Protocol with Statistical Analysis Plan Cover Page:

Official Title:	Nonmyeloablative allogeneic peripheral blood mobilized hematopoietic precursor cell transplantation for severe congenital anemias including sickle cell disease (SCD) and β-thalassemia
NCT number:	NCT00061568
Document Type:	Protocol with Statistical Analysis Plan (SAP)
Document Date:	October 03, 2023

CLINICAL RESEARCH PROTOCOL

Project #03-H-0170
Drug: Sirolimus/Alemtuzumab
IND: Exempt

Date: 03 OCT 2023

Title: Nonmyeloablative allogeneic peripheral blood mobilized hematopoietic precursor cell transplantation for severe congenital anemias including sickle cell disease (SCD) 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®), donor apheresis

Principal Investigator: John F. Tisdale MD, CMTB, NHLBI

Subjects of study:	**Number**	**Sex**	**Age range**
Patients:	60-75	either	≥4
Donors	60-75	either	Age range 2 to 80 years

(accrual will stop after at least 50 successfully transplanted and 15 donors returning for 1 yr. post-transplant testing)

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. Preliminary data have shown a high rate of complete donor engraftment with a relatively low toxicity profile. The decreased risk of transplant-related complications associated with nonmyeloablative transplants expands eligibility to patients with nonmalignant hematological disorders curable by allogeneic transplantation; however, significant toxicity with current regimens persists including severe graft versus host disease (GVHD) leading to significant morbidity and mortality. Moreover, mixed chimerism has been shown to be sufficient to induce clinical remissions in children with nonmalignant hematologic disorders undergoing conventional allogeneic transplantation. Therefore, newer regimens need to be developed that are more applicable to patients with non-malignant disorders in whom no graft vs. leukemia effect is needed, and where mixed chimerism is sufficient for disease amelioration.

In this protocol, we propose transplantation in patients with severe beta-globin disorders including SCD and β- thalassemia, considered at high risk for complications from or ineligible for standard BMT, with allogeneic PBSCs from an HLA identical sibling 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 low dose radiation, Alemtuzumab (Campath®) and Sirolimus (Rapamune®) 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 (filgrastim, G-CSF) mobilized PBSCs will be used to establish hematopoietic and lymphoid reconstitution.

The primary endpoint of this study is treatment success at one year, defined as full donor type hemoglobin on hemoglobin electrophoresis for patients with SCD and transfusion-independence for patients with β-thalassemia. Other end points 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.

TABLE OF CONTENTS

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

1.0 OBJECTIVES

1.1 To evaluate the safety, efficacy, and toxicity of a low intensity nonmyeloablative preparative regimen followed by an allogeneic granulocyte colony-stimulating factor (filgrastim, G-CSF) mobilized peripheral blood stem cell transplant in a population of patients with severe congenital anemias including SCD and β-thalassemia, at increased risk for complications with or ineligible for standard myeloablative allo-transplantation.

1.2 To examine the level of chimerism required to maintain both graft survival as well as hematologic normalcy.

1.3 To determine the incidence and severity of acute and chronic graft versus host disease (GVHD) using a novel nonmyeloablative conditioning regimen

1.4 To determine the rate of graft rejection using a novel nonmyeloablative conditioning regimen

1.5 To determine disease-free survival, overall survival, relapse, transplant-related mortality, and death from all causes using a novel nonmyeloablative conditioning regimen.

1.6 To evaluate the longitudinal effects of a novel nonmyeloablative conditioning regimen on neuropsychological functioning and quality of life (see Addendum B)

2.0 BACKGROUND

2.1 Introduction

Allogeneic bone marrow (and peripheral blood) transplantation (BMT) is the only available cure for SCD and β- thalassemia, but has been infrequently pursued due to its associated complications. 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, only 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, and sirolimus in patients with SCD or β- thalassemia.

