study and as such, we are mandated to retain all samples, once collected, regardless of the age of the subject at the time of collection. Retention of these samples or data does not affect the welfare of subjects.

(3) The research could not practicably be carried out without the waiver or alteration.

a. Considering the length of time between a minor's enrollment and their age of majority, it is possible that more than a few subjects may be lost to follow up. A significant reduction in the number of samples analyzed could impact the quality of the research.

(4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

a. We only plan to request a waiver of re-consent for those subjects who have been lost to follow-up.

At any time during participation in the protocol that new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to all enrolled or prospective patient participants. Documentation will be provided to the IRB and if necessary, the informed consent amended to reflect relevant information.

13.0 CONFLICT OF INTEREST

The Principal Investigator will assure that each associate investigator listed on the protocol title page receives a copy of the NIH Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation will be provided a copy of the document when they are added and a copy of the Conflict of Interest Statement will be forwarded to the Clinical Director. Any potential conflicts will be reported to the IRB and the resolution of the conflict summarized.

14.0 CIMBTR

Data reporting requirements for the NMDP Coordinating Center include: Baseline confirmatory data, pre-conditioning, 100 day, 6 month, 1 year and annually post-transplant outcome data for the recipients' life span. Data reporting requirements for the NMDP Donor Center include: 30 day, 6 months and 1 year post transplant updates.

For the purposes of quality assurance (i.e. accreditation of the NHLBI Transplant program), data without identifiers will be released to the Center for International Blood and Marrow Transplant Research (CIBMTR) according to federally mandated policies and procedures.

15.0 PHARMACEUTICALS

15.1 **Alemtuzumab (CAMPATH-1H, Campath®)**

Supply: Available through the Campath Distribution Program (The Sanofi Foundation for North America 1-877-422-6728). Vials are provided through this program upon completion of a patient specific request form. Prior to submission of a drug request the patient must provide authorization for the release of medical information (NIH-527). Refer to the Pharmacy Department or Clinical Pharmacy Specialist for additional details on drug procurement.

Product description: Alemtuzumab is available in single use vials. Each single use, clear glass vial of Campath contains 30 mg Alemtuzumab in 1 mL of solution (8.0 mg sodium chloride, 1.44 mg dibasic sodium phosphate, 0.2 mg potassium chloride, 0.2 mg monobasic potassium phosphate, 0.1 mg polysorbate 80, and .0187 mg disodium edetate dehydrate). No preservatives are added. Each carton contains three Campath vials (NDC 50419-357-03) or one Campath vial (NDC 50419-357-01).

Storage and stability: Vials of Alemtuzumab should be stored at 2-8°C (36-46°F) and protected from sunlight. The vial should not be frozen; if the vial has been frozen it should be discarded. Alemtuzumab contains no antimicrobial preservative. An internal NIH Pharmacy (Pharmaceutical Development Section) conducted study demonstrated 24 hour stability of alemtuzumab when diluted in 0.9% sodium chloride to a concentration range of 6.67 mcg/mL to 120 mcg/mL at room temperature (Goldspiel JT, et.al. Stability of alemtuzumab solutions at room temperature. Am J Health –Syst Pharm, accepted for publication). Alemtuzumab solutions prepared in the concentration range described above may be stored at room temperature (15-30°C) for up to 24 hours. If diluted outside of this concentration range, alemtuzumab should be used within 8 hours after dilution. Alemtuzumab solutions may be stored at room temperature (15-30°C) or refrigerated. Alemtuzumab solutions should be protected from light.

Preparation: The vial should be inspected for visible particulate matter and discoloration prior to administration. If particulate matter is present or the solution is discolored, the vial should not be used. The vial should not be shaken prior to use. The necessary amount of alemtuzumab should be withdrawn from the vial into a syringe. The vial

contains no preservatives and is intended for single use only; the vial should be discarded with any unused portion after 6 hours. The desired dose is then injected into 100 mL sterile 0.9% Sodium Chloride USP or 5% Dextrose in Water USP. The bag should be gently inverted to mix the solution. The syringe is discarded.
Administration: Alemtuzumab should be administered intravenously only. The infusion should be administered over a 2-hour period.

15.2 **Filgrastim (G-CSF, Neupogen®)**

Supply: Commercially available.

Product description: Filgrastim injection is available in a concentration of 300mcg/ml in 1ml (300mcg) and 1.6ml (480mcg) vials.

Preparation: For subcutaneous administration, the appropriate prescribed dose is drawn up from the vial with no further dilution prior to administration. For intravenous administration, the commercial solution for injection should be diluted prior to administration. It is recommended that the prescribed dose be diluted with dextrose 5% in water (DO NOT DILUTE WITH NORMAL SALINE) to a concentration greater than 5mcg/ml. Filgrastim diluted to concentrations between 5 and 15mcg/ml should be protected from absorption to plastic materials by the addition of Albumin (Human) to a final concentration of 2mg/ml. When diluted in 5% dextrose or 5% dextrose plus Albumin (Human), filgrastim is compatible with glass bottles, PVC and polyolefin IV bags, and polypropylene syringes.

Storage and Stability: Filgrastim for injection should be stored in the refrigerator at 2º to 8ºC (36º to 46ºF). Avoid shaking.

Route of administration: Subcutaneous injection or intravenous infusion over 15-30 minutes.

15.3 **Sirolimus (Rapamune®)**

Supply: Commercially available.

Product description: Sirolimus is available in 0.5, 1, and 2 mg tablets and an oral solution (1 mg/mL concentration) in a 60 mL amber glass bottle.

Preparation: For administration of the oral liquid, the measured dose may be diluted in water or orange juice prior to administration. Empty the correct amount of sirolimus oral liquid into only a glass or plastic container holding at least two ounces of water or orange juice. No other liquids, including grapefruit juice, should be used for dilution. Stir vigorously and drink at once. Refill the container with an additional volume (minimum 4 ounces or 120 mL) of water or orange juice, stir vigorously, and drink at once.

Storage and Stability: Oral tablets should be stored at room temperature: 20-25 °C (68-77 °F). Oral solution should be refrigerated (2-8 °C or 36-46 °F). Once the oral solution bottle is opened, the contents should be used within one month. If necessary, the patient may store the bottles at room temperatures up to 250 °C for a short period of time (e.g. not more than 15 days for the bottles).

Route of administration: Oral.

15.4 **Pentostatin (Nipent®)**

Supply: Commercially available

Product description: Pentostatin is available as a lyophilized powder in vials containing 10 mg of drug.

Preparation: The lyophilized powder will be reconstituted according to manufacturer instructions, into a solution of a 2 mg/ml concentration. The appropriate dose of the reconstituted pentostatin solution will be further diluted with 500 ml of 0.9% sodium chloride and infused over 30 to 60 minutes.

Storage and Stability- Upon reconstitution, the pentostatin can be stored at room temperature but should be used with 8 hours of reconstitution.

Route of administration: Intravenous infusion. Subjects will receive one liter of 0.9% sodium chloride by intravenous infusion as pre-hydration prior to the pentostatin delivery. Pentostatin will be infused over 30-60 minutes.

15.5 **Cyclophosphamide (Cytoxan®)**

Supply: Commercially available.

Product description: Cyclophosphamide is available for oral use as tablets providing 25 mg or 50 mg of cyclophosphamide.

Storage and stability: Oral tablets should be stored at controlled room temperature 15-30ºC (59-86ºF).
Route of administration: Oral.

Filgrastim and sirolimus are used as labeled in the prescribing information. Alemtuzumab, pentostatin, and cyclophosphamide are used as part of this transplant regimen and considered to be exempt from IND based on the fulfilling the following criteria:

- All 3 drugs are lawfully marketed in the US;
- their use is not intended to be reported to FDA as a well-controlled study in support of a new indication and there is no intent to use it to support any other significant change in the labeling of the drug;
- their use does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug product;
- their use is conducted in compliance with the requirements for review by an IRB (21 CFR part 56) and with the requirements for informed consent (21 CFR part 50);
- The investigation is conducted in compliance with the requirements of § 312.7 (i.e., the investigation is not intended to promote or commercialize the drug product).

15.6 **Busulfan**

Busulfan is a bifunctional alkylating agent approved for use as a conditioning agent prior to allogeneic hematopoietic stem cell transplantation. The IV formulation is commercially available as busulfan (Otsuka America Pharmaceutical, Inc.)

- Generic: busulfan
- Classification: alkylating agent
- Action: Alkylates and crosslinks DNA. It has a more marked effect on myeloid cells than on lymphoid cells.
- Source: For patient administration, IV busulfan is purchased by the NIH Clinical Center Pharmacy Department from commercial sources. The drug is supplied as a clear, colorless sterile solution in 10mL single use vials. Each vial of busulfan contains 60mg (6mg/mL) of busulfan.
- Stability and Storage: Unopened vials of parenteral busulfan are stable until the date indicated on the package when stored under refrigeration at 2-6 degrees C (36-46 degrees F).
- Product description: Injection
- Preparation: Parenteral busulfan must be diluted prior to use with either 0.9% sodium chloride or 5% dextrose injection. The diluent quantity should be 10 times the volume of busulfan so that the final concentration of busulfan is approximately 0.5mg/mL. Busulfan should be administered intravenously via a central venous catheter as a 3-hour infusion every 24 hours for a total of 3 doses over 3 days.
- Route: Intravenous
-

16.0 Compensation

Compensation will be provided to donors for their time and inconvenience. They will receive $150 for testing at their 1 year post- transplant visit. 1 night of lodging will be paid for returning for testing. For patient under 18 years old an additional $20 will be paid for escort fee.

