

CLINICAL RESEARCH PROTOCOL

Project # 09-H-0225

Drug: Sirolimus/Alemtuzumab/Cyclophosphamide

IND: IND Exempt

Date: December 19, 2017

Title: Nonmyeloablative Haploidentical Peripheral Blood Mobilized Hematopoietic Precursor Cell Transplantation for Severe Congenital Anemias Including Sickle Cell Disease and β -Thalassemia

Other underlying words: Peripheral blood stem cells, host-donor chimerism, graft-versus-host disease, graft-versus-marrow, Sirolimus (Rapamune®), low dose irradiation, alemtuzumab (Campath®), cyclophosphamide (Cytosan®), donor apheresis

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Subjects of study:	<u>Number</u>	<u>Sex</u>	<u>Age range</u>
Patients:	32-96	either	\geq 18 years

Donors	32-96	either	2-80 years
Accrual Ceiling:	192		
Project involves ionizing radiation?		Yes	
Off site project?		No	
Multi-Institutional project?		No	
DSMB		Yes	

PRECIS

Nonmyeloablative allogeneic peripheral blood stem cell (PBSC) transplants are currently being investigated in phase I/II trials assessing engraftment, efficacy, and toxicity at a number of transplant centers. Our ongoing protocol for patients with severe congenital anemias, particularly sickle cell disease (SCD), and an HLA-matched sibling donor has had excellent preliminary results. None of the patients who engrafted had sickle-related events or any evidence of graft versus host disease (GVHD). There was no significant toxicity associated with the conditioning regimen.

Our main limitation has been a lack of HLA-matched sibling donors in the majority of patients. We performed a study in which patients with severe SCD who lacked a suitable donor underwent a search for a matched unrelated donor or umbilical cord donor. The vast majority of patients were not found to have an appropriate alternative donor. We therefore seek to develop a safe nonmyeloablative regimen to be applied to the haploidentical setting so that family members can serve as donors and greatly expand the donor pool.

In this protocol, we propose PBSC transplantation in patients with SCD and thalassemia, considered at high risk for complications from or ineligible for standard bone marrow transplantation, with allogeneic peripheral blood stem cells from a haploidentical donor using a novel immunosuppressive regimen without myeloablation in an attempt to further decrease the transplant-related morbidity/mortality. The low intensity nonmyeloablative conditioning regimen will consist of a relatively low radiation dose for therapeutic radiation, Alemtuzumab (Campath®), Sirolimus (Rapamune®), and Cyclophosphamide (Cytosan®) as a strategy to provide adequate immunosuppression to allow sufficient engraftment for clinical remission with a lower risk of GVHD development. T-cell replete, donor-derived, granulocyte colony-stimulating factor (G-CSF)-mobilized PBSC will be used to establish hematopoietic and lymphoid reconstitution.

The primary endpoint of this study is the percentage of patients who have sustained donor type hemoglobin without significant GVHD for patients with SCD, or who are transfusion-independent and without significant GVHD for patients with thalassemia. Other endpoints include 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.

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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 an allogeneic haploidentical granulocyte colony-stimulating factor (filgrastim, G-CSF)-mobilized peripheral blood stem cell transplant in (PBSCT) in a population of patients with sickle cell disease (SCD) and β -thalassemia at increased risk for complications, with or ineligible for standard myeloablative allo-transplantation, we hope to:

- 1.1 determine the regimen failure rate, defined as graft rejection, or acute GVHD grade 3 or higher, or extensive chronic GVHD.
- 1.2 examine the level of chimerism required to maintain both graft survival as well as hematologic normalcy using a novel nonmyeloablative conditioning regimen.
- 1.3 evaluate the safety, efficacy, and toxicity, including disease-free survival, overall survival, and transplant-related mortality, of a novel nonmyeloablative conditioning regimen.
- 1.4 evaluate whether post-transplant cyclophosphamide is required and will reduce the incidence and severity of regimen failure using a novel nonmyeloablative conditioning regimen.
- 1.5 evaluate the incidence and severity of regimen failure when various haploidentical donors (i.e. mother versus father, parent versus sibling, and parent versus child) are employed.
- 1.6 evaluate whether cyclophosphamide pharmacokinetics and/or germline allelic variants in drug metabolizing/transporting genes differ in patients with and without renal insufficiency and in patients who do and do not experience graft rejection

2.0 BACKGROUND

Introduction

Allogeneic bone marrow (and peripheral blood) transplantation (BMT) 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 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 graft-versus-host disease (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 Alemtuzumab, low dose radiation, Sirolimus, and Cyclophosphamide in patients with either SCD or thalassemia.

Sickle Cell Disease

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 disease². More recently, sickle hepatopathy and iron overload have been discovered to increase mortality in patients with SCD, as patients with ferritin ≥ 1000 ug/L or direct bilirubin >0.4 mg/dL led to significantly decreased survival as compared to patients with ferritin <1000 ug/L and direct bilirubin <0.4 mg/dL (see Figures 1 and 2, from Jordan Feld and Theo Heller, MD, manuscript in preparation).

Figure 1 Kaplan-Meier survival estimates, by fer2

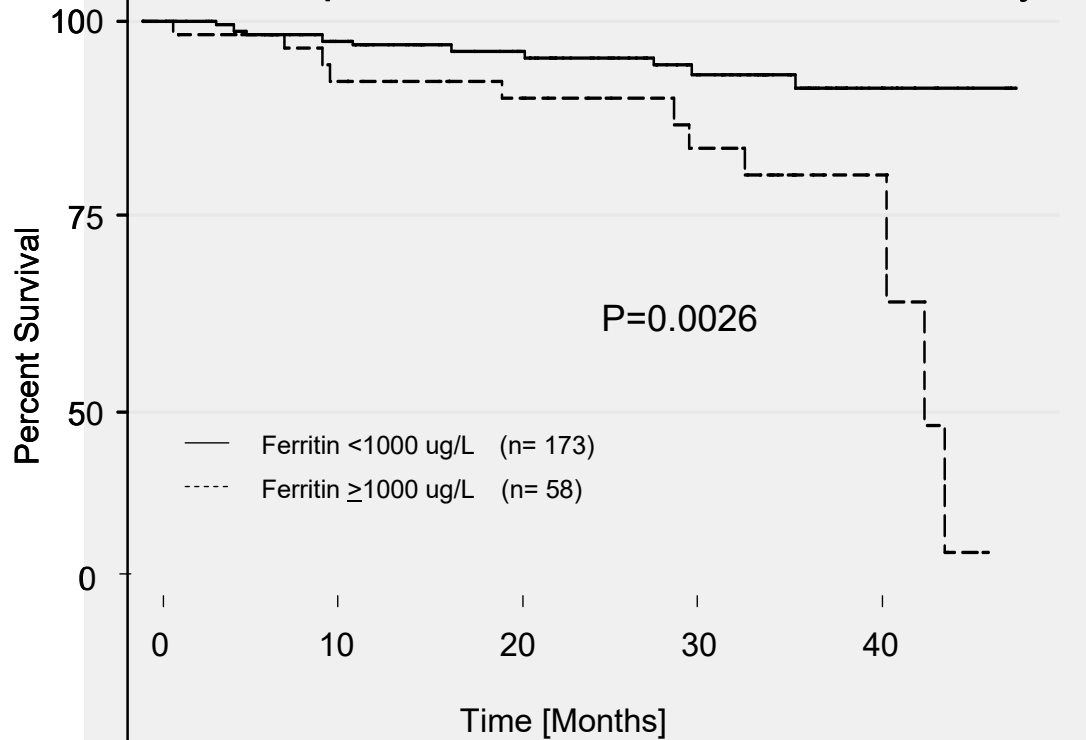
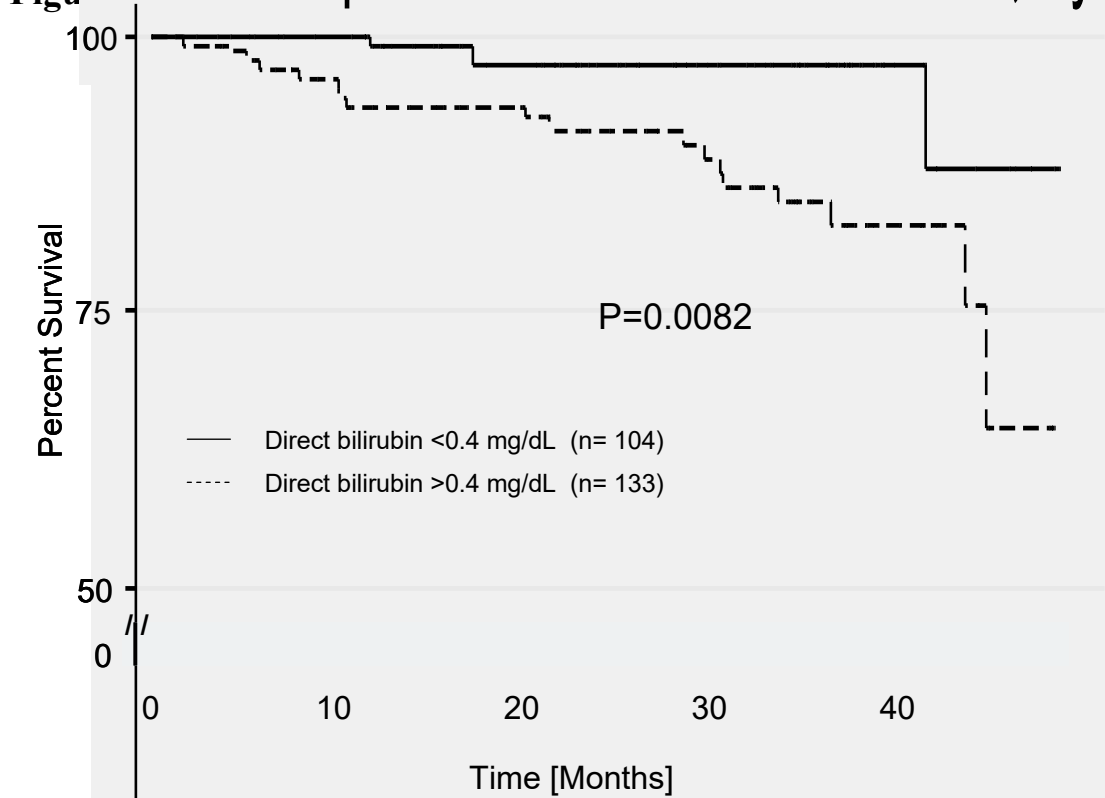


Figure 2 Kaplan-Meier survival estimates, by dir2



In addition, patients with platelet counts in the lowest quartile of the cohort (<267,000/ul) had a 2.7-fold increase in direct bilirubin after controlling for WBC, to exclude the affect of generalized bone marrow suppression as a cause for thrombocytopenia (OR 2.70 95% CI 1.11-6.56, p=0.029).

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 disease³. An overlapping symptom complex also occurs in patients with the double heterozygous forms of SCD, sickle-C, and sickle β -thal⁰ 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 syndrome⁴, 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 lead 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 SCD⁷. 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 72². 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 crisis².

The only established cure for patients with SCD remains allogeneic bone marrow transplantation; however, the procedure has only been applied to highly selected children⁸⁻¹⁰. 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. The results have been very encouraging. However, this protocol has been limited by the availability of HLA-matched sibling donors. Of 59 patients with disease severe enough to be eligible for our protocol, only 13 patients (22%) had a 6/6 HLA-matched sibling donor. Due to the inheritance pattern of SCD, the chance that an HLA-matched sibling will be SCD-free further limits the possibility of an appropriate donor. Conversely, the vast majority of patients will have a haploidentical donor as parents, children, and haploidentical-matched siblings can serve as donors. Therefore, two major goals in the application of BMT are to develop a safer conditioning regimen and to increase the donor pool.

Thalassemia

Thalassemia is the most common genetic disorder worldwide¹¹, 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 expensive and not well tolerated, impacting on quality of life and often results in poor compliance, especially in adolescents.