2.2 Sickle Cell Disease

2.2.1 Pathophysiology

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, and neurologic events, as well as sudden death as the most serious consequences of this disease.[2] More recently, sickle hepatopathy and iron overload have been discovered to increase mortality in patients with SCD, as patients with ferritin $\geq$1000 ug/L or direct bilirubin >0.4mg/dL led to significantly decreased survival as compared to patients with ferritin <1000ug/L and direct bilirubin <0.4 mg/dL.[3]

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 effect 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 and chelation alone, but do not include the impact on quality of life of those with the disease.[2,4] An overlapping symptom complex also occurs in patients with the double heterozygous forms of sickle disease, sickle-SC, and sickle β-thal^0 disease, and in fact these patients cannot be differentiated clinically but only by means of a laboratory test.

2.2.2 Treatment options

Chronic transfusion therapy decreases the incidence of stroke in pediatric patients with abnormal trans-cranial Doppler velocities (internal carotid or middle cerebral artery velocity $\geq$200 cm/s).[5] While transfusions can prevent further neurologic events in patients at risk, iron overload is common, resulting in significant end-organ toxicity. Currently, stroke treatment in SCD has remained mostly with exchange transfusions, and less with hydroxyurea.[6,7,8]

Hydroxyurea results in a significant reduction in the number of painful crises per year and a decreased frequency of acute chest syndrome,[9] and has become the treatment of choice for the majority of individuals with SCD who lack a suitable matched sibling donor. Unfortunately, neither of these approaches is curative, nor do they appear to reverse established end-organ damage.

Several important interventions have led to an improvement in the overall life expectancy of patients with SCD, most notable among these are the use of pneumococcal vaccines and the prophylactic use of penicillin during childhood. Hydroxyurea has also been suggested to improve survival in patients with SCD. However, life expectancy remains significantly shortened compared to the national average with that of an affected male being 47 years versus the national average of 72.[10,11] 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 3764 patients, 18 percent of the patients died with overt organ failure, and early mortality was highest among patients with symptomatic disease, but another 33 percent who appeared to be clinically free of organ failure died during an acute sickle crisis.[2]

The only established cure for patients with SCD is allogeneic bone marrow transplantation; however, the procedure has only been applied in children.[12,13,14,15] In adults, the higher burden of accumulated end-organ damage would be expected to result in higher transplant associated mortality and morbidity, beyond that reported in children, including seizures and intracranial hemorrhages.[16,17] Several recent publications continue to show that adults with SCD have ongoing organ injury and have premature death.[18,19] As a result, this method has 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.

2.3 β-Thalassemia

2.3.1 *Pathophysiology*

Thalassemia is the most common genetic disorder worldwide[20] and is a result of either a defective or absent synthesis of one or more of the globin chain subunits of the hemoglobin tetramer. Inadequate accumulation of the globin subunit results in hypochromia and microcytosis while the unbalanced accumulation of 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 both 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.

2.3.2 Treatment options

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, (less so than with SCD), 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, the estimated probability of being alive at 20 years of age remains low at 67%[21] 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.[22] 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.

Allo-immunization 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.[23,24,25] There are newer oral chelators, deferasirox (Exjade and Jadenu),[26,27] and deferiprone [28,29,30] with each having their own side effect profile that requires periodic monitoring. 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 as in other disorders, BMT is not risk free. 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 or 1, 2 or all three of the factors, respectively. Results are best in Class I children who undergo transplantation early, with a DFS of 90-93% and TRM of 3-4%.[31] 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) are similar at 65 and 62% disease free and overall survival respectively.[32] Adults have a higher rejection rate and also a higher incidence of GVHD making it necessary to devise better conditioning regimens. 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.[33,34] Although there are newer iron chelators available, cost/access to medication and compliance continue to be the main issues in suboptimal chelation. In the present day, the morbidity associated with iron overload remains, and extends into transplant related outcomes. The Pesaro classification is still being used currently.[35,36,37]