Procedure	Inconvenience Unit (IU) or Time	Payment
Neuropsychological Exam	4 hours	$50.00
Neuropsychological Exam (memory testing, processing, verbal and nonverbal reasoning and the quality of life questionnaires)	10 IU	$100.00
Total Compensation		$150.00

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APPENDIX A: ECOG PERFORMANCE STATUS SCALE

GRADE	DESCRIPTION
0	Fully active, able to carry on all pre-disease activities without restriction.
1	Restricted in physically strenuous activities and able to carry out work of a light or sedentary nature, e.g. light housework, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B: TRANSFUSION OF RED CELLS TO RECIPIENTS OF ABO INCOMPATIBLE MARROW

Major ABO incompatible recipie–t - donor

Patient	Donor	Transfused RBC = patients group
O	A, B or AB	O
A	B or AB	A or O
B	A or AB	B or O

Minor ABO incompatibility recipient - donor

Patient	Donor	Transfused RBC = donor group
A, B or AB	O	O
AB	B	B or O
AB	A	A or O

APPENDIX C: TRANSFUSION MEDICINE GUIDELINES FOR APHERESIS PROCEDURES IN SICKLE CELL PATIENTS RECEIVING NONMYELOABLATIVE ALLOGENEIC PBSC TRANSPLANTS

Introduction

Sickle cell disease (SCD) patients are at risk for vaso-occlusive crises such as cerebrovascular accidents and the acute chest syndrome which occur due to the viscosity and sickling properties of sickle hemoglobin S. Pre and peri-transplant maneuvers, such as transfusion or red cell exchanges with allogeneic red cells, may be performed to reduce the risk of ischemic events by reducing the levels of hemoglobin S. Pre-transplant coordination with the Department of Transfusion Medicine (DTM) is critical because these patients may have had prior transfusions and have developed alloantibodies. In addition, the distribution of red blood cell phenotypes in patients with SCD will reflect their ethnic heritage and may differ from that in the NIH donor pool. Obtaining prior transfusion records, prior antibodies and/or transfusion reactions, and recruitment of adequate numbers of compatible units thus requires careful advance planning and knowledge of the patient's phenotype and antibody screen.

Allogeneic transplant patients who receive a lymphocyte-replete PBSC graft will also be at risk for increased red cell requirements if there is an ABO incompatibility with the donor. Minor ABO incompatibility, such as O donors into A, B or AB recipients, is associated with hemolysis due to production of anti-recipient isohemagglutinins by passenger lymphocytes. The appropriate transfusion policy in the peri-transplant period for the ABO group of red cell, plasma and platelet transfusions in patients with ABO incompatible donors is also managed according to standard DTM transplant policies. In addition, the DTM will use red cell serologic testing to carefully monitor

those patients who have minor ABO incompatibility with the donor for evidence of hemolysis in the peri-transplant period using a standard operating procedure.

Major ABO incompatibility, such as A or B donors into O patients, may be associated with a delayed onset of effective donor erythropoiesis resulting in pure red cell aplasia after conversion to sustained donor hematopoiesis. This event appears to be most common after non-myeloablative conditioning regimens that are permissive for persistent production of anti-donor, host-type isohemagglutinins. These patients may have a further increased red cell requirement and need for advance planning and recruitment.

A potential adverse event in hematopoietic transplantation for congenital anemias is rejection of the PBSC graft, especially in patients who have been heavily transfused. Obtaining higher numbers of donor stem cells may reduce the risk of graft rejection. To achieve this goal, the DTM will collect stem cells using a single very large volume apheresis (~4-5 donor blood volumes) on day 5 after filgrastim (G-CSF) administration, which reduces the incidence of thrombocytopenia in the donor associated with apheresis, reduces apheresis time and the time with which central venous catheters remain in place, and produces the same yields as two smaller donor blood volume procedures performed consecutively on days 5 and 6.

All sickle cell transplant candidates and their identified donors will need to have a full red cell phenotype, antibody screen, and quantitative hemoglobin electrophoresis obtained during initial evaluation, well in advance of any apheresis procedures. The DTM will enter appropriate restrictions for blood product transfusion based on this information.

Procedures for Red Cell Exchanges

Sickle cell patients who are not receiving long term transfusion therapy will be evaluated by the DTM fellow/senior staff and considered for a prophylactic red cell exchange prior to transplant to bring the target fraction of hemoglobin S to less than 30% to reduce the incidence of post-transplant stroke and other events that may be associated with high hemoglobin S levels. ADSOL leukoreduced packed red cells will be used for the exchange.

These patients will have a hemoglobin electrophoresis performed to determine their initial fraction of hemoglobin S (% HbS). They must also have a full type and screen performed to identify alloantibodies and allow for recruitment of donors prior to the exchange. The patient total blood volume will be computed from an algorithm using the COBE computer, and the volume of replacement PRBC required for the exchange estimated by utilizing this computer in conjunction with the initial HbS content and the desired end hematocrit and HbS concentration. For this protocol, the calculation of the COBE computer may be verified using the following calculations for determination of the volume of replacement PRBCs needed for the red cell exchange.

> Blood volume x patient hct = Pati'nt's Total Packed Red Cell Volume (PRCV)
> (PRCV) x % HbS = Patient Total Packed RBC Volume of HbS (PRCV-S)
> The volume of ADSOL PRBC needed to bring the residual fraction of red cells to 30% is 1.25 exchange volumes. (= 1.25 x (PRCV-S))

Since increasing the hematocrit in patients with high levels of HbS may precipitate vaso-occlusive crises, the red cell exchange will replace the red cells that are removed with an equal volume of infused red cells. Patients who are significantly anemic may have further transfusions given <u>after</u> the exchange to bring their final hematocrit up to 35%. In these cases, the target % HbS should be 30% after the final transfusions bring the hematocrit up to 35%. The target for the % HbS after the exchange (before additional transfusions) is 35/Hct x 30%, where Hct is the patient hematocrit before the exchange. After the exchange the patient will then receive a volume of red cells equal to approximately (0.35-Hct)(wt)(70 ml/kg).

Patients undergoing red cell exchange may experience citrate toxicity from the anticoagulant used in the apheresis procedure and contained in the ADSOL red cells. A citrate infusion rate will be calculated by the DTM fellow/senior staff based on the flow rate of returned red cells plus 2/3 of the citrate infusion rate. Patients who

receive more than 1.2 mg of citrate per kilogram per minute will receive intravenous calcium through the return line at a rate of 0.5 mg of calcium ion per 21 mg of citrate.

Donor apheresis procedures

I. Donor stem cell mobilization with filgrastim (G-CSF)

After medical evaluation and clearance for suitability as an allogeneic donor, each donor will undergo mobilization with G-CSF, usually as an outpatient. The G-CSF will be administered in a dose of 10 to 16 ug/kg/day for 6-7 days, subcutaneously. The doses for days 1-4 may be given at any time of day, but the doses for day 5 and if necessary, day 6 must be given early in the morning, at least one hour prior to starting apheresis. Day 5 evening dosing may substitute the early morning dosing of day 6. Predictable side effects of G-CSF, including headache, bone pain, and myalgia, will be treated with acetaminophen or ibuprofen. Prophylactic treatment of these side effects with the same medications may be elected. Other side effects will be evaluated and treated accordingly.

II. Donor stem cell collection

The target CD34 dose is 10×10^6/kg, and the minimum is 5×10^6/kg. Donors will receive calcium chloride prophylaxis to prevent citrate toxicity in accordance with standard DTM policies.

The volume processed per apheresis procedure will be determined by DTM medical staff on the day of apheresis, based on peak CD34 cell mobilization response to filgrastim (G-CSF) and the CD34 cell dose needed, based on kilogram weight of recipient. This will range from 15 to 35 liters processed per day for 1 to 3 days, not to exceed a total of 75 liters over 3 days.

The goal is to provide a sufficient number of CD34 cells to ensure engraftment and test the efficacy of this modality against disease relapse. Second cycle of mobilization and collection may be needed in donors whom insufficient number of stem cells were collected. Plerixafor may be added in selected patients according to standard of care/BMT Consortium Guidelines Standard Operating Procedures.

III. Filgrastim (G-CSF) administration.

G-CSF will be administered according to a vial-based algorithm to reduce wastage, improve patient compliance, and increase the total G-CSF dose to lighter weight donors in order to improve CD34 yields.

Donor Weight	**Total filgrastim (G-CSF) Dose (range)**
38-48 kg	600 mcg (12.5 to 15.8 mcg/kg)
49-56 kg	780 mcg (13.9 to 15.9 mcg/kg)
57-60 kg	900 mcg (15.0 to 15.8 mcg/kg)
61-67 kg	960 mcg (14.3 to 15.7 mcg/kg)
68-108 kg	1080 mcg (10.0 to 15.9 mcg/kg)
$\geq$ 109 kg	1200 mcg (11.0 or less)

IV. Ex vivo processing of PBSC and lymphocytes

The target cell doses for the PBSC graft are outlined in the section above.

For this protocol, there will be no T cell depletion of the PBSC or bone marrow. The PBSC and lymphocyte products will be cryopreserved in 5% DMSO/pentastarch for later thawing and infusion. In cases of RBC incompatibility, product manipulations will be done prior to cryopreservation. For minor ABO or other red cell incompatibility, PBSC products may undergo plasma removal, with resuspension in an infusible isotonic solution, according to standard operating procedures in the DTM Cell Processing Laboratory.

All products will be prepared for infusion by SOPs of the DTM Cell Processing Laboratory.

Recipient apheresis procedure

Recipient stem cell mobilization with plerixafor +/- G-CSF

For patients ≥10 years of age, plerixafor will be given at a dose of 240 ug/kg subcutaneously in the early morning, followed by apheresis 4-6 hours after the dose. If the goal CD34 yield is not met, the patient can receive a second dose of plerixafor 240 ug/kg subcutaneously followed by a second apheresis the next day. The patient will return within one week (+/- 2 days) for history and physical exam and complete blood count with differential. If the minimum CD34 count is not reached after 2 aphereses, the patient can return a minimum of 1 month later to be treated again with plerixafor monotherapy or plerixfor with G-CSF based on the patient's previous response.