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. Unfortunately, less than 30% of patients with β -thalassemia major have such suitable donors. Therefore, studies have been performed to assess the safety of using HLA-matched unrelated donors and a myeloablative conditioning regimen for patients with β -thalassemia major.^{20,21} La Nasa and colleagues found a rate of graft rejection of 13%, overall survival 79%, and disease-free survival of 65.8% in 68 patients with a median age of 15 years (range 2 to 37 years)²¹. However, when comparing patients from Classes 1 and 2 to patients in Class 3, overall survival and disease-free survival decreased from 96.7% and 80%, respectively, to 65.2% and 54.5%. In addition, 40% of patients developed grade II-IV acute GVHD, and 18% chronic GVHD.

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.^{22,23}

3.0 SCIENTIFIC AND CLINICAL JUSTIFICATION

In disorders such as hematologic malignancies, the curative effect of bone marrow transplant has been ascribed to the use of myeloablative chemo-radiotherapy and the antileukemic effect of the transplant (the graft-versus-leukemia (GVL) effect).²⁴ 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.^{9,25-27} While it is generally accepted that GVHD is less severe in patients conditioned with low intensity preparative regimens,²⁸ graft rejection is preferable to the development of lethal GVHD in the setting of severe congenital anemias.

Our group currently has an ongoing protocol which uses a combination of 300 cGy TBI, alemtuzumab, and sirolimus to transplant high risk patients with sickle cell disease and β -thalassemia who are 16 years of age and older. To optimize our approach, we use peripheral blood stem cells (PBSC) from sibling donors. Preliminary studies, including our own experience with PBSC transplants and low intensity preparative regimens, indicate that transplant-related mortality and severe acute GVHD is uncommon when matched family donors are used.²⁸ Furthermore, we have established the safety of PBSC mobilization in individuals with sickle cell trait (SCT), likely to represent a sizable fraction of sibling donors for patients with SCD.²⁹ Additionally, for our patients with SCD, we maintain higher platelet counts.³⁰⁻³² Patients with SCD who are not routinely transfused for their therapy undergo exchange transfusion prior to transplant to lower their hemoglobin S to less than 30%.⁸

As of June 1, 2009, ten patients with SCD have been transplanted, with periods of follow-up ranging from 15 to 57 months. Nine patients engrafted, and one patient rejected the graft at two months. That patient subsequently developed recurrent vaso-occlusive crises, though to a milder degree as compared to before transplant, and one episode of acute chest syndrome. None of the patients that have maintained their grafts has experienced any sickle cell-related events. No patients experienced GVHD or required parenteral antibiotics. All of the patients who had pulmonary hypertension prior to transplantation experienced significant improvement or resolution of their pulmonary hypertension. Four patients either have or are continuing to undergo therapeutic phlebotomy with improvement of their transfusional iron-overload. At 2 years post-transplant, the mean % donor myeloid cell level was about 75% for evaluable patients, and the mean % donor lymphoid cell level was about 35%. This degree of donor white blood cell chimerism was sufficient to achieve full erythroid donor chimerism in 8 of 9 engrafted patients and almost full donor chimerism in the 9th patient.

The main limitation for this protocol has been donor availability. More than 100 patients were screened. Of 59 patients with SCD severe enough to be eligible for the protocol, only 13 patients (22%) had a 6/6 histocompatible sibling donor. Two more patients did not qualify due to major ABO mismatch. Our goal is to increase the donor pool so that more patients will have access to this potentially curative therapy. We recently initiated an IRB-approved protocol to establish the feasibility of matched unrelated donor (MUD) and umbilical cord blood (UCB) HSCT. Ten patients who met all study criteria on full screening for our sibling matched HSCT protocol but who did not have a suitable donor were selected for alternative donor searching in the National Marrow Donor Program (NMDP) and Bone Marrow Donors Worldwide (BMDW). We found that only one patient had a greater than 1% probability of having a 6/6 HLA match according to haplogenic. Also, only a median of one suitable ($\geq 2.5 \times 10^7$ total nucleated cells per kilogram body weight and ABO-matched) UCB unit was available per patient.³³ As our sibling-matched HSCT protocol has been successful, we are now interested in developing a safe haploidentical protocol for adult patients with severe congenital anemias so that parents, children, and haploidentical-matched siblings can serve as donors.

Haploidentical HSCT is invariably associated with an increased risk of graft rejection and GVHD. Therefore, as SCD and thalassemia generally are chronic illnesses, this modality has only rarely been reported,³⁴⁻³⁸ as previously the risks of transplantation in the haploidentical setting has been felt to outweigh the benefits. However, since adult sickle cell patients with risk factors such as pulmonary hypertension,^{39,40} renal failure,^{2,41} and frequent hospitalizations for pain crises,^{2,42} or adult patients with thalassemia and severe iron overload and liver fibrosis,⁴³ experience early mortality, and we have had no evidence of GVHD, significant morbidity, or mortality in patients with a matched sibling donor, we believe that the potential benefits outweigh the risks. Given the lower toxicities seen with nonmyeloablative regimens, the possibility of cure with mixed white blood cell chimerism, and the potential improvement of SCD after transplant with even autologous recovery, the extension of low-intensity allogeneic peripheral stem cell transplants to those with sickle cell anemia, sickle-SC, sickle-thal⁰, or thalassemia, who would normally be offered a standard transplant, but are considered at a higher risk due to their age (i.e. 16 years of age or older) or other comorbidities is justified. Our conditioning regimen was designed for slow engraftment and tolerance induction resulting in partial to full white blood cell chimerism with reduced rates of GVHD.

In order to develop a regimen with potential application to individuals with varying degrees of organ dysfunction such as those with SCD or other congenital anemias, we have sought conditioning which could be applied in such a context, avoiding renally excreted drugs, and relying on the immunosuppressive effects of TBI as the basis for such an approach. Patients with SCD or thalassemia who have been frequently transfused may be at an increased risk for graft rejection as compared to patients with hematologic malignancies because frequent exposure to blood products may lead to donor HLA sensitization. A modest increase in the dosage of TBI from 300 cGy used in our HLA-matched sibling protocol to 400 cGy may increase both the degree of myelosuppression and immunosuppression without significantly altering the side effect profile. Moreover, drugs such as antithymocyte globulin which may increase the risk of inciting a sickle cell crisis, or by their side effect profile mimic the symptoms of a pain crisis, need to be avoided specifically in the chosen patient population of this trial.

As in our HLA-matched sibling protocol, immunosuppression with the lymphocyte depleting agent Alemtuzumab will also be employed. Alemtuzumab is a humanized monoclonal antibody directed against CD52 (which is abundantly expressed on all human lymphocytes), and causes T cell activation *in vitro* as well as complement mediated lysis and antibody dependent cellular toxicity. As a result, it depletes both T and B cells efficiently *in vivo*. It is currently being used in clinical trials as monotherapy for certain autoimmune disorders including rheumatoid arthritis and multiple sclerosis,⁴⁴⁻⁴⁷ T and B cell malignancies,⁴⁸ treatment of solid organ rejection,⁴⁹⁻⁵¹ and has been approved for use in chronic lymphocytic leukemia, a B cell malignancy, as a result of its profound immunosuppressive properties.⁵²⁻⁵⁴ More recently, alemtuzumab has been used prospectively to prevent graft rejection in human allotransplantation.⁵⁵⁻⁵⁹ 31 patients have been transplanted using 20 mg of alemtuzumab on Day 0 and +1 of transplantation in combination with low dose CSA, which has been shown to be ineffective when used alone. At 21 months follow-up, only two grafts were lost to rejection.⁵¹ Further, data suggest that the use of alemtuzumab, as compared to fludarabine, reduces the risk of GVHD, even in the unrelated donor setting.⁵⁸ In one study of 44 patients, including eight patients receiving unmanipulated marrow from matched unrelated donors who would therefore be at very high risk for developing GVHD, only two patients had acute GVHD, both of which were grade 2. Only one patient developed chronic GVHD.⁵⁵ Follow-up to this study has included a further 39 patients undergoing unrelated BMT (including patients having failed a prior transplant and/or having a mismatch in either HLA class I or II alleles) for a total of 47 patients, with only three patients developing Grade III to IV acute GVHD, and none developing chronic extensive GVHD.⁵⁸ Other studies have shown that as compared to regimens employing such agents as antithymocyte globulin, calcineurin inhibitors, and methotrexate, alemtuzumab-containing regimens were associated with a decreased risk of severe GVHD⁶⁰⁻⁶². In another study of 12 high risk patients undergoing haploidentical BMT, one patient experienced Grade II and one patient developed Grade III

GVHD; none developed Grade IV GVHD.⁵⁹ This reduced risk of GVHD, as well as its immunosuppressive properties, appears to be due to an in vivo T cell depleting effect on the incoming graft. Unlike ATG, which is a nonspecific antibody directed against lymphocytes and is also used in conditioning regimens, alemtuzumab is also better tolerated and has no risk of causing serum sickness.

To improve the odds for graft acceptance without GVHD, we will employ sirolimus, a novel immunosuppressive agent, instead of the conventional agent, CSA, based on sirolimus' distinct properties as a tolerogenic agent.⁶³ Sirolimus is an immunophilin drug similar to CSA; however, unlike CSA, which inhibits the phosphatase calcineurin and therefore prevents the production of interleukin 2 (IL-2), sirolimus prevents translation of mRNAs encoding cell-cycle regulators. As a result, sirolimus only inhibits the ability of lymphocytes to proliferate in response to IL-2. Powell et al demonstrated that cells cultured and stimulated in the presence of sirolimus became anergic, while cells cultured in the presence of CSA did not.⁶³ These results were also confirmed in our lab using an in vivo model. -In a series of experiments performed by Hale et al., sirolimus also proved superior to CSA at prolonging skin graft survival in Class I and class II disparate, fully mismatched, and xenogeneic recipients, and the use of sirolimus was superior to CSA when added to antilymphocyte globulin and bone marrow in a murine model. Further, mice receiving sirolimus accepted a second same donor skin graft, but rejected third party grafts, demonstrating the development of tolerance⁶⁴⁻⁶⁶. Sirolimus has also been employed in the bone marrow transplant setting, with matched and mismatched sibling and unrelated donors^{67,68}. One study involving 41 patients with hematologic malignancies used sirolimus, tacrolimus, and methotrexate as GVHD prophylaxis. All evaluable patients engrafted, and grades 0-I, II, III, and IV acute GVHD occurred in 75%, 13%, 8%, and 5% of patients, respectively⁶⁷. Another study included 14 evaluable patients with hematologic malignancies, and used sirolimus and mycophenolate mofetil as GVHD prophylaxis. All patients engrafted, and grades II-IV acute GVHD occurred in 21%, and chronic GVHD in 30% of patients⁶⁸.

Sirolimus also has less renal toxicity as compared to CSA. A randomized trial comparing the addition of sirolimus at either 2 or 5 mg vs. azathioprine to CSA and prednisone for prophylaxis of renal allograft rejection showed a significantly lower rate of acute rejection at both doses of the sirolimus as compared to azathioprine (16.9% and 12.0% vs. 29.8%).⁶⁹ In a similar study comparing sirolimus vs. CSA as adjuncts to azathioprine and prednisone, there were similar rates of graft survival and incidence of biopsy confirmed graft rejection (98% vs. 90% and 41% vs. 38%, respectively), but significantly lower serum creatinines in the sirolimus group.⁷⁰ Moreover, in renal transplant studies, sirolimus has been shown to be equally effective in preventing graft rejection and has been approved as an alternative to CSA.