3.0 SCIENTIFIC AND CLINICAL JUSTIFICATION

In disorders such as hematologic malignancies, the curative effect of bone marrow transplantation has been ascribed to the use of myeloablative chemo-radiotherapy and the antileukemic effect of the transplant (the graft-versus-leukemia (GVL) effect).[38] The assumption that the intensive myeloablative preparative regimen is essential for the cure of the malignancy went unchallenged until the demonstration by Kolb et al, (subsequently confirmed by numerous investigators), that donor lymphocytes alone exert a powerful antileukemic effect in the context of patients relapsing with myeloid leukemias after BMT.[39,40,41] This observation has important implications. First, it may be possible to cure some hematologic malignancies with preparative regimens of lower intensity, designed to immunosuppress the recipient to allow lymphocyte and stem cell engraftment without major cytoreduction of the malignancy by myeloablation. Second, such low-intensity preparative regimens appear to have decreased toxicity and may make transplantation appropriate in patients where procedural mortality is usually prohibitive, including patients with more indolent hematologic diseases such as those with severe congenital anemias, as well as patients with co-morbid diseases and older patients. Unlike patients who undergo allogeneic peripheral blood stem cell (PBSC) transplantation for malignant indications, patients with non-malignant disorders such as SCD or β-thalassemia, do not require full and/or rapid donor engraftment for cure of the disease.[42,43,44] While it is generally accepted that graft versus host disease (GVHD) is less severe in patients conditioned with low intensity preparative regimens,[45] graft rejection is preferable to the development of lethal GVHD in the setting of severe congenital anemias.

Several groups, including our own, have begun to investigate this approach to improve the applicability and outcome following allogeneic BMT, and preliminary results were encouraging.[46,47,48] The first so called “mini-transplants” were performed by simply lowering the doses of standard agents; however, these doses were still sufficiently toxic to incur prolonged cytopenias and patients continued to experience significant regimen-related toxicities. Our own experience at the NIH using a nonmyeloablative combination of fludarabine and cyclophosphamide has included over 100 patients with engraftment seen in the majority,[49,50] and extension to patients previously excluded from allogeneic transplant trials such as those infected with the human immunodeficiency virus has been proven feasible.[51] However, GVHD remains a significant problem, occurs at a rate not different from that observed with conventional allogeneic PBSC transplantation, and continues to be the principle cause of death.

In attempts to decrease these toxicities, and to develop immunosuppressive as opposed to myeloablative regimens, many centers have now been exploring different combinations of regimens including the use of low dose radiation. Low dose radiation alone has been shown to have anti-leukemic properties as demonstrated by Shulman et al. where 200 cGy was adequate for inducing remission, albeit short lived, in refractory patients ineligible for other standard treatments.[52] Doses of 100 to 500 cGy have been shown to have very little toxicity in both murine and rhesus transplant models.[53,54,55,56] Even patients with Fanconi anemia who would be considered high risk for radiation induced toxicities, tolerate moderate dose (500 cGy) radiation based regimens.[57] While regarded as a method designed for the creation of “space” within the marrow in bone marrow transplantation (BMT) regimens, radiation has long been the basis of the establishment of

immunosuppressive protocols for both BMT and solid organ transplantation in animal models.[58,59] There are also data in canines demonstrating stable mixed chimerism with the use of 200cGy total body irradiation alone, followed by GVHD prophylaxis with cyclosporine (CsA) and mycophenolate mofetil (MMF).[60] Initial human trials using this regimen of 200 cGy with MMF and CsA were encouraging with approximately 20 percent graft failure*;* and more recent data using fludarabine, an immunosuppressive but nonmyeloablative agent, in addition to 200cGy have shown similar results in 44 patients transplanted to date.[61]

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 total body irradiation as the basis for such an approach. Patients with SCD and β-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 200 cGy used in prior studies to 300 cGy may increase both the degree of myelosuppression and immunosuppression without significantly altering the side effect profile. Moreover, drugs such as anti-thymocyte 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.

Use of alemtuzumab

Further 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,[62,63,64,65] T and B cell malignancies[66] treatment of solid organ rejection,[67,68,69] and has been approved for use in chronic lymphocytic leukemia, a B cell malignancy, as a result of its profound immunosuppressive properties.[70,71]

More recently, alemtuzumab has been used prospectively to prevent graft rejection in human allotransplantation.[72,73,74,75] 31 patients have been transplanted using 20 mg of Alemtuzumab on Day 0 and 1 of transplantation in combination with half dose cyclosporine, which has been shown to be ineffective when used alone. At the 21 months follow-up, only two grafts were lost to rejection.[69] Further, data suggest that the use of alemtuzumab, as compared to fludarabine, reduces the risk of GVHD, even in the unrelated donor setting.[76] In one study of 44 patients, including 8 patients receiving unmanipulated marrow from matched unrelated donors who would therefore be at very high risk for developing GVHD, only 2 patients had acute GVHD, both of which were grade 2. Only 1 patient developed chronic GVHD.[72] Follow up to this study has included a further 39 patients undergoing unrelated bone marrow transplantation (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 GVHD and none developing Grade IV.[76] In another study of 12 high risk patients undergoing haplo-identical BMT, one patient experienced Grade II and one patient developed Grade III GVHD; none developed Grade IV GVHD.[75] 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.