If at least 2 patients do not successfully achieve target CD34 yields after two apheresis procedures with plerixafor monotherapy, subsequent patients including patients who have undergone unsuccessful apheresis with plerixafor monotherapy may be treated with G-CSF in addition to plerixafor. Patients will be treated with hydroxyurea at a dose of at least 20 mg/kg/day prior to initiating G-CSF, and hydroxyurea will be held for at least 2 weeks prior to G-CSF mobilization. The G-CSF dose for each patient will be discussed between the PI and DTM but will range from 5-10 ug/kg (total dose adjusted to the nearest vial). G-CSF will be given subcutaneously for five consecutive days. On the evening of the fourth day, plerixafor at a dose of 240 ug/kg subcutaneously will be given followed by apheresis 10 hours later. If the goal CD34 yield is not met, the patient can receive a 6th dose of G-CSF on the evening of the 5th day as well as a second dose of plerixafor followed by second apheresis 10 hours later. Complete blood count with differential will be frequently obtained while the patient receives G-CSF. The patient will return within one week of apheresis (+/- 2 days) for history and physical and complete blood count with differential.

Recipients younger than 10 years-old will likely undergo bone marrow harvest.

II. Recipient stem cell collection

The minimum CD34 dose is 1 x 10^6/kg, target is ≥2 x 10^6/kg. Recipients will receive calcium chloride prophylaxis to prevent citrate toxicity in accordance with standard DTM policies. The volume processed per apheresis procedure will be determined by DTM medical staff on the day of apheresis based on peak CD34 cell mobilization response to plerixafor and/or G-CSF, the CD34 cell dose needed, and body weight of the recipient. This typically range from 15 to 30 liters processed per day for 1 to 2 days, not to exceed a total of 60 liters over 2 days.

The target for marrow harvest in those <10 years-old is 1x10^8 cells/kg.

APPENDIX D: IDEAL BODY WEIGHT, ADJUSTED BODY WEIGHT, AND PRACTICAL BODY WEIGHT CALCULATIONS

Ideal Body Weight Formula:

Males: 50 kg + (2.3 x the number of inches > 5 feet)
Females: 45 kg + (2.3 x the number of inches > 5 feet)
Pediatrics (age 4-20): consider weight based on the same percentile as stature (height) for age, using CDC weight for stature growth curves.

Adjusted Ideal Body Weight Formula:

[(actual weight – ideal weight) x 25%] + ideal weight

Note: If the patient is ""morbidly obese"" (BMI > 35), adjusted ideal or practical weight (average of ideal and adjusted ideal body weight) may be used after discussion with pharmacist or other transplant providers.

APPENDIX E: Administration Scheme for Busulfan

Busulfan will be administered at a dose based on the calculated AUC of a busulfan test dose of 0.8 mg/kg that will be given 5-14 days prior to the preparative regimen. The busulfan conditioning dose will then be calculated to obtain a targeted AUC of 4800 μM.min. (The PI will have discretion to use a target range of 3600 to 6000 μM.min.) If the test dose cannot be completed or the pharmacokinetic data from the test dose cannot be accurately interpreted, the conditioning busulfan dose will default to a dose of 3.2 mg/kg/dose once daily for 3 consecutive days via IV infusion. The total cumulative dose of the regimen is about 75% of the myeloablative dose. Keppra (levetiracetam) and clonazepam will be initiated at least 12 hours before the first dose of the conditioning dose of busulfan for seizure prophylaxis and both drugs will be discontinued following the morning dose after the last dose of busulfan. To further minimize the risk of seizures and other neurologic complications, magnesium deficiency will be corrected, arterial hypertension will be strictly controlled, and the goal hemoglobin will be 9g/dL and goal platelet count 50K/uL peri-transplant.

Busulfan dosing (default of 3.2mg/kg or by pharmacokinetic testing) will be based on ideal body weight (**Appendix D)** or actual body weight, whichever is lower. For children age 4 through 16, busulfan dosing is based on actual body weight, although the ideal body weight may be used for the test dose if it is lower than the actual body weight. For recipients who are greater than 120% of ideal body weight, busulfan will be dosed on an adjusted ideal body weight (ideal body weight plus 25% of the difference between ideal and actual weight). Four busulfan blood samples will be drawn following the administration of the test dose in a green top (sodium heparin) collection tube (1 mL specimen volume). Optimally, samples should be obtained from a peripheral vein in the arm opposite to the central line where busulfan is infused. If a peripheral vein sample is not feasible, the samples should be obtained from a different central catheter lumen than that used for the busulfan infusion. The first specimen should be drawn immediately after termination of the 2-hour intravenous infusion of 0.8 mg/kg busulfan. Additional specimens should also be drawn at 1 hour, 2 hours and 4 hours after termination of infusion. Each sample should be placed on wet ice immediately after collection. All samples will be sent to outside laboratory for pharmacokinetic monitoring.

If the test dose cannot be completed for unforeseen reasons or if the pharmacokinetic data obtained cannot be accurately interpreted, the busulfan conditioning dose may default to 3.2mg/kg by IV infusion over three hours once daily, based on the ideal body weight or actual body weight, whichever is lower. PK data from the first 1 or 2 days of busulfan dosing may be obtained to adjust dosing for day 3 or 4.

APPENDIX F: Addendum A: Neuropsychological functioning and quality of life in patients with severe congenital anemias (Donors and Recipients)

1.0 Objectives

1.1 Primary Objectives:

1.1.1 To examine the neuropsychological functioning of patients and explore their cognitive strengths and weaknesses among the following main outcome measures:
- Wechsler Abbreviated Scale of Intelligence – Full Scale IQ
- California Verbal Learning Test – Total Trials 1 – 5 T-score
- Digit Span Backwards scaled score
- Test of Everyday Attention Elevator Counting raw score (adults)
- Processing Speed Index
- DKEFS Trailmaking Condition 4 scaled score

1.1.2 To compare, in an exploratory fashion, the neuropsychological functioning between and within the patients and their donor siblings (control group), using the primary measures listed above.

1.2 Secondary Objectives:

1.2.1 To examine patterns of scores on other neuropsychological tests and behavioral questionnaires and on the QOL/pain measures in patients and sibling donors.2 To explore the relationships of neuropsychological and behavioral functioning and QOL with medical, neuroimaging, and family variables.

1.2.3 To explore the sibling donors' experience using the following measures:
- Sibling Pre-Donation Questionnaire
- Sibling Post-Donation Questionnaire

2.0 Background/Scientific and Clinical Justification

2.1 Neuropsychological and emotional functioning in individuals with severe congenital anemiaa. Sickle cell disease

Patients with sickle cell disease (SCD) are at risk for neuropsychological deficits, often related to the occurrence of ischemic injury.[1] [Gold et al, 2008] Impairments often are first evident in childhood. Global cognitive deficits have been found among youth with overt cerebrovascular accidents as well as those with silent infarcts. Some evidence suggests that even children with SCD with no known history of infarcts (i.e., no visible brain abnormalities on MRI scans) perform worse on cognitive measures compared to healthy children. According to existing literature, measures of specific cognitive abilities, such as attention and executive functions, may be more sensitive to the effects of the disease than global cognitive (IQ) scores.[3]

The long-term consequences of such deficits in childhood appear to impact adult functioning in a variety of ways, although neurocognitive outcomes in adults with SCD have not been widely reported in the literature to date.[6] Cognitive test scores continue to be lower in adults with SCD compared to healthy controls in global cognitive functioning and specific areas such as working memory, processing speed, and executive function (Vichinsky et al., 2010 – PMID 20460621). Some research suggests that cognitive difficulties worsen in adulthood and impact activities of daily living (Feliu et al., 2011 – PMID 22035043).

Studies of emotional functioning among children and adults with SCD have produced conflicting reports. While some have found normal levels of emotional well-being[7], others have found a substantial portion of these patients to have internalizing problems, such as anxiety and depression, in the clinical range. Further, symptoms of anxiety and depression are related to lower quality of life among children and adults with SCD. [10,11] Another factor that can impact QOL significantly is pain, which is one of the hallmarks of sickle cell disease.[12] [Ballas et al 2012] Other variables related to poor quality of life in this population include sleep disturbances and disease-related parenting

stress.[10, 13] Additional studies are needed to elucidate the factors predictive of impaired psychological well-being and quality of life among patients with SCD.

b. Beta-thalassemia

Comparatively little is known about the neuropsychological sequelae of beta-thalassemia. Only one report in the literature was identified that used standardized tests to describe the cognitive functioning in children with this disorder.[14] Results indicated that a significant proportion of these youth demonstrate below average global cognitive abilities.[14] A study of adults with beta-thalassemia found low performance relative to healthy controls on tests of executive functioning, attention, visual-spatial skills, and memory.[15] With respect to emotional functioning, research has shown that children and adults with beta-thalassemia have increased levels of depression, anxiety, and poor self-image[16, 17], as well as impaired quality of life.[18]

2.2 Neuropsychological and emotional outcomes in patients undergoing stem-cell transplantation (SCT)

In addition to the neuropsychological complications of SCD and beta-thalassemia, further risk is inherent in the SCT process. Individuals who undergo a SCT may exhibit subsequent impairments due to exposure to neurotoxic agents, including total-body irradiation and high dose ablative chemotherapies, as well as those used for prophylaxis of GVHD.[21-23] Published reports on neuropsychological outcomes in SCT patients have produced mixed results. Numerous studies have reported significant delays, including difficulty with receptive and expressive language, attention[24], processing speed[25], visual and perceptual motor skills[23], memory[24-26], [Iampietro, Giovannetti & Tarazi 2014] and adaptive functioning.[21] Others have described normal neurodevelopment and no declines in functioning among children following BMT or SCT.[21,22,28,29]

The current protocol uses low dose radiation in an attempt to decrease toxicities. Past studies have demonstrated a deleterious impact of higher doses of radiation on cognitive outcomes.[30, 31] However, some negative effects are evident among patients receiving lower doses as well.[32] In addition to dose of radiation, other specific risk factors for neurocognitive deficits post SCT/BMT include younger age at time of treatment[23,33], treatment with both cranial radiation and systemic or intrathecal chemotherapy, poor pre-transplant functioning[22,29], and female gender.[34, 35] Finally, most studies have examined neuropsychological functioning at 1 to 2 years post-transplant [22, 23, 29] but they have not looked at changes in functioning during the transplant process. It is important to identify early cognitive difficulties that might occur in addition to investigating the long-term outcome to better understand the pattern of changes throughout the process.