Cyclophosphamide given from two to three days after bone marrow transplantation has been shown to facilitate engraftment and prevent the development of GVHD by targeting activated lymphocytes.⁷¹⁻⁷⁴ Colson *et al* have shown that post-transplantation high dose cyclophosphamide (200mg/kg) facilitated engraftment of MHC-incompatible marrow grafts in mice after nonmyeloablative conditioning.^{73,74} Post-transplant cyclophosphamide has recently been shown to facilitate engraftment in a partially HLA-mismatched setting. Thirteen patients with high-risk hematologic malignancies were conditioned with fludarabine, 200 cGy TBI, cyclophosphamide 50 mg/kg on day +3, mycophenolate mofetil, and tacrolimus.⁷¹ Eight patients (62%) experienced sustained donor cell engraftment, as compared to 3% when a similar regimen in which cyclosporine replaced tacrolimus in the absence of post-transplant cyclophosphamide was used. Post-transplant cyclophosphamide has also been reported to decrease the incidence of GVHD in a mismatched major histocompatibility complex murine model, in which median survival increased from 25 to 145 days in mice that did not versus did receive post-transplant cyclophosphamide, respectively.⁷² Because of the inherent increased risk of GVHD associated with haploidentical donors as compared to HLA-matched sibling donors, we will explore post-transplant cyclophosphamide in a dose escalating fashion (see below).

An ongoing haploidentical SCT protocol at John Hopkins Hospital for patients with nonmalignant diseases including severe SCD employs pre-transplant cyclophosphamide, fludarabine, 200cGy TBI, 50mg/kg cyclophosphamide which is administered 3 and 4 days post-transplant (100mg/kg cumulative dose), and mycophenolate mofetil and tacrolimus for GVHD prophylaxis⁷⁵. To date, two patients with SCD have undergone SCT. The first patient remains free of SCD and is doing well off of immunosuppressive therapy greater than one year post SCT. However, the second patient developed intractable seizures as a result of posterior reversible encephalopathy syndrome (PRES), which was due to tacrolimus, leading to temporary withholding of tacrolimus therapy and subsequent graft rejection. Unlike the calcineurin inhibitors such as cyclosporine and tacrolimus, rapamycin rarely leads to PRES. Further, in our 10 patients with severe SCD, the conditioning regimen, which includes rapamycin, was well tolerated, and supportive care requirements were minimal. Further, no patient has experienced GVHD or sickle related events.

To determine whether post-transplant cyclophosphamide is beneficial in the setting of low dose TBI and T cell depletion, we embarked upon a series of experiments in the murine model. We first sought to determine the optimal irradiation dose to explore post-transplant cyclophosphamide. We have applied our regimen using mismatched strains, where Balb/C mice serve as donors and C57Bl6 mice are recipients. Recipient mice received conditioning with a pan-lymphocyte suppressive agent, anti-thymocyte serum, rapamycin, and doses of TBI ranging from 100 to 400cGy to determine the level of irradiation necessary for engraftment. From this experiment, we determined that donor white blood cell chimerism levels increased from 10% in mice that received 100cGy to as high as 50% in mice that received 400cGy. We will therefore increase the TBI dose from 300cGy to 400cGy in order to increase the myelosuppression and immunosuppression that may be needed in the haploidentical setting.

Since post-transplant cyclophosphamide is thought to prevent graft rejection and GVHD by deleting alloreactive lymphocytes, we next sought to determine whether cyclophosphamide would be effective in the setting of lymphocyte depletion. Therefore, mice received a lymphocyte-depleting agent, thy-1.2 monoclonal antibody, 400cGy TBI, rapamycin, and doses of post-transplant cyclophosphamide ranging from 0 to 200mg/kg. The level of engraftment did not vary significantly between mice that received cyclophosphamide from 0 to 100mg/kg, with donor myeloid chimerism levels of about 55%. However, mice that received 200mg/kg only achieved donor myeloid chimerism levels of 35% at 35 weeks post-transplant. Further, mortality increased at cyclophosphamide doses above 50mg/kg. In our next series of experiments, we evaluated whether lymphocyte proliferation was sufficiently decreased by rapamycin to inhibit cyclophosphamide's effects post-transplant. We found that rapamycin and post-transplant cyclophosphamide are synergistic, since mice that received 200cGy and either rapamycin or cyclophosphamide failed to engraft, while all mice that received both agents engrafted, with donor white blood cell chimerism levels ranging from 10-60%. Unlike patients with hematologic malignancies, patients with congenital anemias do not require high doses of chemotherapy to treat their disease. Therefore, they may not be as prone to developing GVHD, and instead the propensity towards graft rejection and GVHD may balance such that patients may not require additional immunosuppression. Further, since we demonstrated that rapamycin and post-transplant cyclophosphamide are synergistic, but may not be beneficial in the setting of profound lymphocyte depletion, which occurs with alemtuzumab, we will only add cyclophosphamide in a dose-escalating fashion if the first cohort of patients experiences graft rejection or severe GVHD. This treatment protocol will therefore include 400cGy TBI, alemtuzumab, sirolimus, and if necessary due to the development of graft rejection or severe GVHD in our patients, post-transplant cyclophosphamide in a dose-escalating fashion.

Pharmacokinetic evaluation of cyclophosphamide and its metabolites, such as 4-hydroxycyclophosphamide and 2-dechloroethylcyclophosphamide, have been performed⁷⁶⁻⁸⁰. However, sufficient data do not exist in patients who receive cyclophosphamide post-PBSCT. Various genotyping assays for single nucleotide polymorphisms in CYP450 genes (for example CYP3A4/5, CYP2B6, and CYP2C9) and in MDR1/ABCB1 (for example C3435T) have been

shown to affect the clearance of cyclophosphamide^{76,77,81}. Differences in cyclophosphamide clearance in African-American as compared to Caucasian patients are thought to be related to genotypic differences⁷⁷. Further, studies performed in patients with renal insufficiency show that decreased kidney function alters cyclophosphamide clearance^{76,82}. Lastly, studies have not evaluated whether cyclophosphamide clearance affects the incidence of graft rejection in patients who administer cyclophosphamide post-transplant.

4.0 STUDY DESIGN

A haploidentical relative donor will receive filgrastim (G-CSF) 10 to 16 µg/kg/d subcutaneously or intravenously for up to 6 days with apheresis collections of peripheral blood hematopoietic progenitor cells (PBPC) on day 5 (and day 6 if required). The product will be collected and frozen at least two weeks prior to the recipient beginning his/her conditioning.

The patient will receive a preparative regimen of Alemtuzumab (Campath®) to be infused on days -7 to -3, followed by 400 cGy TBI delivered in two divided fractions on days -2 and -1. The PBPC graft targeted to deliver $\geq 5 \times 10^6$ CD34⁺ cells/kg will be infused on day 0. Cyclophosphamide (Cytosan®) initially will not be administered. However, if patients develop graft rejection or severe (Grade 3 to 4 acute or chronic extensive) GVHD, the subsequent cohorts of patients will receive cyclophosphamide at 50 mg/kg or 100 mg/kg (see section 6.2). Sirolimus (Rapamune®) will be started at a loading dose of 5mg PO q4h x three doses one day after the completion of cyclophosphamide (on day +4 in cohorts 1 and 2 or day +5 in cohort 3) and continued the following day at 5mg PO q24h to maintain trough levels between 10-15ng/ml. On days +14, +30, +60 and +100, and periodically after day +100, the chimeric status of patients will be assessed by microsatellite analysis of the peripheral blood. More frequent monitoring may be required.

The design of the study incorporates the following features:

- 1) This is a phase I/II pilot study to determine the safety and therapeutic potential of a new transplant approach (disease-free survival, overall survival) and to evaluate its toxicity profile (immediate toxicity, graft-versus-host disease, graft rejection, mortality) in a patient population with severe congenital anemias.
- 2) The patient cohort to be studied: Those patients with severe sickle cell disease or β -thalassemia, who have risk factors for high mortality and morbidity related to their disease (see inclusion criteria in section 5.1)
- 3) Nonmyeloablative Transplant Conditioning Regimen - Immunosuppression without myeloablation: Patients will receive conditioning sufficient to allow donor lympho-hematopoietic engraftment without complete marrow ablation. If the graft is rejected, the patient will reconstitute autologous marrow function. We will use a combination of low dose irradiation (400 cGy TBI), Alemtuzumab (Campath®), and sirolimus with or without Cyclophosphamide (Cytosan®).
- 4) Peripheral blood hematopoietic progenitor cell (PBPC) transplant: An unmanipulated peripheral blood stem cell collection from a filgrastim (G-CSF) stimulated haploidentical relative donor should improve the chance of engraftment because of the high stem cell dose ($\geq 5 \times 10^6$ /kg CD34⁺ cells) and the presence of donor lymphocytes. To further reduce the risk of GVHD, patients will receive sirolimus after the transplant. The sirolimus will be tapered as necessary to minimize any graft-versus-host disease while still maintaining adequate chimerism. Patients will also receive post-transplant cyclophosphamide as needed if patients in the first cohort develop graft rejection or severe GVHD.

5.0 PATIENT SELECTION

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 sickle cell disease (HB SS, SC, or S β^0 -thal) at high risk for disease-related morbidity or mortality, defined by having severe end-organ damage (A, B, C, or D) or potentially modifiable complication(s) not ameliorated by hydroxyurea (E):

- 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 $< 50\text{mL}/\text{min}$ OR requiring peritoneal or hemodialysis⁸³⁻⁸⁵; OR
- C. Pulmonary hypertension defined as tricuspid regurgitant jet velocity (TRV) of $\geq 2.5 \text{ m/s}$ ^{39,40} at baseline (without vaso-occlusive crisis); OR
- D. Sickle hepatopathy defined as EITHER ferritin $>1000\text{mcg}/\text{L}$ OR direct bilirubin $>0.4 \text{ mg}/\text{dL}$ AND platelet count $<250,000/\text{uL}$ at baseline (without vaso-occlusive crisis, Jordan Feld and Theo Heller, MD manuscript in preparation)
- E. Any one of the below complications:

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 maximal tolerated dose of hydroxyurea* ²
Acute chest syndrome	2 prior ACS	any ACS while on hydroxyurea* ⁸⁶

*hydroxyurea at maximum tolerated dose for at least 6 months

5.1.1.2 Patients with 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 ≥ 18 years
- 5.1.2.2** Haploidentical relative donor available
- 5.1.2.3** Ability to comprehend and willing to sign an informed consent
- 5.1.2.4** Negative β -HCG

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

- 5.2.1** 6/6 HLA-matched with or without an ABO minor mismatched sibling donor
- 5.2.2** ECOG performance status of 3 or more (See Appendix A)
- 5.2.3** 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.
- 5.2.4** Major anticipated illness or organ failure incompatible with survival from PBSC transplant
- 5.2.5** Pregnant or lactating
- 5.2.6** Major ABO mismatch (See Appendix B and C)

5.3 Inclusion criteria - donor

- 5.3.1 Haploidentical relative donor
- 5.3.2 Weight ≥ 20 kg (insofar that the weight difference between recipient and donor does not exceed a reasonable likelihood of being able to obtain an adequate cell dose from the donor within two aphereses)
- 5.3.3 Fit to receive filgrastim (G-CSF) and to give peripheral blood stem cells (blood counts and blood pressure within DTM standards)
- 5.3.4 No history of congestive heart failure or unstable angina, and no history of stroke)
- 5.3.5 Ability to comprehend and willing to sign an informed consent; assent obtained from minors

5.4 Exclusion criteria- donor: (any of the following would exclude the donor from participating)

- 5.4.1 Pregnant or lactating
- 5.4.2 HIV positive
- 5.4.3 Hemoglobin S $\geq 50\%$, or beta thalassemia intermedia

6.0 TREATMENT PLAN

Sickle cell patients with pulmonary hypertension will meet with a Pulmonary Medicine Consult to determine appropriate management prior to SCT.

Iron chelation must be discontinued ≥ 48 hours before initiating the conditioning regimen. Hydroxyurea must be discontinued one day prior to initiating the conditioning regimen.