Use of sirolimus

Finally, to improve the odds for graft acceptance without GVHD, we will employ sirolimus, a novel immunosuppressive agent, instead of the conventional agent, CysA, based on sirolimus' distinct properties as a tolerogenic agent.[77,78] Sirolimus is an immunophilin drug similar to CysA; however, unlike CysA which inhibits the phosphatase calcineurin and therefore prevents the production of interleukin 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 interleukin-2. We demonstrated that cells cultured and stimulated in the presence of sirolimus became anergic, while cells cultured in the presence of CysA did not.[77]

To confirm these results in an in vivo model, we sought to compare the use of sirolimus to the standard post transplant immunosuppressant, CysA, in a nonmyeloablative setting. We therefore transplanted F1 hybrid mice into their parental strain – a model designed to promote graft rejection-using only low dose radiation and sirolimus. C57Bl6 recipient mice were injected with either sirolimus at 5 mg/kg or CysA, beginning one day before cell infusion.[78] On day 0, they were given 300 cGy TBI followed by 100 x 10^6 splenocytes obtained from G-CSF mobilized BalbC/C57Bl6 hybrid donors.

The immunosuppressants were continued for four weeks beyond cell infusion, and chimerism was monitored using flow cytometric analysis. The mice transplanted with sirolimus showed moderate donor engraftment by week 2 which subsequently increased over time even after the discontinuation of the sirolimus, reaching levels of greater than 75%. In contrast, those treated with CysA lost their engraftment even while receiving the CysA. Application of this regimen to transgenic mice expressing human sickle hemoglobin exclusively produced correction of anemia and red cell sickling even at very low levels of donor chimerism.[78]

In a series of experiments performed by Hale et al., sirolimus also proved superior to CysA 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 CysA when added to ALG and BM in a murine model. Further, mice receiving sirolimus accepted a second same donor skin graft, but rejected third party grafts, demonstrating the development of tolerance.[79,80,81] Sirolimus has also been employed in the bone marrow transplant setting, with matched and mismatched sibling and unrelated donors.[82] 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, respectively.[82] 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 patients.[83]

Sirolimus also has less renal toxicity as compared to CysA. A randomized trial comparing the addition of sirolimus at either 2 or 5mg vs. azathioprine to CysA and prednisone for prophylaxis of renal allograft rejection showed a significantly lower rate of acute rejection episodes at both doses of the sirolimus as compared to azathioprine (16.9% and 12.0% vs. 29.8%).[84] In a similar study, comparing sirolimus vs. CysA 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 creatinine in the sirolimus group.[85] 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 CysA.

Our group is currently using a combination of 300 cGy TBI, alemtuzumab, and sirolimus to transplant high risk patients with SCD and, β-thalassemia who are 16 years of age and older. 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.[86] Additionally, for patients with SCD, higher platelet counts are maintained.[87,88,89] 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%.[12]

Given the lower toxicities seen with nonmyeloablative regimens, the possibility of cure with mixed chimerisms, 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 homozygous SCD, sickle SC, sickle β–thal^0, and β-thalassemia, who would normally be offered a standard transplant but are considered at a higher risk due to their age (i.e. 18 or older) or other co-morbidities is justified. We will use a combination of low dose irradiation, Alemtuzumab, and sirolimus as our conditioning regimen. This combination is designed for slow engraftment and tolerance induction resulting in partial to full chimerism with reduced rates of graft versus host disease. To optimize our approach, we will use peripheral blood stem cells from sibling related donors, as preliminary studies, including our own experience with PBPC (peripheral blood progenitor cell) transplants and low intensity preparative regimens, indicate that transplant-related mortality and severe acute GVHD is uncommon when matched family donors are used.[45]