For transplant patients, the demands of SCT including physical isolation, absence from school or work, and neurocognitive and physical changes may result in impaired quality of life. Studies of psychological sequelae of BMT/SCT suggest increased symptoms of anxiety, depression, anger, sadness, hypervigilance, hostility, and social withdrawal[36-39] accompanied by reports of increased fatigue, sleep disturbance, appetite loss, and decreased activity.[36, 39-41] Many studies report peaks in these symptoms during the weeks immediately following transplantation. However, others have reported peaks in emotional distress immediately before transplant, suggesting that patients may enter the hospital with an already heightened level of distress. [39, 42] Some pediatric longitudinal studies have reported that symptoms of psychological distress typically return to admission levels 4 to 6 weeks post-SCT and dissipate several months post-transplant.[36, 39, 41] Other studies have found persistent psychological distress 6 to 12 month's post-BMT.[28, 43] Specific risk factors for impaired quality of life include increased worry, reduced communication (e.g., with family and physicians), poor family cohesion, older age, and increased medical symptom severity post-BMT.[36, 39, 44]

2.3 Functioning of sibling donora. Use of sibling donors as a comparison group in research

A thorough understanding of the neuropsychological functioning and QOL of patients who undergo SCT is made complicated by the numerous variables that can impact cognitive outcomes, including factors related to the disease and its associated treatment. Differentiating these potential causal factors from genetic and environmental factors is extremely challenging and often impossible. One method of resolving this problem involves comparing the functioning of patients with their biological siblings. The authors of a meta-analysis[3] noted the appropriateness of using a sibling control group for examining the cognitive effects of SCD while accounting for the unique roles of family environment and genetics. One recent study of children treated with SCT was able to demonstrate that the

cognitive deficits in the patient group were not accounted for by environmental factors, since the siblings scored significantly higher and in the normal range.[45] Further studies utilizing this method are needed in order to characterize the neuropsychological profile of individuals undergoing SCT.

b. Coping of sibling donors

With respect to the emotional well-being of sibling donors, a recent review article noted several studies that identified these youngsters as being at risk for depression, withdrawal, behavioral problems, and lowered self-esteem.[46] Being a stem cell donor can be a stressful experience for pediatric siblings and can potentially lead to the development of long-term distress responses. Outcomes following SCT appear to have a direct affect on the sibling stem cell donors' psychosocial adjustment. Feeling responsible for the transplant outcome is of notable concern for sibling donors. As the outcome of a SCT relies in part on the histocompatibility of the donor and recipient, when the recipient experiences transplant complications, sibling donors may believe that they caused the complications because their marrow or stem cells were not "good enough".[47] The literature has found that: (a) education about the HSCT process alone is inadequate preparation[48,49], (b) children should receive preparatory information and have opportunities to express concerns and questions in order to manage anxiety and guilt[50], (c) children should have the opportunity to express emotions throughout the SCT process[51], and (d) parents should be involved in the donor's preparation and follow-up to ensure family communication during SCT.[49] A positive response to the donor experience, such as improved family relationships, along with heightened intimacy between recipient and donor has also been described in previous research on conventional BMT donors [52, 53]. Whether or not the transplant is successful, each sibling's family life will be interrupted by the transplant experience. Therefore, for clinical purposes, obtaining a psychosocial assessment of the sibling donors strengths and vulnerabilities prior to and following the transplant process and having a solid understanding of how the sibling might cope if the transplant is unsuccessful are essential components to the donation process. However, as no studies were identified that examined the experiences specific to SCD in sibling donors, this study will explore in a qualitative, preliminary manner, the sibling donor experiences (secondary objective 1.2.3).

3.0 Eligibility Assessment

3.1 Eligibility Criteria

3.1.1 Every patient who enrolls on the transplant protocol will be eligible to participate in the neuropsychological and QOL/pain assessments.

3.1.2 All sibling donors who enroll on the transplant study will be eligible to participate in the neuropsychological and QOL assessments.

3.2 Exclusion Criteria

3.2.1 Presence of psychotic symptoms, extreme behavioral difficulties, and/or severe cognitive impairment that, in the judgment of the Principal or Associate Investigator(s), would compromise the patient's or sibling's ability to engage in the neuropsychological testing or QOL assessment or is likely to interfere with the study procedures or results.

4.0 Study Design

4.1 Neuropsychological and QOL/pain assessment in patients with severe congenital anemias

We plan to examine the longitudinal neuropsychological profiles of children (ages 16 and 17) and adults (18+) undergoing SCT by assessing the domains of global cognitive functioning, memory, attention, processing speed, executive functioning, academic functioning, and fine motor skills using a battery of age-appropriate neuropsychological measures. As part of this battery of tests, we also will assess psychological and behavioral functioning. All of the neuropsychological tests and questionnaires have been validated for use in children and/or adults with chronic illness. Some of the tests have Spanish versions, and any Spanish-speaking patients enrolled on the protocol will be administered these measures and/or administered the nonverbal measures by translating the instructions into Spanish. The neuropsychological tests will be administered by members of the Health Psychology

and Neurobehavioral Research Group consisting of licensed psychologists, psychology associates, or supervised graduate students in psychology.

The comprehensive neuropsychological test battery will be administered to patients at baseline (Week –1) and 12 and 24 months post-SCT, and a brief monitoring battery will be given at 100 days post-transplant . The estimated total time for administration of the tests in the comprehensive battery ranges from 90 to 105 minutes, depending on patient age. The parent report questionnaires that are part of the comprehensive battery will take about 60 minutes to complete and can be done while the patient is being evaluated. The brief monitoring battery takes approximately 30 minutes to administer, and the parent questionnaires that are part of the brief monitoring battery take about 25 minutes to complete. Patients will not be tested when they are febrile or are not able to eat by mouth. Breaks will be taken as needed throughout the test session.

A brief assessment of quality of life and pain may be administered before transplant, and at (+/- 7 days) 1 month, 2 months, 100 days, 6 months, 12 months, 18 months, and 24 months post-transplant, as designated in the assessment schedule. Questions will focus on areas such as physical activity, emotional well-being, social functioning, and academic achievement. Since health related quality of life has been reported to be negatively impacted by high reported frequency of pain as well as high ratings of current pain, we will complement the quality of life measurements with a separate pain questionnaire that includes two visual-analogue items. The QOL/pain assessments will take approximately 10 minutes to complete.

4.2 Neuropsychological and QOL assessment in sibling donors
In addition to evaluating the patients, we will assess sibling donors of all ages as well. The same comprehensive neuropsychological battery and QOL measures will be administered to the siblings at baseline and 12 months post-transplant. The pain questionnaires that are part of the patients' QOL assessment will be omitted for the siblings' QOL assessment.

4.3 Clinical assessment of sibling donors
Each sibling donor will have a pre-transplant clinical assessment conducted by a member of the Psychosocial Support and Research team, administered for clinical purposes. This individual, either a social worker, psychologist, child psychiatrist, or supervised graduate student, will also provide a brief telephone follow-up at one month post stem cell donation with both the sibling and the siblings' parent (for siblings living with their parents and/or under age 25) to assess for any subsequent distress associated with the donation process.

Sibling donors will also be administered a short qualitative pre-donation and post-donation questionnaire designed for this study to explore the siblings' experience being a stem cell donor. The pre-donation questionnaire will be administered at the visit pre-donation and the post-donation questionnaire will be administered at 12 months post-transplant. These questionnaires are designed for sibling donors ages 7 and older and are conducted via an interview format with a member of the Psychosocial Support and Research team. Each child will be asked if they are comfortable participating in the interview without a parent present. If they are not, the parent will be invited to be present.

4.4 MRI studies
Each patient will undergo a brain MRI at pre-transplant, and at 12 and 24 months post-transplant. A neurologist or neuroradiologist will review and categorize each scan as (1) normal (no CNS pathology), or (2) evidence of cerebral infarction. It is anticipated that the majority of participants will not require sedation in order to tolerate neuroimaging. However, all participants requesting sedation will be offered monitored anesthesia care through the Department of Anesthesia and Surgical Services (DASS), Clinical Center, NIH, and a separate consent will be completed as needed.

4.5 Off Study Criteria
- 4.5.1 Completion of study.
- 4.5.2 Parent or patient/sibling request.

4.5.3 Participant withdraws as a patient from an NHLBI protocol either due to untreatable progressive disease or death.
4.5.4 Participant is documented to have a serious mental disorder/psychiatric condition that warrants therapeutic intervention and which in the judgment of the Principal or Associate Investigators or consulting psychiatrist would compromise the patient's ability to engage in testing or otherwise interfere with the study procedures or results.
4.5.5 Study cancelled or stopped.
4.5.6 Study discontinuation at the request of the patient, parent of the patient/sibling, or at the discretion of the Principal Investigator or Study Chairperson if they determine that study withdrawal is in the patient's best interest.