6.1 HLA typing

A haploidentical donor shall be selected which shares one haplotype in common with the recipient such that HLA compatibility will be a minimum of 5 out of 10 HLA loci matched. The HLA loci to be tested will be HLA A, B, Cw, DRB1, and DQB1. A minimum number of mismatches is desirable; however if several options are available the selection of a donor will be based on the loci where the mismatch occurs and the relative importance of its potential immunological function. Donor-recipient pairs will initially be typed molecularly to provide a low resolution typing (antigen-level) to aid in the selection of the potential donor. Upon review of the familial inheritance pattern, a qualified HLA staff member will review haplotype inheritance. If a donor(s) exists in the family that has a 5/10 haplotype possibility with the recipient, the HLA laboratory will initiate KIR typing to potentially aid in the selection of a donor. After consultation with NHLBI physicians and qualified HLA personnel, a donor(s) will be selected for further testing. High resolution (allele-level) typing will be performed. Final selection of a donor will be in consultation with NHLBI physicians and qualified HLA personnel. Documentation of final donor selection will be taken for each case in regards to final decision factors.

6.2 Exchange Transfusion (See AppendixC)

Within 2 weeks prior to conditioning, those patients with SCD who are not routinely (exchange) transfused will 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.

6.3 Collection of Autologous Peripheral Blood Stem Cells (PBSC) (See AppendixC)

Collection of autologous PBSC for backup in case of graft rejection and delayed autologous recovery will 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 solo agent to mobilize stem cells in patients with severe β -thalassemia, including those with and without prior splenectomy⁸⁷. The target stem cell dose will be $\geq 2 \times 10^6$ CD34 cells/kg, and the minimum dose for proceeding to transplant will be $\geq 1 \times 10^6$ CD34 cells/kg. If use of plerixafor does not result in collection of a sufficient number of CD34 cells, G-CSF will 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 thalassemia⁸⁷.

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 SCD⁸⁸. However, as of 4/1/16, >40 subjects with sickle cell trait have undergone mobilization with G-CSF at the NIH without any events that would suggest a vaso-occlusive crisis or any other sickle-related complication. Further, a recent study compared 12 subjects with sickle cell trait and 12 matched controls who received G-CSF⁸⁹. There was no significant difference in the rate of adverse events between the 2 groups. Plerixafor has not been studied in patients with SCD, so the risks are unknown. Bone marrow harvest has also not been studied in patients with SCD. The risk of general anesthesia may be significant in patients with severe disease, and fluid shifts associated with removal of bone marrow may also lead to significant morbidity. Therefore, the risks of autologous cell collection outweigh the benefits in patients with SCD, and hence autologous cells will not be collected in that patient population.

6.4 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.

Admission, exchange transfusion if necessary

Days -7 to -3 Alemtuzumab (Campath®) IV given in an escalating dose schedule over a total of 5 days as follows:

Day -7: Diphenhydramine 1mg/kg (maximum 50mg) I.V. and Acetaminophen 10-15mg/kg (maximum 650mg) P.O., 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) I.V. and Acetaminophen 10-15mg/kg (maximum 650mg) P.O., 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) I.V. and Acetaminophen 10-15mg/kg (maximum 650mg) P.O., then followed 30 minutes later by Alemtuzumab 0.3 mg/kg, in 100mL normal saline infused over 2 hours

Day -2: Total Body Irradiation (TBI), dose of 200 cGy delivered as per the Department of Radiology standard of practice

Day -1: Total Body Irradiation (TBI), dose of 200 cGy (cumulative dose of 400 cGy in two divided fractions given on Day -2 and -1) delivered as per the Department of Radiology standard of practice

Day 0: Infusion of unmanipulated filgrastim (G-CSF, Neupogen®)- mobilized peripheral blood stem cells

Day +3 to Day +6: Cyclophosphamide 50 mg/kg/dose will be given on Day +3 to cohort 2 patients and 50mg/kg/dose will be given on Day +3 and Day +4 (total dose 100 mg/kg) to cohort 3 patients (see below)

by IV infusion over 60 minutes. Dosing will be based on practical body weight for “morbidly obese” patients (BMI \geq 35, see Appendix E). Mesna and intravenous hydration will be given concurrently with the cyclophosphamide doses as based on current BMT consortium guidelines. Patients will be advanced to subsequent cohorts if the protocol reaches stop criteria for regimen failure (see section 14.0).

Cohort Number	Cyclophosphamide Dose (mg/kg/dose)	Day Post Transplant	Cumulative Cyclophosphamide Dose (mg/kg)
1	0	N/A	0
2	50	+3	50
3	50 50	+3 +4	100

Sirolimus (Rapamune®) will be started at 5mg PO q4h x three doses one day after the completion of cyclophosphamide (on day +4 in cohorts 1 and 2 patients and on day +5 in cohort 3 patients). Sirolimus (Rapamune®) will be continued at 5mg PO q24h starting on day +5 in cohorts 1 and 2 patients and on day +6 in cohort 3 patients. Trough levels will be maintained between 10-15 ng/ml.

6.5 Total body irradiation

TBI will be delivered in two fractions of 200 cGy on day -2 and -1 for a total dose of 400 cGy. Equally weighted opposed lateral beams will be used to encompass the total body with the patient positioned supine. Treatment will be delivered at an SAD of 6 meters. The prescription point will be the midplane at the maximal hip separation. The dose rate to midplane will be between 8-13 cGy per minute. Head and neck compensation will be used to increase homogeneity. Adjustments to treatment technique but not dose prescription may be made at the discretion of the treating radiation oncologist if deemed necessary.

6.6 Central venous line placement

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

6.7 Supportive Care

6.7.1 Blood product support

Filtered and irradiated blood products will be used in all patients, regardless of CMV status. 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 9-10g/dL in patients with SCD. Otherwise, packed red blood cell and platelet transfusions will be given according to BMT consortium guidelines.

6.7.2 Infection Prophylaxis and Treatment

- Penicillin VK 250 mg PO BID from day 0 until pneumococcal vaccination is complete post-transplant.

- EBV, CMV, HHV6, and adenovirus monitoring and treatment:
 - Subjects will be monitored for EBV, CMV, HHV6, and adenovirus PCR in the blood at baseline, and then weekly until discharge from the hospital, then at least every 2-3 weeks until day 100. Thereafter, monitoring will be performed as clinically indicated.
 - EBV reactivation, CMV reactivation, and adenovirus infection will be treated according to BMT consortium guidelines
 - HHV6 reactivation will not be treated unless compelling evidence exists for clinical disease related to HHV6. Such patients may receive foscarnet upon the advice of NIH infectious disease consultants
- Family members, including donors, will be offered influenza vaccination, as seasonally indicated. Patients will be offered influenza vaccination, as seasonally indicated, when they are at least 6 months post-transplant per CDC HSCT guidelines.
- Prophylaxis and treatment of infections will otherwise be administered according to BMT consortium guidelines

6.7.3 Anti-emetics

Anti-emetics will be administered according to NIH Pharmacy Department guidelines or with consultation of a clinical pharmacist.

6.8 Peripheral blood progenitor cell transplant (see Appendix C)

The target collection number for progenitor cells is $\geq 10 \times 10^6$ CD34+ cells/kg. 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. In pediatric subjects, defined as less than 40 kg, a maximum of 8 blood volumes will be processed per day, for up to 2-3 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 $\geq 5 \times 10^6$ 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. Minor ABO incompatible grafts will have plasma removed per Transfusion Medicine protocol.

6.9 GVHD prophylaxis

Sirolimus (Rapamune®) will be started on day +4 in cohorts 1 and 2 patients and on day +5 in cohort 3 patients with a loading dose of 5mg PO q4h for three doses, then 5mg PO q24h starting on day +5 in cohorts 1 and 2 patients and on day +6 in cohort 3 patients. Sirolimus will then be titrated to trough levels of 10-15ng/ml. 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.

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

6.10 Nutrition

Parenteral nutrition will be instituted if daily caloric intake <1000KCal /day in patients.

6.11 Hospital Discharge

Patients will be discharged when the following criteria are fulfilled:

- Patient afebrile, positive weight balance; no parenteral feeding required
- Platelet transfusion requirement absent or manageable as an outpatient
- Patient or family able to care for central line

6.12 Contraindication Listings

Filgrastim (G-CSF) will be listed as a 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.

7.0 STUDY PARAMETERS

Primary endpoint

The percentage of patients at 1 year post-transplant with sustained donor type hemoglobin on hemoglobin electrophoresis for patients with sickle cell disease and who are transfusion-independent for patients with thalassemia and who do not have severe graft-versus-host disease. Therefore, as seen in the table, regimen failure is defined as the absence of donor hemoglobin and/or history of severe GVHD at 1 year post-transplant.

	Yes donor hemoglobin	No donor hemoglobin
No GVHD	Desired outcome	Regimen failure
Yes GVHD	Regimen failure	Regimen failure

Secondary endpoints

- 1) The level of chimerism required to maintain both graft survival as well as hematologic normalcy. The chimeric status of patients will be measured on days +14 (or when subject starts to engraft), +30, +60 and +100, and periodically after day +100, by microsatellite analysis of the peripheral blood. More frequent monitoring may be required.
- 2) Incidence of acute and chronic GVHD
- 3) Disease-free survival and overall survival
- 4) Relapse rate and graft rejection rate
- 5) Transplant-related mortality
- 6) Determine whether post-transplant cyclophosphamide is required and will reduce the incidence and severity of regimen failure
- 7) Determine whether specific haploidentical donors (i.e. parent versus sibling versus child) will decrease the incidence of regimen failure

- 8) Determine whether cyclophosphamide pharmacokinetics and/or germline allelic variants in drug metabolizing/transporting genes differ in patients with and without renal insufficiency and in patients who do and do not experience graft rejection

These secondary endpoints may be achieved by monitoring the following parameters:

- 1) CD34⁺ cell dose, CD3⁺ cell dose
- 2) Degrees of donor-recipient lymphoid, myeloid, and erythroid chimerism by microsatellite PCR analysis and normal hemoglobin quantitation-either by gel electrophoresis or if necessary by flow analysis – using peripheral blood as appropriate.
- 3) Neutrophil recovery (days to neutrophil count of $0.5 \times 10^9/l$ and $1.0 \times 10^9/l$).
- 4) Platelet recovery (days to platelet count of $50 \times 10^9/l$, days to transfusion independence)
- 5) Red cell recovery (days to transfusion independence)
- 6) Non-hematologic effects attributable to the preparative regimen
- 7) Transplant related mortality by 1 year
- 8) Hemoglobin F and S levels on hemoglobin electrophoresis in appropriate patients
- 9) Development of further neurologic disease
- 10) Gonadal organ function as reflected by hormone levels and normal menstrual cycle
- 11) Quality of life
- 12) Neuropsychologic testing
- 13) Assessment of immune reconstitution
- 14) Assessment of cytokines and lymphocyte function

8.0 RESEARCH STUDIES

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

For pediatric patients, 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.

8.1 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 in the circulation. Approximately 10 mL will be drawn around days 14, 30, 60, 100, and every 6-12 months thereafter.

8.2 Bone Marrow Samples

A volume (up to 25 ml) of bone marrow aspirate will be collected for research studies at the pretransplant evaluation, day 100 post transplant, and/or when full donor erythroid chimerism is attained or if clinically indicated. These will be used to help elucidate the contribution of the progenitor cells to the circulating component

8.3 Mobilized Hematopoietic Stem Cells

Following cryopreservation of mobilized, unmanipulated autologous “back-up” PBSCs in patients with thalassemia the “empty” bag and tubing will be washed and the recovered cells will be collected and stored. These cells will be used for gene therapy research involving genetic correction of the DNA in order to produce

normal hemoglobin.

8.4 Transthoracic Echocardiography

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

8.5 Dual X-ray Absorptiometry (DEXA Scan)

Through bone density scans recommended by routine endocrinology consults in 20 subjects with sickle cell disease, we discovered that more than half of our patients were osteopenic or osteoporotic before transplant. Therefore, to formally study the bone effects from sickle cell disease and to monitor the effect of transplant on bone disease, we would like to perform DEXA scans in all patients pre-transplant and every 1-2 years post-transplant depending on how severe their bone disease is.