If there is the same or even less toxicity but successful engraftment in these higher risk patients as we anticipate, this technique could be offered to all those who have histocompatible donors regardless of risk factors as the risk benefit ratio would clearly be in favor of this potential cure. As a secondary endpoint, we will also examine the levels of donor myeloid chimerism sufficient to provide full erythroid chimerism, i.e. the level of overall engraftment needed to achieve disease amelioration for SCD, and β-thalassemia. This information is also necessary for future applications of gene replacement therapy which is the ultimate goal for the cure of SCD and β-thalassemia.

Continuation of this protocol

Unfortunately, the treatments for these beta globin disorders have not changed substantially in the last decade and allo-HSCT remains the most accessible curative option. While gene transfer and gene editing approaches are beginning to show promise, the availability to these clinical trials are limited.

Most of the efforts in allo-HSCT in SCD and β-thalassemia continue to be in children, thus this is the major reason to continue accrual. We have had anecdotal reports from Israel, Saudi Arabia, London, Buffalo, and Alberta, who used this same approach and demonstrated the efficacy of this approach (although unpublished). Recently the transplant group at the University of Illinois in Chicago published their results using our approach,[90] and confirmed safety and efficacy in 13 patients. Interestingly, ABO incompatibility was not an exclusion in their protocol, and two patients with major ABO incompatibility were among the 12 successfully engrafted. Thus, we hope to continue enrollment, maximizing safety while gaining knowledge, particularly for the secondary endpoints.

4.0 STUDY DESIGN

The current overall protocol success rate is 85-90% in individuals ≥16 years old. While the accrual in younger children is expected to be low, it is still of research interest to test in pediatric population to see if fertility preservation can be achieved. This protocol also serves to capture children who are ineligible for standard myeloablative transplant because they met exclusion criteria with poor lung, kidney, or liver function. Similarly, with β-thalassemia, some with Pesaro Class 2 and all with Class 3 are excluded from myeloablative transplant. Thus this study will continue to be open for children with SCD or β-thalassemia.

We also plan to continue accruing different genotype/phenotypes of SCD, as both are β-globin disorders and continue to be reported together in the current literature. Having separate studies for each of the sickle subtypes is not standard practice in transplant centers: similarly, in all AML are included in one treatment or transplant study, regardless of 7 subtypes; or treating aplastic anemia, regardless if it is hepatitis associated, drug induced, or from telomere disease.

A human lymphocyte antigen (HLA)-matched sibling donor will receive filgrastim (G-CSF) 10 to16 µg/kg/d subcutaneously or intravenously for up to 6 days with apheresis collections of PBPC on day 5 (and day 6 if required) as a standard procedure under a separate NHLBI protocol (94H0010 or 20H0099). The product will be collected and frozen at the NIH Department of Transfusion Medicine (DTM) 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 300 cGy TBI given as a single dose on day –2. Sirolimus (Rapamune®) at a dose of 5mg/day in patients >16 years of age or 1mg/m2/dose to a maximum daily dose of 5mg in patients <16 years of age to maintain trough levels between 5-15 ng/ml will be started on day –1. The PBPC graft targeted to deliver ≥5 x 10^6 $CD34^+$ cells/kg will be infused on day 0.

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, GVM effect) 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 SCD and β-thalassemia, who have risk factors for high mortality and morbidity related to their disease (see inclusion criteria in section 5.1.1 and 5.1.2).

3) 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, Alemtuzumab (Campath®), and sirolimus.

4) Peripheral blood hematopoietic progenitor cell (PBPC) transplant: An unmanipulated peripheral blood stem cell collection from a filgrastim (G-CSF) stimulated HLA-matched donor should improve the chance of engraftment because of the high stem cell dose (≥5 x 10^6/kg CD34+ cells) and the presence of donor lymphocytes. To reduce the risk of GVHD, patients will receive sirolimus before and after the transplant. The sirolimus will be tapered as necessary to minimize any graft versus host disease while still maintaining adequate chimerism.