5.0 Data Collection

5.1 Neuropsychological Assessments
The neuropsychological assessments will be administered by members of the Health Psychology and Neurobehavioral Research Group of the Pediatric Oncology Branch (POB) of the National Cancer Institute (NCI). This group is comprised of licensed psychologists Psychology Associates, and graduate level psychology students. The Psychology Associate and graduate students are supervised by one of the licensed psychologists. The psychologists have extensive experience with neuropsychological testing with patients of all ages.

The neuropsychological test data are handled in compliance with Clinical Center policies, the Health Insurance Portability and Accountability Act (HIPPA), and the ethical principles relating to psychological data as outlined by the American Psychological Association. These data are stored in the NCI neuropsychological database that is currently located on the secure and well-maintained Titan mainframe computer system at the NIH Computer Center that can be accessed from the psychologists' desktop computers. Given that the mainframe is scheduled to be shut down in the near future, the database is in the process of being transferred to a SAS system on the secure Psychology server. The secure neuropsychological database includes names of the NIH patients but the accompanying lines of data are not interpretable without a separate password-protected SAS program. No identifying information will be included in any presentation of findings, including publications in peer-reviewed journals or conference presentations.

5.2 QOL/Pain Assessments
The QOL/pain assessments will be conducted by a member of the Neuropsychology Testing and Research Group or the sickle cell medical team. These data will be entered into the NCI neuropsychological database along with the results from the neuropsychological testing.

5.3 Statistical Considerations
The two primary statistical objectives are (1) to explore the cognitive strengths and weaknesses by examining mean scores in congenitally anemic patients' neuropsychological functioning and changes over time, and (2) to compare the neuropsychological functioning between patients and their sibling donors on six primary outcome measures. Given the small sample size expected, data analyses for these objectives will be done in an exploratory fashion and results will be used as hypothesis-generating for future studies. The specific test scores that will be used to address this objective are: the WASI Full-Scale IQ (global intelligence), the CVLT Trials 1 – 5 T-score (verbal learning and memory), the Digit Span Backward scaled score (working memory), the TEA Elevator Counting raw score, the Processing Speed Index (processing speed), and the DKEFS Trailmaking Condition 4 scaled score (executive function). Possible strengths and weaknesses in functioning will be identified as individual scores that are one standard deviation above or below the normative mean. The success rate will be reported by age group, but the study does not have sufficient power to test for differences among age groups.

To assess the secondary objective 1.2.1 of examining patterns of scores on other neuropsychological tests and on the QOL/pain measures in patients and siblings, a large number of secondary evaluations will be performed in an exploratory fashion. Specifically, repeated measures ANOVAs will be performed to look at differences between and within groups over time, and the results interpreted as secondary and hypothesis generating. The following

measures will be included in these analyses: neuropsychological test results (e.g., WASI Verbal and Performance IQ, WRAT-IV Reading and Arithmetic standard scores, Cancellation scaled score, Grooved Pegboard dominant hand z-score); psychological functioning (parent report questionnaire: BASC-II Composite and subscales for adolescents ages 16 – 21 years), adaptive functioning (parent report questionnaire for adolescents and young adults ages 16 – 25): Vineland Daily Living and Socialization domain standard scores), and behavior (self-report questionnaires: Conners Adult ADHD Rating Scale subscales or parent report questionnaire: Conners Parent Rating Scale subscales). Possible strengths and weaknesses in functioning will be identified as individual scores that are one standard deviation above or below the normative mean.

Another secondary objective (1.2.2) is to assess the relationships of neuropsychological functioning and QOL to medical, neuroimaging, and family variables. We will perform Pearson r or Spearman rank correlations between the following variables: cognitive test results (e.g., IQ, memory, attention, processing speed, and executive function), QOL and pain scores, and hematocrit levels. Patients' MRI scans will be categorized as normal or as having evidence of a cerebral infarction. The scans that show evidence of an infarction will be further divided into those with a clinical history of an overt CVA, and those with no clinical history of a CVA (these will be considered to have had silent infarcts). This classification will result in three final categories: (1) normal, (2) overt CVA, and (3) silent infarction. A multivariate ANOVA will be conducted to compare neuropsychological function between these three groups. Also, among patients whose scans show evidence of infarctions, exploratory analyses will be conducted to assess the relationship between lesion location (e.g., frontal vs. parietal, cortical vs. subcortical) and neuropsychological test scores.

For the secondary objective that explores the sibling donors' experience of the transplantation process, responses to the pre- and post-donation questionnaires will be reported descriptively. Quantitative data will be reported in terms of frequencies of responses to individual questions relating to their satisfaction, understanding, and decision-making around donation of stem cells. Qualitative responses to the open-ended questions about their feelings about having a sibling with a severe congenital anemia and the donation experience will be coded and organized using NVivo8.0 qualitative software[54] and a content analysis will be conducted.

Because of the large number of parameters being evaluated to assess the secondary objectives, it is impractical to correct for multiple comparisons. However, we will set more stringent criteria for statistical significance by setting the alpha level at .01. Moreover, results of these analyses will be considered exploratory and hypothesis-generating for future studies. Careful discussion regarding interpretation of results in this context will be included in any written reports based on these data.

6.0 Human Subjects Protection

6.1 Evaluation of Risks and Benefits:
The primary goal of this neuropsychological/QOL study is to assess the neurocognitive functioning, emotional well-being, and QOL of children prior to, during, and following stem cell transplantation. Minimal risk is involved in participating in these assessments, and potential benefits may be derived. After the testing, patients (and/or parents, as appropriate) will be provided with feedback about the patient's neurocognitive strengths and weaknesses, which can be helpful in educational and career planning. We can also provide specific recommendations to facilitate academic achievement, optimal job performance, and improvements in adaptive functioning. Most people find neuropsychological testing to be interesting and enjoyable. However, some parts may be challenging and some people may become frustrated or tired. Similarly, it may be upsetting for some participants to answer questions about their emotional functioning. If a participant becomes significantly distressed during testing, the evaluator will initiate a break. Any patient or sibling can choose to stop the testing if he or she does not want to continue and participants do not have to answer every question. A licensed psychologist or supervised psychology associate will be available to speak with any participants who become distressed at any point during or following the testing. Referrals to local mental health providers will be made if necessary.

Another part of this study involves the clinical assessment and monitoring of the sibling donors to be conducted by a clinical social worker. Expected benefits include facilitating communication about psychological concerns in

these donor siblings. If the participant reports discomfort in discussing these issues, the interview will end. The siblings and their parents will be informed that not participating in this clinical assessment will not affect their treatment or status on the protocol in any way.

6.2 Consent and Assent Process and Documentation

Since the neuropsychological and QOL/pain assessments will be done as part of the primary medical protocol, no separate consent for these studies will be obtained. The consent and assent forms for the patients and siblings enrolling on the medical protocol include information about the neuropsychological and QOL/pain evaluations. The consent and assent forms for the sibling donors include additional information about the clinical assessment that will be conducted with those participants.

Neuropsychological Test Battery*

Domain	Test	Time (min)	Comprehensive (Baseline, 1 yr, 2 yrs)	Monitor (100 days)
General Intelligence	Wechsler Abbreviated Scale of Intelligence# (4 subtests)	45	√	
Memory & Learning	California Verbal Learning Test-II#	20	√	
Working Memory	Digit Span (WAIS-IV)#	5	√	√
Attention	TEA Elevator Counting Subtest# WAIS Cancellation subtest	5 5	√ √	 √
Processing speed	Processing Speed Index# (WAIS-IV Coding, Symbol Search subtests)	5	√	√
Executive function	DKEFS Trailmaking Conditions 2 – 4#	5	√	√
Fine Motor	Grooved Pegboard	5	√	√
Academic Function	Wide Range Achievement Test-IV (Reading/Arithmetic)	15	√	
Behavior	Parent Report Questionnaires: BRIEF-Parent/Adult BASC-II Vineland Scale (Daily Living/Social) Conners Parent Rating Scale Patients BASC-II (16-17 yrs) or BSI (adults 18+) CAARS	 10 15 15 5 10 5	 √ √ √ √ √ √	 √ √
Background Information	Background Information Form (baseline and update)	5	√	√

Estimated time of the comprehensive assessment:[$] 115 minutes
Estimated time of the monitoring battery:[$] 35 minutes (all ages)

* Other tests may be administered to more comprehensively assess the patient as deemed appropriate by the examiner.
# These tests comprise the primary measures specified in objective 1.1.1. The remaining tests are referred to in secondary objectives 1.2.1 and 1.2.2.
$ The actual times may be longer depending on the examinee's learning style and behavior as some are slower to respond than others and some may need more breaks.

Quality of Life (QOL)/Pain Assessment

Domain	Test	**Time** (min)
QOL	Pediatric Quality of Life Inventory (Peds QL) (–6 - 25 years) Medical Outcomes Study (MOS) SF-36 (23+ years)	5 5
Pain	PedsQL Pain Perception Questionnaire–e - VAS only (16 – 18 years) Short Form McGill Pain Scale – VAS only (>18 years)	5 5

Estimated time of QOL assessment: 10 minutes

Schedule of Neuropsychological and Quality of Life/Pain Assessments

	Baseline	**1 mo.**	**2 mo.**	**100 days**	**6 mo.**	**12 mo.**	**18 mo.**	**24 mo.**
Patients:								
Neuropsych. Testing	C			M		C		C
QOL/Pain	√			√		√		√
Sibling Donors:								
Neuropsych. Testing	C					C		
QOL	√					√		

C = Comprehensive Test Battery
M = Monitoring Test Battery

Clinical Assessment of Sibling Donors*

- Pre-transplant

Parental interview

- Parental perceptions of donation process for family
- Level of discussion that has occurred with donor, recipient

Donor Interview

- Assess donors, age-appropriate understanding of the process
- Dispel any misconceptions
- Ensure the absence of coercion, confirm perception of voluntary choice
- Anticipatory guidance regarding process
- Administer the pre-donation questionnaire (see page 10)

- Post-transplant
  - Assess donors' perception of the process 1 month following procedure
  - Obtain additional parental report 1 month post-donation
  - Assess any disturbance in donors' mood, sleep, appetite, symptoms of post-traumatic stress
  - If transplant is unsuccessful/patient dies, additional follow-up with donor if feasible
  - Administer post-donation questionnaire 12 months post donation (see pages 13-17)

* Adapted from Phipps, S., 2009

Donor Questionnaire Pre-Transplant

We are asking you to answer these questions because you have a sibling with a severe anemia condition, like Sickle Cell Disease or beta-Thalassemia. The first few questions are about becoming a stem cell donor.