8.6 Assessment of quality of life

PROMIS (patient reported outcome instrument) is commercially available and has been applied in many diseases and conditions, and will be a useful tool to assess the overall physical and mental health perception in our patients before and after BMT. PROMIS (appendix G) will be administered pre-BMT, day 30, day 60, day 100, 6 months, one year, two years, three years, four years, and five years post BMT.

8.7 Neuropsychologic testing

Neuropsychologic testing, performed by our psychologists or other members of the neuropsychology group, will include but not be limited to the Wechsler Abbreviated Scale of Intelligence and the California Verbal Learning Test-C or CVLT-II at baseline and 2 years (+/- 6 months) post BMT. In addition, a brief monitoring battery assessing selective domains such as attention, executive function, processing speed, as well as emotional functioning with the Brief Symptom Inventory, will be administered at 100 days (+/- 3 months) post BMT.

8.8 Immune reconstitution

Lymphocyte subpopulations and immunoglobulin levels (IgG, IgA, and IgM) will be quantified before transplant, and approximately 30 days, 60 days, 100 days, 6, 12, and 18 months post-transplant, and yearly thereafter until results have normalized. Approximately 7 mL of blood will be collected at each time point. Lymphocyte subpopulations and other immune cells may also be characterized by flow cytometry for specific markers associated with developmental stage, function and alloreactivity which would require an additional 1-2 blood samples (approx. 30 mL each).

8.9 Assessment of cytokines and lymphocyte function

Donor serum and lymphocytes will be collected pretransplant. Patient serum and lymphocytes will be collected pretransplant and at 6 and 12 months post-transplant. Approximately 30 mL will be collected. Engrafted patients who discontinue sirolimus will have additional samples collected just prior to and 6 months after discontinuing sirolimus. In patients that develop graft rejection or GVHD, patient serum and lymphocytes will be collected once within 2 weeks of the onset of clinical symptoms, once just prior to discontinuing immunosuppression or at 12 months post-transplant, whichever is longer, and once 6 months after immunosuppression is discontinued or at

18 months post-transplant, whichever is longer. Serum tumor necrosis factor, interferon-gamma, and interleukin-17 will be quantified. Levels may continue to be followed every 6 to 12 months thereafter based on the results during the first 18 months.

Donor/host alloreactivity will be assessed at each time point by CFSE dilution assay in the presence and absence of sirolimus in vitro. We will also measure mTOR signaling in patient T cell samples 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. Patient effector T cell subsets will be measured at each time point by intracellular cytokine staining for interferon gamma, interleukin-4, interleukin-17, and FoxP3.

CMV pp65 specific T cell responses will 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 will measure influenza specific T cell responses by intracellular cytokine staining for interferon-gamma, interleukin-4, interleukin-17, and FoxP3 in response to influenza peptides.

8.10 Evaluation for markers of graft rejection

Blood samples (approx. 20 mL) will be collected serially at baseline and at 1 week, 2 weeks, 1 month, 2 months, 100 days, 6 months, 18 months, 24 months, and yearly thereafter post-transplant. Cytokine and immunophenotyping studies will be performed to evaluate for biomarkers associated with primary graft failure, acute rejection, and chronic rejection. Samples may be collected more frequently as necessary based on our preliminary results. Donor-derived cell-free DNA levels will also be measured to determine whether acute elevation predicts subsequent graft rejection⁸⁹.

8.11 Pharmacokinetic studies

Blood samples (approx. 3 mL each time) will be drawn at the beginning of and 0.5, 1, 4, 6, 12, and 24 hours after the first cyclophosphamide infusion and 1 and 24 hours after the second cyclophosphamide infusion. An additional sample will also be drawn pre-and post- hemodialysis after the first cyclophosphamide infusion. These samples will be used to evaluate cyclophosphamide, 4-hydroxycyclophosphamide and 2-dechloroethylcyclophosphamide levels. These data will be used to create plasma concentration versus time profiles for each drug or metabolite.

8.12 Pharmacogenomic studies

Peripheral blood will be drawn prior to transplant to perform genotyping for single nucleotide polymorphisms in CYP3A4/5, CYP2B6, CYP2C9, and C3435T. If the original sample has insufficient DNA for analysis, then an additional sample will be obtained from patients prior to transplant.

9.0 Human Specimen Use, Disposition, Tracking and Storage of Samples and Data

During the course of participating on this study, blood, tissue and data will be collected for correlative laboratory research studies. Specimens collected strictly for research purposes will not be read by a pathologist.

Biospecimen management:

Specimens and their derivatives (e.g., genomic material, cell lines) will be coded and stored in conformity with DIR Policy (e.g., BSI). Biospecimens and/or data will not be sent outside of the NIH without IRB approval and an executed agreement.

Storage: Jonathan Powell, MD, PhD, Johns Hopkins University will receive patient samples to assess cytokine and lymphocyte function as described in section 8.7, Melanie Joy, PharmD, PhD, University of Colorado will receive patient samples to evaluate cyclophosphamide pharmacokinetics as described in section 8.9, William D. Figg, Sr. PharmD, MBA, CRC/NCI/OCD will receive patient samples to assess cyclophosphamide pharmacogenomics studies as described in section 8.11, and John Sninsky, PhD. CareDx, Brisbane, California will receive samples to assess donor-derived cell-free DNA levels. All other research samples will be stored in the principal investigator's laboratory. Samples will never be labeled with the patient's name. Samples will be assigned a unique code known only to the principal and associate investigators, which will serve as a link to the patient's clinical information collected as part of this research protocol. Therefore confidentiality is protected.

De-identified peripheral blood and clinical data may be sent to Dr. Allistair Abraham at Children's National Medical Center to compare donor/recipient lymphocyte contribution and NK cell phenotypes.

Data sharing and future use of data:

Research data may be shared with qualified non-collaborator recipients following publication of the primary research results after removal of PII and IRB or OHSRP approval. Future research use of data not defined in the research protocol may occur only after IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protections (OHSRP). Refusal of a research subject participant to permit future use of data--other than required in the protocol or by the FDA--will be honored. Limitations in data sharing and future use of data due to contractual obligations (e.g., CRADAs) or intellectual property proceedings (such as patent filings) will be honored.

Future use of biospecimens:

Following analyses of biospecimens for primary research purposes as described in the protocol, remaining samples suitable for future research must be stored in manner that conforms with DIR policy (such as BSI) or in a publicly accessible research biospecimen repository following IRB approval. Biospecimens may be destroyed only when permitted by the clinical director and approved by the IRB. Any future research use of biospecimens not defined in the research protocol will occur only after IRB review and approval or an exemption from OHSRP. Biospecimens will not be sent outside of the NIH for future research use without IRB approval and an executed agreement. Refusal of a research subject participant to allow for future use of biospecimens--other than required in the protocol or the FDA--will be honored.

Tracking: Samples will be ordered and tracked through 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.

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 breach 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 that complies with NIH security standards and NHLBI DIR policy or locked research file system and ensuring data accuracy, consistency, and timeliness. The principal investigator, associate investigators, fellows, and research nurses will assist with the data management efforts. All human subjects personally identifiable information (PII), as defined in accordance to the Health Insurance Portability and

Accountability Act (HIPAA), eligibility and consent verification will be recorded in conformity with DIR policy. Primary and final analyzed data will have unique codes so that research data can be attributed to an individual human subject participant. Data will be abstracted from NIH progress notes as well as from progress notes forwarded from home physicians. Laboratory data will be entered manually or imported electronically from CRIS into an in-house database. Laboratory values from referring home physicians will be manually entered into the system.

End of study procedures: Data will be stored in locked cabinets and in a password protected database in conformity with NHLBI DIR policy or in a publicly accessible research repository until they are no longer of scientific value. Data may be destroyed only when permitted by the clinical director and approved by the IRB.

Loss or destruction of data: Should we become aware that a major breach in the plan to protect patient confidentiality and trial data has occurred, the clinical director and IRB will be notified.

Publication Policy: Given the research mandate of the NIH, patient data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protection (OHSRP).

10.0 CLINICAL EVALUATION - PATIENT

10.1 Pre-study evaluation

- 10.1.1** History, physical examination to establish baseline symptoms, height and weight
- 10.1.2** ECOG performance status
 - 10.1.2.1** High resolution molecular HLA- A, -B, -Cw, -DRB1, and -DQB1 typing of patient and as many family members as possible and/or necessary to confirm haploidentical matching of the donor
 - 10.1.2.2** Hemoglobin electrophoresis and/or flow cytometric analysis of hemoglobin A, F and SS, SC or S- β -thal⁰ as appropriate (for both patient and donor)
 - 10.1.2.3** Antibody screen for HBV, HCV, HIV, HTLV-I/II, CMV, EBV, toxoplasma, syphilis. Consider PPD test for patients from areas where tuberculosis is prevalent
- 10.1.3** Coagulation screen, CBC with differential
- 10.1.4** Comprehensive metabolic panel
- 10.1.5** Extended red cell phenotyping
- 10.1.6** HLA antibody screen
- 10.1.7** β -HCG serum pregnancy test for females of childbearing potential
- 10.1.8** Chest radiograph, pulmonary function test
- 10.1.9** Sinus, chest, abdomen and pelvis CT scans as clinically indicated
- 10.1.10** Brain MRI
- 10.1.11** Cardiac function: EKG, ECHO, 24 hour holter
- 10.1.12** Troponin T and/or Troponin I
- 10.1.13** Lymphocyte subpopulation analysis and serum immunoglobulin levels
- 10.1.14** Research sample for cytokine and lymphocyte function analyses
- 10.1.15** Nutritional assessment
- 10.1.16** 24 Hour Urine collection for assessment of creatinine clearance, urinalysis, urine for protein and creatinine ratio
- 10.1.17** Dental review
- 10.1.18** Endocrine consultation and testing which will include DEXA Scan, thyroid panel, insulin-like growth factor 1, morning cortisol, ACTH stimulation test, fasting glucose, fasting insulin, oral glucose tolerance test, hemoglobin A1C (or serum fructosamine level in patients with SCD), 25 hydroxy vitamin D, and in male patients, testosterone, luteinizing hormone level (LH),

and follicle stimulating hormone (FSH), and in female patients, LH, FSH, anti-mullerian hormone, and estradiol

10.1.19 Social worker interview

10.1.20 Ophthalmology consultation

10.1.21 Interview with members of primary care team and visit to unit

10.1.22 Consent form signed

10.1.23 Social work interview

10.1.24 Complete lipid profile with triglycerides

10.1.25 Pulmonary hypertension evaluation by tricuspid regurgitant velocity (TRV) analysis using standard transthoracic echocardiography (pulmonary hypertension is defined as a TRV \geq 2.5 m/s)

10.1.26 Quality of life assessment

10.1.27 Neuropsychologic testing

10.1.28 Genotyping studies for single nucleotide polymorphisms in CYP3A4/5, CYP2B6, CYP2C9, C3435T, and others that may be important in drug metabolism

10.2 Inpatient monitoring

All patients with a sickling disorder undergoing transplant will be listed as having a contraindication to the use of filgrastim (G-CSF).

Once daily: CBC with differential, comprehensive metabolic panel

Twice weekly: reticulocytes, pre-albumin, sirolimus level, and coagulation screen

Weekly: CMV, EBV, adenovirus, and HHV6 surveillance

Every two weeks: serum cholesterol, triglycerides

After the last dose Campath: Troponin T and/or Troponin I, ECHO, 24 hour holter

10.3 Follow-up to day 100: outpatient

At least every 2-3 weeks up to 100 days then on return follow-up visits and when clinically indicated: CBC, hemoglobin electrophoresis to assess HbS and Hb F levels in appropriate patients, coagulation screen, comprehensive metabolic panel, sirolimus level; CMV, EBV, adenovirus, HHV6 surveillance. A complete physical exam will be repeated at each visit. Quality of life assessments will be administered pre-BMT, day 30, day 60, and day 100. Neuropsychologic testing will be performed at 100 days post BMT.

Monthly (+/- 7 days): Serum cholesterol, triglycerides.