5.0 ELIGIBILITY ASSESSMENT

5.1 Inclusion criteria – recipients (must fulfill <u>one disease</u> category in 5.1.1 and <u>all</u> of 5.1.2)

5.1.1 Disease specific:

5.1.1.1 Patients with sickle cell disease at high risk for disease related morbidity or mortality, defined by having irreversible end-organ damage (A, B, C, D, or E) or potentially reversible complication(s) not ameliorated by hydroxyurea (F):

A. Stroke defined as a clinically significant neurologic event that is accompanied by an infarct on cerebral MRI OR an abnormal trans-cranial Doppler examination (≥200 m/s); OR
B. Sickle cell related renal insufficiency defined by a creatinine level ≥1.5 times the upper limit of normal (see table below) and kidney biopsy consistent with sickle cell nephropathy OR nephrotic syndrome OR creatinine clearance <60mL/min/1.73m^2 for patients ≤ 16 years of age or < 50mL/min for patients ≥16 years of age OR requiring peritoneal or hemodialysis. .[91,92,93] OR

Age (Years)	Upper limit of normal serum creatinine (mg/dl)
≤ 5	0.8
5 < age ≤ 10	1.0
10 < age ≤ 15	1.2
> 15	1.3

C. Tricuspid regurgitant jet velocity (TRV) of ≥2.5 m/s in patients ≥18 years of age at least 3 weeks after a vaso-occlusive crisis;[94,95] OR
D. Recurrent tricorporal priapism defined as at least two episodes of an erection lasting ≥4 hours involving the corpora cavernosa and corpus spongiosa;[96] OR
E. Sickle hepatopathy defined as EITHER ferritin >1000mcg/L OR direct bilirubin >0.4 mg/dL at baseline in patients ≥ 18 years of age; OR
F. 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[97]	More than 1 hospital admission per year while on maximal tolerated dose of hydroxyurea*[2]
Acute chest syndrome (ACS)	2 prior ACS while > 3 years of age and adequately treated for asthma	any ACS while on hydroxyurea*[98]
Osteonecrosis of 2 or more joints	And significantly affecting their quality of life by Karnofsky score 50-60 (see Appendix A)	And on hydroxyurea* where total hemoglobin increase less than 1 g/dL or fetal hemoglobin increases < 2.5 times the baseline level
Red cell alloimmunization	Transfusion-dependent	Total hemoglobin increase < 1 g/dL while on hydroxyurea*

*hydroxyurea at maximum tolerated dose

5.1.1.2 Patients with β-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[31])
- hepatomegaly of greater than 2 cm below the costochondral margin

5.1.2 Non-disease specific:

5.1.2.1 Ages ≥4 years
5.1.2.2 6/6 HLA matched family donor available
5.1.2.3 Ability to comprehend and willing to sign an informed consent, assent obtained from minors
5.1.2.4 Negative serum β-HCG, when applicable
5.1.2.5 Pediatric patients < 16 years of age must decline myeloablative bone marrow transplantation

5.2 **Exclusion criteria –recipient (**any of the following would exclude the subject from participating)
5.2.1 ECOG performance status of 3 or more, or Lanksy performance status of <40 (See Appendix A).
5.2.2 Diffusion capacity of carbon monoxide (DLCO) <35% predicted (corrected for hemoglobin and alveolar volume).
5.2.3 Baseline oxygen saturation of <85% or PaO2 <70
5.2.4 Left ventricular ejection fraction: <35% estimated by ECHO.
5.2.5 Transaminases > 5x upper limit of normal for age
5.2.6 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
5.2.7 Major anticipated illness or organ failure incompatible with survival from PBSC transplant.
5.2.8 Pregnant or lactating
5.2.9 Major ABO mismatch (See Appendix B and C)

5.3 Inclusion criteria - Donor
Donor deemed suitable and eligible, and willing to donate per clinical evaluations, who are additionally willing to donate blood for research and undergo a neuropsychological test. Donors will be evaluated in accordance with existing Standard NIH Policies and Procedures for determination of eligibility and suitability for clinical donation under a separate NHLBI protocol. Note that participation in this study is offered to all donors, but is not required for a donor to make a stem cell donation, so it is possible that not all donors will enroll onto this study.