1) Who first told you that you were a donor match?
   - Your parent(s)
   - Your sibling
   - Other relative. Specify:
   - Medical team member. Specify:
   - Other. Specify:

2) When you learned that you could be a donor, was the information told to you:
   - Very easy to understand
   - Pretty easy to understand
   - Neither easy nor hard to understand
   - Pretty hard to understand
   - Very hard to understand

3) Who made the decision that you would be a donor?
   - You
   - You and your parent(s)
   - Your parent(s)
   - Other. specify:

4) How much pressure, if any, do you feel from other people to be a donor?
   - A lot of pressure
   - A little pressure
   - No pressure (SKIP to question 9)

5) If you feel pressure, who did you feel pressure from?
   - Your parents
   - Your sibling who needed the transplant
   - Other relatives. Specify:
   - The medical team. Specify:
   - Other. Specify:

6) How willing are you to be a donor?
   - Very willing
   - Pretty willing
   - Neutral
   - Pretty unwilling

Very unwilling

7) Do you feel you can refuse if you want to?
   Yes
   No
   Not sure

8) What do you think will happen if you decide not to be a donor?

_____
_____
_____
_____

In your own words, can you tell me what it means to be a stem cell donor for your sibling?

_____
_____
_____
_____

The following questions are about learning that your brother or sister has a severe anemia condition (e.g., Sickle cell disease).

9) Who first told you that your brother or sister has a severe anemic condition?
   Your parent(s)
   Your sibling
   Other relative. Specify:
   A medical team member. Specify:
   Other. Specify:

10) How old were you when you first learned about your brother/sister's illness? What exactly were you told?

_____
_____
_____

What do you remember feeling when you learned this information?

_____
_____
_____

11) What questions do you remember asking when you learned that your sibling has this illness? Looking back are there other questions that you would have liked to ask?

_____
_____

_____

_____

12) How has your sibling's illness affected your life?

_____

_____

_____

13) How has having a sibling with a severe anemia affected your family? Who has been affected the most?

_____

_____

_____

14) How has having a sibling with a severe anemia affected your life? (check all that apply)

I feel closer to my brother/sister
I appreciate my life more
Our family has been more stressed
Our family has gotten closer. Please explain:
I worry a lot more. Please explain:
Other. Please explain:

15) Is there anything else you would like to share with us about having a sibling with a severe anemia?

_____

_____

THANK YOU!

Sibling Post-donation Questionnaire

Thank you for participating in this study. We are asking you to answer these questions because you have been a donor to _____. We would like to learn about your experience being a donor for your sibling.

1) Who first told you that you were a donor match?
   - Your parent(s)
   - Your sibling
   - Other relative. Specify:
   - Medical team member. Specify:
   - Other. Specify:

2) When you learned that you would be a donor, was the information you received:
   - Very easy to understand
   - Pretty easy to understand
   - Neither easy nor hard to understand
   - Pretty hard to understand
   - Very hard to understand

3) Who made the decision that you would be a donor?
   - You
   - You and your parent(s)
   - Your parent(s)
   - Other. Specify:

4) How much pressure, if any, did you feel from other people to be a donor?
   - A lot of pressure
   - A little pressure
   - No pressure (SKIP to question 6)

5) If you did feel pressure, who did you feel pressure from?
   - Your parent (s)
   - Your sibling who needed the transplant
   - Other relatives. Specify:
   - The medical team. Specify:
   - Other. Specify:

6) At the time that you became a donor, how willing were you to be a donor?
   - Very willing
   - Pretty willing
   - Neutral
   - Pretty unwilling

Participant ID:_____

Very unwilling

7) Could you have refused if you wanted to?
   Yes
   No

8) What do you think would have happened if you had refused to be a donor?
   _____
   _____
   _____

Now I will ask you some questions about the preparation you received for the stem cell donation

9) Which of the following provided you with the most helpful information about stem cell collection? (check all that apply)
   Written consent form
   Verbal assent
   Meeting with the apheresis team
   Meeting with the medical team
   Medical play
   Meeting other donors
   Other. Specify:

10) Would any of the following have helped you better prepare for the donation? (Check all that apply)
   Meeting with other donors
   Meeting with a social worker or counselor individually
   Meeting with a social worker or counselor as a family watching a video describing the aphaeresis procedure and the transplant process
   Pamphlets and booklets on being a stem cell donor with descriptions of aphaeresis and stem cell transplant outcomes
   Multiple preparation sessions with the medical team
   Other. Specify:
   None of the above

11) Were you given a chance to ask questions about the procedure before you agreed to participate?
   No
   Yes
   Don’t remember

12) Did you ask questions?
   No

Yes (what questions:_____)

13) How nervous did you feel about the apheresis?

Not at all (SKIP to 15)
A little
Somewhat
Pretty nervous
Extremely nervous

14) What made you nervous about the apheresis? (check all that apply)

G-SCF shots
Needles
Pain
Being in a hospital
Seeing blood
Central venous line placement
Possible damage to you from the apheresis
Undergoing anesthesia
Missing school
Outcome of the transplant
Possible harm to ill brother or sister (transplant recipient)
Other. Specify:

15) What do you remember about the donation?

_____

_____

Did you experience any of the following complications/problems **during** the stem cell donation? (check all that apply)

16)

Discomfort. If yes, explain:
Difficulty using the bed pan. If yes, explain:
Other. Explain:
I did not experience any complications during this time

17) Who was present during the procedure? (check all that apply)

Your parent(s)
Your sibling
Other relatives. Specify:
Medical team. Specify:
Other. Specify:

18) Did the procedure feel like you expected it to feel?

No. What was different? Yes

19) Was there any information that was omitted during the pre-donation discussions that would have helped you with the apheresis?

No

Yes. Specify:

20) Now that you know what to expect, would you go through the donation process again?

No. Why not?

Yes. Why?

21) If you had a friend who was going through the procedure, what advice would you give him/her?

_____

_____

_____

Who do you think should decide if siblings should be donors

22)

The donor him/herself

The parent(s) of the donor

Both the donor and the parent(s) together

Other. Specify:

23) At what aged do you think is it ok to become a donor? _____ years

24) How important do you think it is for a donor to be well informed about the donor experience

Not important at all

Not very important

Pretty important

Very important

25) How much support did you feel you had from your family and friends?

Very little support

Some support

A lot of support

26) Did you experience any of the following complications/problems in the week <u>after</u> the donation? (check all that apply)

Pain

Sever bruising from line placement

Multiple collections
Inability to return to work/school/daily activities due to pain from procedure
Hospitalization required following the procedure
Other. Specify:
I did not experience any complications during this time

27) What was the best part about being a stem cell donor for your sibling?

_____
_____

28) What would you say was the worst part about being a stem cell donor for your sibling?

_____
_____

29) Now I am going to ask you some questions about your feelings about the transplant and having a sibling with a severe anemia (e.g., Sickle cell disease).

30) When thinking about the transplant, would you say…?

The transplant was totally successful
Most of the transplant was successful. Explain:
Not sure if the transplant was successful or not. Explain:
Most of the transplant was unsuccessful. Explain:
The transplant was not at all successful

31) How has the donor experience affected your life?

_____
_____

32) Since the transplant, have you had problems with

Feeling sad. Explain:
Feeling anxious or nervous. Explain:
Having less of an appetite or eating more than you did before the transplant?
Other emotional symptoms. Explain:

33) How has having a sibling with a severe anemia affected your life? (check all that apply)

I feel closer to my sibling
I appreciate my life more
Our family has been more stressed Explain:
Our family has gotten closer. Explain:
Other. Explain:

34) Is there anything else you would like to share with us about having a sibling with a severe anemia?

_____

_____

THANK YOU!

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APPENDIX G: Addendum B to protocol 14-H-0077: Reproductive questionnaires in patients with severe congenital anemias before and after transplant

1. Objectives

1.1. To obtain detailed medical, surgical, medication, social, family, and other relevant history, through the use of questionnaires, to complement endocrine related laboratory testing before and after hematopoietic stem cell transplant (HSCT)
1.2. To accumulate possible qualitative changes before and after stem cell transplant in the hypothalamic, pituitary, adrenal, and gonadal organs, with the emphasis on reproductive potential.
1.3. To estimate the magnitude and need of fertility related issues among transplanted patients.

2. Background and Scientific Rationale

Male and female patients with sickle cell disease (SCD) or thalassemia frequently suffer from endocrine and reproductive health problems, which can have multiple and complex etiologies. The underlying cause(s) can not only be related to the diseases themselves, but are frequently also a consequence of the treatment(s), which the patients have received in the past or are receiving currently. This area is under-studied in the literature; searching for "sickle cell" and "fertility" yielded only 87 entries in Pubmed with 19 relevant reports. Searching for "sickle cell" and "reproduction" yielded 1464 entries, spanning 1960's to 2013. 258 entries were about prenatal or newborn screening, 297 were about pregnancy management or pregnancy outcomes, 29 were about contraception, and only 39 entries were relevant to the reproductive potential in sickle cell population. Thus these reports (19+39) are limited to address the causes of infertility (male or female factors), the effects of hydroxyurea, blood transfusion (and iron overload), iron chelators on gonadal function, or what magnitude and effects does chronic vaso-occlusion have on gonadal reserve. Half of the combined 58 articles discussed priapism, but only on medication or surgical management [1-3] – not on fertility potential. There were only case reports of fertility or fertility rates in women[4,5], but again are limited in addressing the causes of fertility potential with SCD and/or treatment[6].