Peripheral blood will be drawn on days +14 (or when subject starts to engraft), +30, +60 and +100 to assess for donor-host chimerism in the lymphoid, myeloid, and erythroid cell lines including the use of Hb S and Hb F levels, F cell and F retic% analyses.

100 Days (+/- 1 week) after the last dose Campath: ECHO, Troponin T and/or Troponin I

10.4 Beyond day 100

At 6, 12, 18, 24, 36, 48 and 60 months (+/- 1 month): CBC, Hb S and Hb F levels, comprehensive metabolic panel, serum cholesterol, triglycerides, chimerism studies, and pulmonary function testing. At 6, 12, and 18 months blood will be drawn into heparinized tubes for preparation of plasma and lymphocytes for in vitro studies, and serum immunoglobulin levels and lymphocyte subpopulation analysis will be performed. Bone marrow

aspirate samples will be obtained at day 100 post-transplant and/or when full donor erythroid chimerism is attained or if clinically indicated. Patients will be screened for CMV beyond day 100-post transplant for a minimum of 6 months. Quality of life testing will be performed at 6 months, 1 year, 2 years, 3 years, 4 years, and 5 years post BMT. Neuropsychologic testing will be performed at 2 years post BMT.

At 12, 24, 36, 48, and 60 months: thyroid stimulating hormone, free thyroxine, insulin-like growth factor 1, morning cortisol level, fasting glucose, fasting insulin, hemoglobin A1C (or serum fructosamine level in patients whose donors have sickle cell trait), 25 hydroxy-vitamin D, and in male patients, testosterone level, luteinizing hormone level (LH), and follicle stimulating hormone level (FSH), and in female patients, LH, FSH, anti-mullerian hormone level, and estradiol level. DEXA Scans will be performed every 1 to 2 years depending on the severity of the bone disease.

After 5 years, follow-up visits are not mandatory, but yearly communication with the patient and the referring physician will continue in order to monitor any adverse events that may be related to the transplant procedure.

11.0 CLINICAL EVALUATION AND PLAN - DONOR

11.1 Pre-study consult and evaluation

- 11.1.1** HLA-A,-B,-Cw,-DRB1, -DQB1 typing of as many family members as necessary
- 11.1.2** Confirm HLA haploidentity of donor with patient
- 11.1.3** History and physical examination
- 11.1.4** Hemoglobin electrophoresis
- 11.1.5** Hepatitis B, C, HIV, HTLV-I/II, CMV antibodies, RPR
- 11.1.6** CBC with differential, coagulation screen, comprehensive metabolic panel
- 11.1.7** Extended red cell phenotyping, HLA Ab screening
- 11.1.8** Chest x-ray
- 11.1.9** Fit to donate: Orientation- visit to Department of Transfusion Medicine for inspection of veins to determine the need for a central line for apheresis
- 11.1.10** Consent to undergo filgrastim (G-CSF) mobilization (see below)
- 11.1.11** Donors will have a follow-up visit within 2 weeks of starting G-CSF
- 11.1.12** Donors who consent will have blood drawn for preparation of plasma and lymphocytes for in vitro studies.

11.2 PBPC collections (see AppendixC)

The donor will receive 10 to 16 mcg/kg filgrastim (G-CSF) subcutaneously or intravenously daily, starting at least one week before the anticipated transplant date (see AppendixC).

The collections will then be cryopreserved. Should this allograft contain less than the minimum dose of CD34+ cells/kg, the donor may undergo a second apheresis on day 6 or a repeat G-CSF mobilization (a minimum of 2 weeks later – see section 6.6) at a higher dose of G-CSF with the pooled products of the two mobilizations being given to the patient at the time of transplant.

In the event of relapsed disease, the donor may be asked to undergo a repeat G-CSF mobilization and PBPC collection as detailed above.

12.0 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:

12.1 Viral reactivation: Patients will be treated according to BMT consortium guidelines (see section 6.5.2)

12.2 Acute GVHD

Sirolimus may be continued and other immunosuppressive drugs may be used as clinically appropriate.

12.3 Chronic GVHD

Sirolimus may be continued and other immunosuppressive drugs may be used as clinically appropriate.

12.4 Graft rejection

Patients with graft rejection may be treated with standard of care treatment options with or without stem cell rescue (2nd transplant, using a conventional conditioning regimen) or referred back to their primary physician depending on what is considered to be in the best interest of the patient. Patients who undergo repeat transplant as part of another protocol can be followed under this protocol to continue immunosuppressive therapy as necessary and to be monitored for long-term complications. This transplant protocol uses a nonmyeloablative preparative regimen. Therefore, autologous recovery (hence relapse of disease) is anticipated in patients who fail to engraft (see 11.5). To avoid the rare situations where patients fail to engraft and recover autologous hematopoiesis, autologous HSCs will be collected from patients with thalassemia for rescue.

12.5 Relapse of disease

Patients with disease relapse may be treated with standard of care treatment options with or without stem cell rescue (2nd transplant, using conventional conditioning regimen) or referred back to their primary physician depending on what is considered to be in the best interest of the patient.

12.6 Use of Immunosuppressive drugs

In the event that a subject has an adverse reaction to sirolimus, alternative immunosuppressive drugs may be used as clinically appropriate.

13.0 HUMAN SUBJECT PROTECTIONS

13.1 Rationale for subject selection

All patients with confirmed thalassemia or sickle cell disease, 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).

13.2 Participation of children

13.2.1 *As stem cell transplant recipients*

As the risk of our regimen in the haploidentical transplant setting is not known and is likely to be higher than our 6/6-HLA matched sibling transplant regimen, we will not include children on this protocol until the risk is more established.

13.2.2 *As donors of stem cells*

Allogeneic bone marrow (and peripheral blood) transplantation (BMT) is the only available cure for sickle cell disease or thalassemia, and is therefore considered an accepted standard clinical intervention for these diseases. The donor would be donating stem cells to his/her family member regardless of the objectives of this research protocol.

We are, however, excluding participation as donors children who weigh <20 kg. The risks of the apheresis procedure are related to the weight of the child, more precisely his/her extracorporeal volume, which is weight-dependent. The risks have to do with (1) need for a central line, (2) need for an allogeneic red cell prime, and (3) need for systemic heparinization because the subject is too small to get citrate:

> 25 kg: the procedure and associated risk is the same as that in an adult, however a central line is almost always needed.

20 to 25 kg: A central line is required. Donors may or may not need a red cell prime (at the discretion of the Apheresis department). Although there may be a need for heparinization, generally donors are anticoagulated with citrate.

<20 kg: All donors in this weight range are excluded from participation as they would require a central line, red cell priming, and systemic heparinization.

13.2.3 *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 12.6).

13.3 Hazards and discomforts- recipient

13.3.1 *Related to the transplant*

The mortality from conventional BMT may be as high as 40%. Although our data as well as that of others suggest a significant reduction in transplant-related mortality with nonmyeloablative PBSC transplantation, the procedure nevertheless carries significant risk. It is therefore only appropriate to carry out this experimental procedure in the context of debilitating or life-threatening conditions and with full informed consent from the patient, donor, and immediate family. We have sought to develop a conditioning regimen which avoids the use of renally excreted drugs, and relies on the immunosuppressive effects of TBI as the basis for such an approach. A modest increase in the dosage of TBI from the 300 cGy used in our prior study to 4 00 cGy may increase both the degree of myelosuppression and immunosuppression without significantly altering the side effect profile. The

specific hazards of this study using a nonmyeloablative preparative regimen and high PBPC content graft are graft rejection, graft-versus-host disease, disease relapse, and infectious complications. The major discomforts are those of nausea, anorexia, diarrhea, fever, malaise, and intolerance of the isolation period. The ten patients with SCD that were treated according to our HLA-matched sibling protocol at the NIH did not experience significant toxicity related to the transplant regimen or GVHD.

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

13.3.2 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/ μ L) 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/ μ L. The median time to recovery of CD4⁺ counts to 200/ μ L 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 administered prior to recovery from lymphopenia 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)/ μ L, at 2 months after discontinuation of Alemtuzumab therapy, 207/ μ L, and 6 months after discontinuation, 470/ μ L. 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. We will also closely monitor subjects for cardiac symptomology and ask them to immediately report any cardiac symptoms (palpitations, irregular pulse, difficulty in breathing, dizziness, swelling in the ankles, chest discomfort or pain).

13.3.3 *Related to Sirolimus:*

The anticipated toxicities of sirolimus in this trial are those related to its immunosuppressive properties, such as an increased likelihood of infection, and mucosal (including mouth, gastric, small bowel, or large bowel) ulcers, which may bleed. Other possible toxicities are listed here and include those reported with $\geq 3\%$ and $<20\%$ incidence in patients in any Sirolimus treatment group in the two controlled clinical trials for the prevention of acute organ graft rejection:

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;

Nervous System: anxiety, confusion, depression, dizziness, emotional lability, hypertonia, hypesthesia, hypotonia, insomnia, neuropathy, paresthesia, somnolence, stroke;

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, 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.

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.

13.3.4 Related to 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.

13.3.5 Related to Plerixafor

Side effects reported in healthy volunteers who received subcutaneous injections of plerixafor with doses ranging from 40 to 480 µg/kg included abdominal distension, abdominal pain, diarrhea, flatulence, nausea, vomiting, and decreased appetite⁹⁰⁻⁹². Injection reactions also occurred including erythema, burning, bruising, pain, pruritis, and swelling. Other adverse events included dizziness, headache, disorientation, paresthesia, chest tightness, palpitations, tinnitus, vertigo, and ear congestion. None of the adverse events were severe. The addition of G-CSF (10 µg/kg/day for 5 days) to plerixafor (160 mcg/kg) in healthy volunteers led to an increase in CD34 yield without an increase in adverse events⁹³.

Another study reported nine patients with thalassemia (5 splenectomized and 4 non-splenectomized) who received plerixafor alone (240 µg/kg/day for two consecutive evenings), and one splenectomized patient who was previously insufficiently mobilized with G-CSF alone was re-mobilized with plerixafor and G-CSF⁸⁷. Twenty-three patients (13 splenectomized and 10 non-splenectomized) received G-CSF alone. No severe adverse events were reported. The most common adverse events related to plerixafor were mild and included nausea, diarrhea, and injection site erythema. There was a $5.8 \pm 7.8\%$ (range 0-19%) mean increase in splenic volume with plerixafor, which was significantly lower than the 60% mean increase in splenic volume in patients mobilized with G-CSF. Eight of nine patients treated with plerixafor achieved a CD34 cell yield of $>4 \times 10^6/\text{kg}$ with one or two aphereses. The patient who received plerixafor and G-CSF yielded $6.5 \times 10^6/\text{kg}$ CD34+ cells in one apheresis without an increase in adverse events and without excessive leukocytosis.

13.3.6 Related to G-CSF

In the above study, 23 patients with thalassemia (13 splenectomized and 10 non-splenectomized) were treated with G-CSF alone (2.5-10 µg/kg/day for 4-7 days). The most common adverse events were bone pain, low-grade fever, and grade 1 thrombocytopenia during apheresis⁸⁷. There were no serious adverse events. However, patients with intact spleens experienced a 60% mean increase in splenic size. In splenectomized patients, a G-CSF dose of 10 µg/kg led to hyperleukocytosis (white blood count $71 \times 10^3/\mu\text{L}$) after 2 doses. A subsequent decrease in G-CSF dose to 2.5 to 5.0 µg/kg/day led to insufficient CD34 mobilization. When patients were pre-treated with hydroxyurea (up to 25mg/kg/day in splenectomized and 20mg/kg/day in non-splenectomized patients) and then started G-CSF (total mean daily dose $7.5 \pm 1.28 \mu\text{g/kg}$) 12 to 15 days after hydroxyurea cessation, they experienced sufficient CD34 yields without excessive leukocytosis and there was

a decrease in the degree of splenic enlargement. There is one report of a patient being treated with plerixafor and G-CSF as stated above⁸⁷. Another study reported G-CSF given safely to 20 patients with thalassemia at doses ranging from 10-16µg/kg/day⁹⁴. They did not report whether their patients had intact spleens.