5.4 Exclusion criteria- donor:
None

6.0 CLINICAL EVALUATION OF THE RECIPIENT

Samples will be ordered and tracked through the CRIS Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

6.1 Pre-study evaluation
6.1.1 History, physical examination to establish baseline symptoms, height and weight
6.1.2 ECOG or Lanksy performance status
6.1.3 Low resolution molecular HLA- A, B, DR typing of patient and as many family members as possible to confirm complete matching of the donor, HLA confirmatory testing of recipient once donor is identified

6.1.4 Hemoglobin electrophoresis and/or flow cytometric analysis of hemoglobin A, F and SS, SC or Sβthal^0 as appropriate (for both patient and donor).
6.1.5 Antibody screen for HBV, HCV, HIV, HTLV-I/II, CMV, EBV, toxoplasma, syphilis. Consider PPD test for patients from areas where tuberculosis is prevalent.
6.1.6 Coagulation screen, CBC with differential
6.1.7 comprehensive metabolic panel
6.1.8 Extended red cell phenotyping, lymphocyte phenotyping TBNK, d-dimer
6.1.9 HLA antibody screen if indicated
6.1.10 β-HCG pregnancy test for females of childbearing potential
6.1.11 CXR, pulmonary function testing (in patients ≥ 5 years of age): vital capacity FEV-1, DLCO
6.1.12 Sinus, chest, abdomen, and pelvis CT scans as clinically indicated
6.1.13 Brain MRI for appropriate patients
6.1.14 CNS Doppler flow analysis (to be performed at Children's National Medical Center Department of Radiology) for appropriate patients
6.1.15 Cardiac function: EKG, trans-thoracic ECHO, 24 hour holter
6.1.16 Troponin T
6.1.17 Nutritional assessment.
6.1.18 Dental review
6.1.19 Social worker interview
6.1.20 Ophthalmology consultation
6.1.21 Endocrine consultation and testing which will include bone density 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 at least 12 years of age, testosterone, luteinizing hormone level (LH), and follicle stimulating hormone (FSH), and in female patients at least 10 years of age LH, FSH, anti-mullerian hormone, and estradiol
6.1.22 Interview with members of primary care team and visit to unit.
6.1.23 Consent/Assent documents signed.
6.1.24 Complete lipid profile with triglycerides
6.1.25 Pulmonary hypertension evaluation by tricuspid regurgitant velocity (TRV) analysis using standard trans-thoracic echocardiography
6.1.26 Bone marrow aspirate and/or biopsy to rule out other hematologic disorders
6.1.27 Quality of life assessment (see Addendum A)
6.1.28 Neuropsychologic testing (see Addendum A)
6.1.29 Reproductive health assessment (Addendum B)
6.1.30 To evaluate sickle cell nephropathy in patients with SCD: cystatin C, c reactive protein (CRP), and the following urine studies: urinalysis, phosphate, uric acid, protein to creatinine ratio, albumin to creatinine ratio, and 24 hour urine for creatinine, protein, microalbumin, phosphate, uric acid, creatinine clearance, and urine protein electrophoresis (UPEP)
6.1.30 Evaluation of splenic function via abdominal ultrasound and/or quantitative Howell-Jolly body analysis in appropriate patients
6.1.31 Hepatology, Transfusion Medicine, Pulmonary, and other consult as indicated
6.1.32 STR profile

6.2 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, temperature, pulse, blood pressure, respiratory rate, weight

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

Weekly: CMV/EBV surveillance

Every two weeks: serum cholesterol, triglycerides

After the last dose Campath: Troponin T, ECHO, 24 hour holter as clinically indicated

6.3 Follow up to day 100: outpatient

Once a week up to 30 days, blood samples may be collected to test for alemtuzumab levels.

At least weekly up to 30 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, temperature, pulse, blood pressure, respiratory rate, weight; CMV/EBV. A complete physical exam will be repeated at each visit.

Monthly (+/- 7 days): Serum cholesterol, triglycerides, pregnancy test
Peripheral blood will be drawn on days, +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.