Additionally, the literature about effects of hydroxyurea on fertility in male patients with SCD is mixed. Some report irreversible reduction in sperm production even with hydroxyurea discontinuation[7,8], while others report modest recovery [9]. There are no reports on the effects of hydroxyurea on gonadal function in women with SCD.

Currently HSCT is the only cure for patients with SCD and thalassemia. The most common type of HSCT employs full or myeloablative conditioning, which renders 60-85% of all transplant recipients infertile [10-12]. Reduced intensity or non-myeloabaltive regimens such as the one on this protocol theoretically should be less toxic on gonadal function. Different drug combinations and varying dosing schedule make the results from other transplant reports difficult to apply to this study[13,14]. Furthermore, our regimen uses total body irradiation, where testes are partially shielded but not ovaries, and alemtuzumab which targets CD52 on lymphocytes and spermatocytes [15]. These are unique features that make studying post-transplant endocrine effects (fertility potential) especially important.

When we previously approached the reproductive endocrinology group in NICHD about how best to follow our patients, they offered to evaluate our patients on as needed basis and pointed out their limitation – namely only for female patients as they are medically and surgically trained as obstetricians and gynecologists. There is currently no andrology support at this time. In order to determine whether the measures we initiate in association with HSCT contribute to negatively impact the patients' endocrine health in general and their reproductive health in particular, we propose to start with a questionnaire, designed for male and female patients, which complements the endocrine related laboratory testing we are currently performing in this protocol (sections 6.1.21 and 6.4). Our interest is

focused on determining whether our treatment (HSCT and associated medications) worsen the patients' endocrine balance, and if yes, to what extent. By ruling out other potential confounding factors, we will be able to better understand the role that our treatment may play.

3. Methods

There are several publicly available fertility related questionnaires (see web links after references). They include questions that are standard in the reproductive science field, as they address the patients' family, social, medical, and surgical histories, their medications, and prior treatments. With regard to the social history part of the questionnaire, a patient's lifestyle may provide clues or suggest any endocrine or reproductive health problems, as would be the case e.g. if patients were exposed to hazardous agents at work, or if they used certain recreational drugs.

There are no questionnaires that are specific for SCD or any other disorder; all the questionnaires are general – one for all patients. Thus we reviewed all the available questionnaires, took all the standard questions, and added relevant questions for SCD and hemoglobinopathies. Our implementation method includes the following:

3.1. The questionnaire is voluntary, as other addenda to this protocol are voluntary. Subjects 18 years old and older are our intended focus: male questionnaire (3 pages), female questionnaires (3 pages).
3.2. For prospective patients, we plan to administer the questionnaire pre-HSCT, 1 year post-HSCT, and 2 year post-HSCT. The remaining 19 subjects (50 accrual ceiling – 31 transplanted subjects) should allow us to identify if there are any qualitative changes.
3.3. For patients who have already been transplanted, we also plan to administer the questionnaire annually for 2 times with the annual follow-up visits (for example, year 3 and 4 or year 6 and 7 depending on the patient). The answers from these patients would help to estimate the need of fertility related issues post-transplant.
3.4. The questionnaires are to be administered by the protocol providers or research coordinators with the patients to ensure uniformity. The questionnaires are not meant be filled out by the patients alone.
3.5. While it is common to obtain detailed history from partners of patients, we are omitting this aspect for several reasons. This effort is a pilot study with fact finding intent, thus we would like to focus on the patients first. Many of our patients currently do not have a stable partner to consider family planning, and we want to avoid re-administering the questionnaire if the partner were to change. Partner consenting to this addendum, possible impact of participating in questionnaire on the ongoing relationship, and confidentiality also make administering questionnaires to partners impractical for our team at this time.

4. Potential outcomes/results and data collection/analysis

4.1. Previously we had 2 male subjects that reported decreased libido and erections in the first 6 months after transplant. As a result, we have sent them to for endocrine evaluation and related lab testing. We also had 1 female subject who has been trying to get pregnant, and we have sent her to see medical and reproductive endocrinology for evaluation. Since often these patients have many transplant or medical issues, fertility related history is often neglected. This questionnaire helps to create a more complete set of data of fertility related issues/symptoms before and after transplant.

As we have done for the 3 patients listed in 4.1 of this section, when we identify area(s) of need in the patients in medical or reproductive endocrinology, we will inform the patients and refer them for suitable consultation either here at the Clinical Center or near patients' home residence, depending on the patients' input. If social work, psychologic, and other types of counseling is needed, we will find the most suitable consultation and/or referral.

4.2.

4.3. Responses for men and women will be cataloged separately and proportion of patients with certain SCD treatment (e.g. how many red cell transfusions), symptoms (e.g. priapism or oligomenorrhea), or previous medical illness (e.g. infections) will be tabulated.

4.3.1. Responses from prospective patients will estimate the magnitude of fertility related needs or patterns before and after transplant, how/if they change after transplant short and long term.

4.3.2. Responses from already transplanted patients will add to the estimate of fertility need after transplant, and make the long term data immediately available.

4.4. Much of the data will be descriptive. The history portions of the questionnaires are intended to be compared qualitatively, and likely will be analyzed non-parametrically, similarly to this report[16].

When possible we will try to correlate the history portions with the laboratory testing, focusing on the reproductive of the hypothalamic-pituitary-adrenal-reproductive axes. Some examples include do lower libido correlate with lower sex hormone levels, what are FSH and LH levels in women with irregular menses and how do they change over time

4.5. ?

References for Addendum B

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Web links to publically available fertility questionnaires

1. http://www.cdc.gov/reproductivehealth/Global/PDFs/RHAToolkitQuestionnaireUpdatedSeptember2011_FINAL_Tag508.pdf
2. http://shirenaturalfertility.com.au/wp-content/uploads/2011/06/First-Visit-Form.pdf
3. http://coe.ucsf.edu/ivf/forms/FORM_411-Male_Reproductive_Health_Questionnaire.pdf
4. http://urology2008-2012.ucsf.edu/patientGuides/pdf/maleInf/MaleIntake.pdf
5. http://www.racha.org.kh/rc2008/105/RPH-024-Eng.pdf
6. http://www.utswmedicine.org/hospitals-clinics/pob-2/ob-gyn/reproductive-health-questionnaire.pdf
7. www2.massgeneral.org/Vincent/images/ivf_infertilityquestionnaire1.pdf
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9. www.uwmedicine.org/patient-care/our-services/medical-services/gynecology/documents/male-infertility-history.pdf
10. Obgyn.ucla.edu/workfiles/new_patients/patient_history_obgyn.pdf
11. www.uamshealth.com/upload/docs/clinics/women%20health/fertility-history-uams.pdf
12. www.shadygrovefertility.com/your_first_visit

Female Reproductive Health Questionnaire

Question	Picklist	Format
1. Married or committed relationship?	No	dropdown
	Yes	dropdown
Yes - Number of years:		text
2. At what age did you have your first menstrual period?		string
3. How are your menstrual periods?	Regular (21-35 days)	dropdown
	Irregular - Since when?	dropdown
Irregular - Since when?		text
4. When was the first day of your most recent menstrual period?		string
5. How would you describe your bleeding?	Light (< 3 pads)	dropdown
	Average (3 - 5 pads)	dropdown
	Heavy (> 5 pads)	dropdown
6. Do you bleed or spot between periods or after intercourse?	No	dropdown
	Yes	dropdown
Yes - Since when?		text
7. When was your most recent:		
Pap Smear? (mm/dd/yyyy)		string
Mammogram? (mm/dd/yyyy)		string
Pelvic ultrasound? (mm/dd/yyyy)		string
8. Did you ever have an abnormal Pap Smear?	No	dropdown

	Yes	dropdown
Yes - When?		text
9. How many times per week (on average) do you have intercourse?		text
10. Do you use contraception?	No	dropdown
	Yes	dropdown
Yes - What type and since when?		text
11. Have you ever attempted to become pregnant?	No	dropdown
	Yes - successful	dropdown
	Yes - unsuccessful	dropdown
Yes - successful - How many times have you been pregnant?		text
12. Did you become pregnant naturally?	No	dropdown
	Yes	dropdown
13. Did you delivery at term?	No	dropdown
	Yes	dropdown
No - Please explain:		text
14. Did you ever have a miscarriage?	No	dropdown
	Yes	dropdown
Yes - How many times?		text
During which trimester of pregnancy?		text
15. Have you been evaluated for infertility in the past?	No	dropdown
	Yes	dropdown

Yes - Comment (if any):		text
16. Did you have infertility treatment?	No	dropdown
	Yes	dropdown
Yes - Type/treatment:		text
17. If you have children:		
A. Did you have them with your current partner?	No	dropdown
	Yes	dropdown
Yes - Comment (if any):		text
B. Are your children healthy?		
Children with current partner:	No	dropdown
	Yes	dropdown
Children with a previous partner:	No	dropdown
	Yes	dropdown
C. If they have health problems what type of health problems do they have?		
Children with current partner: Please describe:		text
Children with a previous partner: Please describe		text
18. Has your weight changed more than 15 lbs in the last year?	No	dropdown
	Yes	dropdown
Medical History		header
1. Thyroid Disorder	No	dropdown
	Yes	dropdown