13.3.7 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.

13.3.8 Related to Radiation

Side effects of radiation have been well described.⁹⁵ 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.⁹⁶ Importantly, the majority of the nonneoplastic effects were sub clinical and/or reversible.⁹⁷ 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^{98,99}. 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. From our own experience, one patient with severe sickle cell disease who underwent an HLA-matched sibling peripheral blood stem cell transplant and received 300cGy TBI had a healthy baby 3 ½ years post transplant. 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.¹⁰⁰ 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,^{101,102} but the highest risk factor was felt to be the presence of chronic GVHD and long term treatment with cyclosporine.^{103,104} Therefore the actual risk cannot be quantified for the low dose of 400cGy to be used in this trial; however the risk is presumed to be lower. The additional radiation exposure from DEXA Scans is negligible and is not expected to significantly increase the risk presented by TBI.

13.3.9 Related to Antimicrobials in general:

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

13.3.10 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.

13.3.11 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.

13.3.12 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.

13.3.13 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.

13.3.14 Related to MRI with contrast

If contrast is ordered, Gadolinium, an FDA approved medication will be used to improve MRI images. About 98% of patients receiving gadolinium have no symptoms related to the injection of this medication. Mild symptoms that may occur include: coldness in the arm at injection, a metallic taste, headache, and nausea. In an extremely small number of patients, more severe symptoms have been reported including: shortness of breath, wheezing, and lowering of blood pressure.

The US Food and Drug Administration has issued a warning that administration of gadolinium, the contrast imaging agent that may be used in this protocol, has been associated with development of a disease called nephrogenic systemic fibrosis. The syndrome is rare (approximately 200 cases reported worldwide as of December, 2006 out of several million administrations of gadolinium), but disabling and in some cases, fatal. All cases to date have occurred in patients with severe renal disease, including patients on dialysis. We will ask the patient whether they have or have had kidney disease or diabetes, whether they take diuretics (water pills) for any medical condition and whether they have received x-ray dye or drugs recently that might affect

their kidney function. Depending on their history and creatinine levels, it will be determined whether or not they may receive gadolinium.

13.4 Hazards and discomforts- donor

13.4.1 *Related to filgrastim (G-CSF)*

The hazard to the donor is low. The discomfort from G-CSF mobilization and apheresis for collection of blood stem cells are probably lower than those associated with marrow harvesting.

G-CSF has been given to large numbers of normal donors without major side effects or long term consequences. The immediate side effects of G-CSF in 50-75 % of recipients are bone pain, fatigue, insomnia, myalgia, and headache. These are usually mild and are self-limiting. Reversible thrombocytopenia, with platelet counts falling to the region of 100,000/mm³ is frequent, and may decrease to less than 60,000/mm³ after a third apheresis. Few patients have been reported to experience non-fatal splenic rupture after more prolonged treatment with higher doses of G-CSF. One of these two patients had concurrent mononucleosis, a second cause for splenic rupture. Patients with ongoing ischemic heart disease have been reported to have angina seemingly temporally related to G-CSF administration and apheresis. There are also case reports of patients developing lung infiltrates, changes in pulmonary function tests, and acute respiratory distress. In addition, rarely subjects have experienced severe pulmonary toxicity including pulmonary hemorrhage and pulmonary edema, which was fatal in one case.¹⁰⁵⁻¹⁰⁷ Therefore, a baseline chest x-ray will be performed on all potential donors prior to G-CSF administration. Leukocytosis is expected after administration of G-CSF. Other adverse reactions associated with the use of G-CSF as cited in the literature include fever (rare), transient but reversible increases of alkaline phosphatase, lactate dehydrogenase, and uric acid levels, and exacerbation of preexisting rashes.

13.4.2 *Related to central line placement*

It is estimated that about 50% of the donors will require intravenous central line placement to successfully complete apheresis. Intravenous line placement in the femoral vein using a temporary double-lumen Arrow catheter carries a small risk of bleeding, bruising, infection, or pain, and a very low risk of accidental injury to the adjacent artery and nerve. These risks are minimized by using only trained experienced staff for the procedure.

13.4.3 *Related to apheresis*

Adverse reactions related to apheresis include hypotension resulting from transient blood volume loss and cutaneous paresthesia from the use of anticoagulant. The former toxicity can be corrected by postural changes and volume replacement. The latter is manageable with slowing the rate of anticoagulant infusion and/or providing calcium supplement. In exceptional instances, the donor may be required to donate PBPC a third time or to give bone marrow. Donation of PBPC on three successive days significantly increases the risk of thrombocytopenia (<100,000/ul). However, thrombocytopenia is transient and unlikely to cause clinical sequelae. There is no additional risk to the donor giving marrow after PBPC donation (than would normally be associated with bone marrow harvesting).

13.5 Risks in relation to benefit

As of September 2016, this study is now closed to new subject accrual and continues in subject follow up. The level of risk has not changed, since subjects remain active on study.

13.5.1 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-related mortality, thus making more patients candidates for potentially curative therapy.

Therefore the research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102)

13.5.2 For adults participating as stem cell donors

Normal haploidentical relatives will be co-enrolled into this study as stem cell donors. Neither adult nor child donors are considered research subjects because they undergo standard of care donation procedures. As the stem cell collection aspect of this protocol is not investigational, the risks of the stem cell collection procedure would not be considered risks of the research for the adult donors. Therefore, participation as a stem cell donor is considered exempt from the criteria set forth in 45 CFR 46, Subpart D.

13.5.3 For pediatric subjects participating as stem cell donors

As the stem cell collection procedure is not considered part of the research for the pediatric donor participants, the risks of the stem cell collection procedure would not be considered risks of the research for the pediatric donors. Therefore participation as a stem cell donor on this protocol is considered exempt from the criteria set forth in 45 CFR 46, Subpart D.

13.5.4 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.

Therefore, participation of pediatric donors in laboratory research on this protocol involves not greater than minimal risk (45 CFR 46.404).

13.6 Informed consent

The investigational nature and research objectives of this trial, the procedure and its attendant risks and discomforts, and alternative forms of therapy will be carefully explained to the subject, and a signed informed consent document will be obtained prior to entry onto this study. The principal investigator or an associate investigator will lead this discussion and may obtain written informed consent. Informed consent will be

obtained using forms approved by the Institutional Review Board (IRB) in accordance with good clinical practice for research involving human subjects. Each patient will be informed of the right of the patient to withdraw from the trial at any time without prejudice. After this explanation and before entering the trial, the patient will voluntarily sign and date an informed consent.

If the donor 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. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent.

Waiver of Consent for Minors when they reach the age of majority

For minors who were initially enrolled in this study we request a waiver of informed consent when they reach the age of majority consistent with 45CFR46.116 (d), specifically:

(d) An IRB may approve a consent procedure which does not include, or which alters, some or all of the elements of informed consent set forth in this section, or waive the requirements to obtain informed consent provided the IRB finds and documents that:

1) The research involves no more than minimal risk to the subjects; 2) The waiver or alteration will not adversely affect rights and welfare of subjects; 3) The research could not practicably be carried out without the waiver or alteration; 4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

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.

14.0 DATA AND SAFETY MONITORING

14.1 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. Accrual and safety data will be reviewed annually by the IRB. Prior to implementation of this study, the protocol and the proposed patient consent forms will be reviewed and approved by the properly constituted IRB operating according to the 45 CFR 46. 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 or follow up of subjects.

DSMB: The NHLBI Data Safety and Monitoring Board will review the protocol at 6-12 month 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.

Safety Monitoring/Recording/Reporting of Events:

Event Characterization and Reporting to the IRB and Clinical Director (CD)

Approved by HSRAC on September 30, 2013

Date effective: October 28, 2013

All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems, and serious protocol deviations, will be reported to the IRB and Clinical Director as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and Clinical Director as soon as possible but not more than 14 days after the PI first learns of the event. Not serious protocol deviations will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event. In accordance with NHLBI policy, SAEs that do not meet the criteria of Unanticipated Problem (UP) must be reported to the IRB Chair and Clinical Director within 14 days of learning of the event.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

14.2 Adverse Events

Adverse Events (AEs) are defined as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), symptom, or disease which either occurs during the study participation, having been absent at baseline or if present at baseline, appears to worsen.

The Principal Investigator will be responsible for assessing AEs. Information on AEs will be solicited from subjects through questions from study personnel and information volunteered by the subject.

All AEs (serious and non-serious) will be recorded from start of study treatment through final study visit and reported to the IRB at the time of continuing review.

Suspected adverse reaction: Suspected adverse reaction means any adverse event for which there is a reasonable possibility that one of the study drugs caused the adverse event. For the purposes of safety reporting, ‘reasonable possibility’ means there is evidence to suggest a causal relationship between one of the study drugs and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse reaction: An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the package insert or is not listed at the specificity or severity that has been observed; or is not consistent with the risk information described in the protocol or elsewhere in the current application. “Unexpected”, also refers to adverse events or suspected adverse reactions that are mentioned in the package insert as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Adverse events used to evaluate the safety of this protocol regimen will be collected to include any unfavorable and unintended signs, symptoms or diseases which either occurs during the study, having been absent at baseline or if present at baseline appear to worsen. 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>.

Adverse event recording will start after the initial dose of the drug is administered. All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until satisfactory resolution. AEs should be reported up to 30 days following the last dose of study drugs. An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms

- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

14.3 Adverse Events - Recipients

Adverse events used to evaluate the safety of this protocol regimen will be collected to include any unfavorable and unintended signs (including abnormal laboratory findings), symptoms or diseases (i.e. incidence of GVHD, graft rejection, regimen-related toxicities, or infectious complications), which either occur during the study, having been absent at baseline or if present at baseline, appear to worsen and are determined to be possibly, probably or definitely related to this investigational treatment in accordance with the NHLBI hematology guidelines for adverse events reporting.

14.4 Adverse Events - Donors

The following are expected outcomes for the donor that are listed in the protocol and informed consent but will not be reported to the IRB unless they meet the criteria of an SAE:

- Common side effects of G-CSF administration (bone pain, fatigue, arthralgias, headache, insomnia, fever, worsening of pre-existing rashes, increases of alkaline phosphatase, lactate dehydrogenase and/or uric acid levels, elevated blood leukocyte count, or thrombocytopenia)
- Hypotension during apheresis

The following expected outcomes for the donor would not be reported to IRB at each occurrence unless they meet the criteria of an SAE. The PI will incorporate these events into the protocol and consent as appropriate. They will be reported in summary form at the time of continuing review and at termination of the protocol:

- Ischemic chest pain during G-CSF administration
- Splenic enlargement/ infarct
- Cutaneous vasculitis
- Bone pain, muscle aches, or headaches not controlled with non-narcotic analgesics

14.5 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

14.6 Attribution of Adverse Events:

Relationship	Attribution	Description
Unrelated to investigational agent/intervention ¹	Unrelated	The AE <i>is clearly NOT related</i> to the intervention
	Unlikely	The AE <i>is doubtfully related</i> to the intervention
Related to investigational agent/intervention ¹	Possibly	The AE <i>may be related</i> to the intervention
	Probably	The AE <i>is likely related</i> to the intervention
	Definitely	The AE <i>is clearly related</i> to the intervention

NOTE: 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).

14.7 Serious Adverse Events (SAE)

A serious adverse event is defined by federal regulation as any AE that results in any of the following outcomes:

- death
- life threatening adverse event
- any event that requires or prolongs in-patient hospitalization
- any event that results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- any congenital anomaly/birth defect diagnosed in a child of a subject who participated in this study.
- other medically important events that in the opinion of the investigator may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above.

We request a waiver from reporting SAEs to the IRB for subjects who are off treatment due to disease relapse, that are attributable to the subject’s sickle cell disease (e.g. hospitalization for pain crisis) or pre-existing illnesses (e.g. autoimmune disease), unless reaching the threshold of a UP.

Following the completion of 5 years of follow-up care post BMT, only SAE’s that are related to the bone marrow transplant procedure will be recorded and reported to the IRB.

14.8 Reporting of Serious Adverse Events:

PI: All serious adverse events will be reported to:

Courtney Fitzhugh, M.D.
Bldg 10, Room 9N116
Phone: 301-402-6496
Email: fitzhughc@mail.nih.gov

IRB: Serious adverse events will be reported to the IRB using the Serious Adverse Event Reporting Form (see Appendix F)

DSMB: Reports of serious adverse events that are unexpected and thought to be related to the experimental portion of the study will be forwarded immediately to the Data and Safety Monitoring Board (DSMB). All SAEs will be included for review semiannually by the DSMB.

If the serious adverse event is thought to be related to the experimental component of the protocol, accession to the protocol will be stopped until a full discussion with the IRB has been held. If however the severe adverse event involves a recipient, the protocol will continue to accrue donor subjects.

14.9 Unanticipated Problems and Protocol Deviations:

An unanticipated problem is any incident, experience, or outcome that is:

1. unexpected in terms of nature, severity, or frequency in relation to:
 - a) the research risks that are described in the IRB-approved research protocol and informed consent document, Investigator's Brochure or other study documents, and
 - b) the characteristics of the subject population being studied, and
2. related or possibly related to participation in the research, and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (An AE with a serious outcome will be considered increased risk.)

A protocol deviation is any change, divergence, or departure from the study design or procedures of an IRB-approved research protocol.

15.0 STATISTICAL CONSIDERATIONS

We propose to test a novel allogeneic PBSCT regimen using haploidentical family donors, incorporating escalating doses of cyclophosphamide to improve study outcomes, in high risk patients with SCD and β -thalassemia. The primary endpoint is to assess the percentage of patients at 1 year post-transplant with sustained donor type hemoglobin on hemoglobin electrophoresis for patients with sickle cell disease and who are transfusion-independent for patients with thalassemia and who do not have severe acute GVHD (grade 3 and higher), or extensive chronic GVHD. Our sample size is calculated based on the anticipated success rate of 60-80% (regimen failure rate of 20%, with the acceptable upper limit of 40%). These numbers are based on prior studies in very high risk β -thalassemia adult patients undergoing matched unrelated myeloablative or haploidentical transplants^{20,21}. There are no studies available in SCD for comparison.

Sample Size

To test the regimen with escalating doses for reducing the therapy failure, we will apply a sequential design to rapidly discover beneficial dose. We plan to monitor the study most frequently for every 4 accrued patients and stop a cohort as soon as we have evidence of futility. For sequential design of a single group, the sample size was calculated to be 32 patients per cohort with the following parameters: 7 interim looks per cohort, 79.5% power, a regimen failure rate of 20%, and the upper limit of the 95 percent confidence interval (one-sided) not exceeding 40%. The significance level is 0.05. This study involves 1 and possible 2 additional cohorts. The sample size was calculated with EAST 5.2 (Cytel Inc. Cambridge, MA), Lan-Demets spending function was used to determine the stopping boundaries and estimate the sample size adjusting for interim analyses.

Sequential Cohorts (maximum of 32 patients per cohort)

Cohort 1: No cyclophosphamide

Cohort 2: 50 mg/kg cyclophosphamide (Day +3)

Cohort 3: 50 mg/kg cyclophosphamide (Day +3 and +4, 100 mg/kg cumulative dose)

Stopping Rules⁹⁴

Each cohort will be monitored after every 4 accrued patients, and stopped if the regimen failure rate is equal to the stopping boundaries listed in the table below. If the stopping boundaries are reached, the next subject will be advanced to the subsequent cohort, and the stopping boundaries will start over with the next cohort.

Group Monitoring Plan

Number of patients accrued	Number of regimen failures
4	3
8	5
12	6
16	7
20	8
24	10
28	11
32	12

Statistical analysis plan

For each cohort, the regimen failure rate will be calculated and a one-sided binomial test will be used to determine if the upper limit of failure rate is over 40%. The significance levels for 7 interim looks and final analysis will be 0.0097, 0.0110, 0.0123, 0.0134, 0.0143, 0.0151, 0.0158 and 0.0164.

The Kaplan–Meier method will be used to analyze incidence of acute and chronic GVHD, disease-free survival and overall survival, relapse rate and graft, rejection rate, transplant-related mortality. The Log rank test will be used to test the difference among different types of donors.

The change of biomarkers at specific time point will be compared with paired t-test. The repeated measures ANOVA will be used to assess the time course of biomarkers.

16.0 ON STUDY DATE: Date of consent signing

17.0 OFF STUDY CRITERIA

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).

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 receive alternative treatments or will be referred back to his/her referring physician depending on what is considered to be in the best interest of the patient.

18.0 PHARMACEUTICALS

Alemtuzumab (Campath®)

Generic: alemtuzumab

Classification: monoclonal antibody

Action: Monoclonal antibody directed against CD52 antigen, a surface glycoprotein expressed by lymphocytes

Availability: Commercial: Berlex Laboratories

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.

Storage: Stored at 2 to 8 degrees Celsius (36 to 46 degrees Fahrenheit) and protected from direct sunlight. Protect from freezing; discard if frozen.

Stability: Diluted solution for administration can be stored at room temperature (15 to 30 degrees Celsius) or refrigerated, and should be used within 8 hours after dilution; protect solution from light.

Product Description: Injection

Preparation: The drug product should be visually inspected for 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. Contents should be injected into 100mL sterile 0.9% sodium chloride and the bag should be gently inverted to mix the solution. Alemtuzumab should be used within 8 hours after dilution.

Route: Intravenous

Cyclophosphamide (Cytoxan®)

Generic: cyclophosphamide

Classification: alkylating agent

Action: Alkylates and crosslinks DNA

Availability: Commercial

Storage: Stored at room temperature.

Stability: Once reconstituted as directed, solutions of cyclophosphamide are stable for 24 hours at room temperature, or 6 days when refrigerated at 2-8° C.

Product description: Injection

Preparation: Cyclophosphamide powder for injection should be reconstituted with sterile water for injection to yield a concentration of 20 mg/mL as described in the product labeling. Once reconstituted, the prescribed dose will be further diluted in 250 mL of 0.9% sodium chloride injection or 5% dextrose in water for intravenous administration over 60 minutes.

Route: Intravenous

Filgrastim (G-CSF; human recombinant granulocyte colony-stimulating factor)

Generic: human recombinant granulocyte colony-stimulating factor

Classification: glycoprotein

Action: Regulates the production of neutrophils from bone marrow progenitor cells and mobilizes primitive hematopoietic stem cells from the bone marrow into the circulation.

Availability: Commercial

Storage: The intact vials of G-CSF should be stored under refrigeration (2 - 8°C).

Stability: G-CSF in the intact vial is stable for 36 months when stored in a refrigerator at 2 - 8°C. A single brief exposure (up to 7 days) to elevated temperatures (< 37°C) does not affect the stability. G-CSF should not be frozen, and vials which have been frozen should not be used.

Product description: injection

Route: G-CSF may be given subcutaneously or IV over 30 min in a single daily dose.

Plerixafor (AMD3100, Mozobil®)

Generic: plerixafor

Classification: hematopoietic stem cell mobilizer

Action: Reversibly inhibits binding of stromal cell-derived factor-1-alpha (SDF-1 α), expressed on bone marrow stromal cells, to the CXC chemokine receptor 4 (CXCR4), resulting in mobilization of hematopoietic stem and progenitor cells from bone marrow into peripheral blood.

Availability: Commercial

Storage and stability: Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F). [see USP Controlled Room temperature] Each vial of plerixafor injection is intended for single use only. Any unused drug remaining after injection must be discarded.

Production description: Plerixafor injection is a sterile, preservative-free, clear, colorless to pale yellow, isotonic solution for subcutaneous injection. Each mL of the sterile solution contains 20mg of plerixafor. Each single-use vial is filled to deliver 1.2mL of the sterile solution that contains 240 μ g of plerixafor and 5.9mg of sodium chloride in Water for Injection adjusted to a pH of 6.0 to 7.5 with hydrochloric acid and with sodium hydroxide, if required.

Route: subcutaneous injection.

Hydroxyurea (Hydrea, Droxia®)

Generic: hydroxyurea

Supply: commercially available

Classification: antineoplastic agent, antimetabolite

Action: Selectively inhibits ribonucleoside diphosphate reductase, halting the cell cycle at the G1/S phase.

Storage and stability: Oral capsules should be stored at controlled room temperature 15-30°C (59-86°F).

Product description: Hydroxyurea is available for oral use as capsules providing 200 mg, 300 mg, 400 mg and 500 mg of hydroxyurea. The 200 mg, 300 mg, and 500 mg capsules are stocked by the NIH Clinical Center Pharmacy.

Route: oral administration

Sirolimus (Rapamune®)

Generic: sirolimus

Classification: mammalian Target of Rapamycin (mTOR) inhibitor

Action: Inhibits T-lymphocyte activation and proliferation and also inhibits antibody production.

Availability: Commercial

Storage: Tablets should be stored at 20° to 25° C. Cartons should be used to protect blister cards and strips from light. Sirolimus should be dispensed in a tight, light-resistant container.

Stability: Studies on batches of sirolimus, which had been stored at 5° C and 25° C for 18 months in a package simulating the bulk storage package, were performed and did not reveal any significant changes in sirolimus stability.

Product description: Tablet or oral solution

Route: Sirolimus is administered orally as either 1mg tablets or oral solution available in 1mg/ml.

19.0 Center for International Blood and Marrow Transplant Research (CIMBTR)

Subjects will be offered co-enrollment on protocol 07-I-0183: The collection of research samples and data for repository from unrelated hematopoietic stem cell transplantation recipients for the national marrow donor program. If consented to participate on this ancillary study the *National Marrow Donor program (NMDP)* will be forwarded transplant outcome data. 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), anonymized data will be released to the CIBMTR according to Federally mandated policies and procedures.

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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 recipient - 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

TRANSFUSION OF PLATELETS TO RECIPIENTS OF ABO INCOMPATIBLE MARROW

Give donor group or volume reduce

Additional pre- and post transplant monitoring will be performed to monitor donor erythropoiesis and immune hemolysis as described in Appendix D.

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 peritransplant 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. Pretransplant 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. 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 (see Appendix C), is associated with hemolysis due to production of anti-recipient isohemagglutinins by passenger lymphocytes. 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 full 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. As this study requires full donor erythroid chimerism to ensure patient cure, donors with major ABO mismatch will be excluded from the study. Regarding patients with minor ABO incompatibility, immediate complications at the time of infusion can be managed by standard DTM policies for manipulation of the graft to remove plasma (see Appendix C). The appropriate transfusion policy in the peritransplant 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 peritransplant period using a standard operating procedure. As major ABO incompatibility may lead to pure red cell aplasia and therefore absence of erythroid cells which are necessary to cure patients with congenital anemias, those with major ABO incompatibility will be ineligible for the protocol.

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 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 = Patient'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 after 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 $35/Hct \times 30\%$, 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 $(0.35-Hct)(wt)(70 \text{ ml/kg})$.

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. 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 $10 \times 10^6/kg$, and the minimum is $5 \times 10^6/kg$. 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. In pediatric subjects, defined as less than 40 kg, a maximum of 8 blood volumes will be processed per day for up to 1-3 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.

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)
≥ 109 kg	1200 mcg (11.0 or less)

IV. Ex vivo processing of PBPC and lymphocytes

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

For this protocol, there will be no T cell depletion of the PBPC or bone marrow. The PBPC 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, PBPC products will 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 standard operating procedures of the DTM Cell Processing Laboratory. Donors with major ABO mismatch will be ineligible for the protocol.

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

Recipient apheresis procedures

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

Plerixafor will be given at a dose of 240 ug/kg subcutaneously in the evening, followed by apheresis 10 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 10 hours later. 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 plerixafor 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 will 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 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.

II. Recipient stem cell collection

The minimum CD34 dose is 1 x 10⁶/kg, target is >2 x 10⁶/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 and the CD34 cell dose needed. This will 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.