Yes - What type of disorder?		text
When where you diagnosed? (mm/yyyy)		string
Treatment?		text
2. Fibroids endometriosis polycystic ovary syndrome or other GYN pathology?	Yes	dropdown
	No	dropdown
Yes - please describe:		text
3. Frequent urinary tract infections?	No	dropdown
	Yes	dropdown
Yes - Since when? (mm/yyyy)		text
How frequently per three months (on average)?		text
Treatment:		text
4. Sexually transmitted disease?	No	dropdown
	Yes	dropdown
Yes - Type:		text
Treatment:		text
5. Cancer?	No	dropdown
	Yes	dropdown
Yes - Which year? (YYYY)		string
Type:		text
Treatment:		text
6. Neurological Problems?	No	dropdown
	Yes	dropdown

Yes - Type:		text
Treatment:		text
Treatment: From when to when?		text
7. Have you ever taken hydroxyurea?	No	dropdown
	Yes	dropdown
Yes - From when to when?		text
At what dose?		text
If you stopped taking the drug what was reason for it?		text
Surgical History		header
1. Dilatation and curettage?	No	dropdown
	Yes	dropdown
Yes - Date/year (if known): (mm/yyyy)		string
What was the reason?		text
2. Cervical cone biopsy?	No	dropdown
	Yes	dropdown
Yes - Date/year (if known): (mm/yyyy)		text
What was the reason?		text
3. Surgery of the ovary?	No	dropdown
	Yes	dropdown
Yes - Date/year (if known): (mm/yyyy)		string
What was the reason?		text
4. Surgery of the Fallopian tube?	No	dropdown
	Yes	dropdown

Yes - Date/year (if known): (mm/yyyy)		string
What was the reason?		text
5. Surgery of the uterus?	No	dropdown
	Yes	dropdown
Yes - Date/year (if known): (mm/yyyy)		string
What was the reason?		text
6. Laparoscopy?	No	dropdown
	Yes	dropdown
Yes - Date/year (if known): (mm/yyyy)		string
What was the reason?		text
Exposure History		header
1. Do you currently smoke tobacco?	No	dropdown
	Yes	dropdown
Yes - Since how many months		string
Years:		string
How much (per day):		string
2. If you quit smoking how long has it been?		
# of months		string
# of years		string
3. Do you use any of the following:		
Alcohol?	No	dropdown
	Yes	dropdown
Consumption	Less than 2 drinks/day	dropdown

	More than 2 drinks/day	dropdown
Marijuana?	No	dropdown
	Yes	dropdown
Frequency:	Up to 3x/month	dropdown
	More than 3x/month	dropdown
Other types of recreational drugs?	No	dropdown
	Yes	dropdown
Yes - Type:		
Frequency:	Up to 3x/month	dropdown
	More than 3x/month	dropdown
4. What is your job?		text
For how long have you had this job? (years)		text
What was/were your previous job(s)?		text
5. Do you consider your job stressful?	No	dropdown
	Yes	dropdown
Stress level:	Low stress	dropdown
	Moderately stress	dropdown
	High stress	dropdown
	Extremely stress	dropdown
6. Do you perform regular vigorous exercise?	No	dropdown
	Yes	dropdown
Yes - How frequently per week (on average):		text
What type(s)?		text

Family History		
1. Do you have a positive family history of infertility?	Yes	dropdown
	No	dropdown
	Dont know	dropdown
Yes - Who?		text
Cause/problem (if known):		text
2. Are there any adopted children in your family?	No	dropdown
	Yes	dropdown
Yes - Who?		text
3. Any miscarriages in the immediate family?	No	dropdown
	Yes	dropdown
Yes - Comment (if any):		text

Male Reproductive Health Questionnaire

1. Married or committed relationship?	No	dropdown
	Yes	dropdown
Yes - Number of years:		text
2. How many times per week (on average) do you have intercourse?		text
3. Have you ever attempted to impregnate your partner?	No	dropdown
	Yes	dropdown
Yes - How many months trying to impregnate your partner?		string
Years?		string
4. Prior pregnancies between you and your partner?	No	dropdown
	Yes	dropdown
Yes- Number of pregnancies carried to term and delivered:		
Number of miscarriages:		string
Number of abortions:		string
5. Have you had prior infertility treatments?	No	dropdown
	Yes	dropdown
Yes - Type:		text
Method(s) used?		text
6. Do any of the following concern you:		
...your ability to get an erection?	No	dropdown
	Yes	dropdown
Yes - comment (if any):		text
...your ability to maintain an erection?	No	dropdown

	Yes	dropdown
Yes - Comment (if any):		text
...ejaculating before your partner is ready?	No	dropdown
	Yes	dropdown
Yes - Comment (if any):		text
Medical History		Header
1. Undescended testicles at birth?	No	dropdown
	Yes	dropdown
Yes - Which side?	Right	dropdown
	Left	dropdown
2. Mumps after puberty with painful testes?	No	dropdown
	Yes	dropdown
Yes - Comment (if any):		text
3. Cancer?	No	dropdown
	Yes	dropdown
Yes - Which year? (YYYY)		string
Type:		dropdown
Treatment:		text
4. Neurological Problems?	No	dropdown
	Yes	dropdown
Yes - Type:		text
Treatment:		text
5. Infection of the urinary tract?	No	dropdown

	Yes	dropdown
Yes - Type:		text
Treatment:		text
6. Infection of the prostate (prostatitis)?	No	dropdown
	Yes	dropdown
Yes - Comment (if any):		text
7. Infection of the epididymis?	No	dropdown
	Yes	dropdown
Yes - Comment (if any):		text
8. Sexually transmitted disease?	No	dropdown
	Yes	dropdown
Yes - Type:		text
Treatment:		text
9. Do you or did you ever have priapism (painful erections)?	Yes	dropdown
	No	dropdown
Yes - Frequency:		text
Severity of episodes:	Mild	dropdown
	Moderate	dropdown
	Severe	dropdown
Age at first episode:		text
Treatment:		text
Requirement for interventions (e.g. drainage)	No	dropdown
	Yes	dropdown

Frequency:	Once	dropdown
	Less than 3 times	dropdown
	Three or more times	dropdown
10. Have you ever taken hydroxyurea?	No	dropdown
	Yes	dropdown
Yes - From when to when?		text
At what dose?		text
If you stopped taking the drug what was reason for it?		text
11. Are you bothered by problems with urination?	No	dropdown
	Yes	dropdown
Yes - Type:		text
Treatment:		text
12. Injury to the testicles that needed hospitalization?	No	dropdown
	Yes	dropdown
Yes - Type:		text
Treatment:		text
Surgical History		Header
1. Hernia operation?	No	dropdown
	Yes	dropdown
Yes - Date/year (if known): (mm/yyyy)		string
2. Bladder or penis operation as a child?	No	dropdown
	Yes	dropdown

Yes - Date/year (if known): (mm/yyyy)		string
3. Pelvic or back surgery?	No	dropdown
	Yes	dropdown
Yes - Date/year (if known): (mm/yyyy)		string
4. Testis surgery?	No	dropdown
	Yes	dropdown
Yes - Date\year (if known): (mm/yyyy)		string
5. Vasectomy?	No	dropdown
	Yes	dropdown
Yes - Date/year (if known): (mm/yyyy)		string
6. Infertility surgery?	No	dropdown
	Yes	dropdown
Yes - type		string
Exposure History		Header
1. Do you currently smoke tobacco?	No	dropdown
	Yes	dropdown
Yes - Since how many months		string
Years:		string
How much (per day):		string
2. If you quit smoking how long has it been?		
# of months		string
# of years		string
3. Do you use any of the following:		

Alcohol?	No	dropdown
	Yes	dropdown
Consumption	Less than 2 drinks/day	dropdown
	More than 2 drinks/day	dropdown
Marijuana?	No	dropdown
	Yes	dropdown
Frequency:	Up to 3x/month	dropdown
	More than 3x/month	dropdown
Other types of recreational drugs?	No	dropdown
	Yes	dropdown
Yes - Type:		text
Frequency:	Up to 3x/month	dropdown
	More than 3x/month	dropdown
4. What is your job?		text
For how long have you had this job? (years)		string
What was/were your previous job(s)?		text
5. Do you consider your job stressful?	No	dropdown
	Yes	dropdown
Yes - Stress level:	Low stress	dropdown

	Moderately stress	dropdown
	High stress	dropdown
	Extremely stress	dropdown
6. Are there any radiation or harmful chemical(s) used on the job?	No	dropdown
	Yes	dropdown
Yes - type:		text
Length of exposure:		text
7. Any exposure to prolonged heat in work / hobbies?	No	dropdown
	Yes	dropdown
Yes - Type:		text
Length of exposure:		text
ENDOCRINE HISTORY / REVIEW OF SYSTEMS		
Have you ever ben told (or know) that you have any of following:		
1. Difficulty with smell?	No	dropdown
	Yes	dropdown
Yes - Comment (if any):		text
2. Difficulty with vision (besides needing glasses)?	No	dropdown
	Yes	dropdown
Yes - Comment (if any):		text
3. Problems with growth when you were young?	No	dropdown
	Yes	dropdown
Yes - Comment (if any):		text

4. Did your voice change later than your friends?	No	dropdown
	Yes	dropdown
Yes - Comment (if any):		text
5. Any tenderness to your breasts?	No	dropdown
	Yes	dropdown
Yes - Comment (if any):		text
Family History		Header
1. Do you have a positive family history of infertility?	Yes	dropdown
	No	dropdown
	Dont know	dropdown
Yes - Who?		text
Cause/problem (if known):		text
2. Are there any adopted children in your family?	No	dropdown
	Yes	dropdown
Yes - Who?		text
3. Any miscarriages in the immediate family?	No	dropdown
	Yes	dropdown
Yes - Comment (if any):		text
4. Did your mother ever take DES (diethylstilbesterol)?	No	dropdown
	Yes	dropdown
	Dont know	dropdown