100 Days (+/- 1 week) after the last dose Campath: ECHO (if clinically indicated), Troponin (if clinically indicated), d-dimer, and in patients with SCD Cystatin C, CRP, and the following urine studies: urinalysis, phosphate, uric acid, protein to creatinine ratio, and albumin to creatinine ratio

Days 100 (+/- 7 days): Neuropsychologic testing, Quality of life testing (See Addendum B)

6.4 Beyond day 100

At 6, 12, (optional) 18, 24 (+/- 1 month), 36, 48, and 60 months (+/- 6 month): CBC, Hb S and Hb F levels, comprehensive metabolic panel. Serum cholesterol, triglycerides, echo, and pulmonary function tests if clinically indicated.

At 6, 12, and (optional) 18 months (+/- 1 month): Blood will be drawn for preparation of plasma and lymphocytes for in vitro studies. Based on the results, studies may be repeated every 6 to 12 months thereafter

At 6 and 12 months (+/-1 month): Lymphocyte subpopulations and immunoglobulin levels will be checked, and the tests will be performed yearly until normalization.

At 6 months in patients with SCD (when possible): Cystatin C, CRP, and the following urine studies: urinalysis, phosphate, uric acid, protein to creatinine ratio, and albumin to creatinine ratio

At 12 months in patients with SCD (when possible): Cystatin C, CRP, and the following urine studies: urinalysis, phosphate, uric acid, protein to creatinine ratio, albumin to creatinine ratio, Bone marrow aspirate and/or biopsy samples will be obtained at day 100 post-transplant and/or when full donor erythroid chimerism (patient may refuse) is attained or if clinically indicated.

At 12, 24 (+/- 1 month), 36, 48, and 60 months (+/- 6 months): bone density scan (if indicated), 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 at least 12 years of age, testosterone level, luteinizing hormone level (LH), and follicle stimulating hormone level (FSH), and in female patients at least 10 years of age LH, FSH, anti-mullerian hormone level, and estradiol level. All these endocrine labs may be repeated as clinically indicated.

At 12, 24 (+/- 1 month), 36, 48, and 60 months (+/- 6 month): CNS Doppler flow for appropriate patients (to be performed at Children's National Medical Center Department of Radiology)

At 12, and 24 months (+/-1 month): Quality of life testing, neuropsychologic testing, brain MRI (see Addendum B)

At 12 and 24 months (+/-1 month): Reproductive health assessment (see Addendum C)

Splenic function will be measured at 2 years post-transplant in appropriate patients.

At 36, 48, and 60 months' post-transplant, there is a window of +/- 6 months for their annual visit. After 5 years, follow-up visits are not mandatory, but yearly communication with the patient and the referring physician may continue.

After 60 months (5 years post-transplant), follow-up visits are not mandatory, but yearly communications with the patient and the referring physician will continue to monitor adverse events that may be related to the transplant procedure.

7.0 TREATMENT PLAN FOR THE RECIPENT

Patients with fever or suspected minor infection should await resolution of symptoms before starting the conditioning regimen. Iron chelation must be discontinued $\geq$ 48 hours before initiating the conditioning regimen. Hydroxyurea must be discontinued one day prior to initiating the conditioning regimen

7.1 Central venous line placement

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

7.2 Infection prophylaxis

Penicillin VK 250 mg PO BID from day 0 until day 100 until pneumococcal vaccination complete post-transplant. Prophylaxis and treatment of infections will otherwise be administered according to BMT consortium guidelines.

If patients develop significant transaminitis with ALT and/or AST $\geq$ 2x their baseline value, nystatin may be substituted for fluconazole as anti-fungal prophylaxis.

Donors will be offered the influenza vaccination at the NIH Clinical Center as seasonally indicated pre-transplant (see section 10.5). Patients will be offered influenza vaccination, as seasonally indicated, when they are at least 6 months post-transplant per CDC HSCT guidelines.

7.3 Fever regimen (See BMT Consortium Guidelines)

7.4 Exchange transfusion (See Appendix C)

Prior to the transplant, those patients with SCD who are not routinely (exchange) transfused will undergo an exchange transfusion per DTM procedure for a goal HbS near 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.

7.5 Bleeding prophylaxis

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

7.6 Preparative regimen (see Appendix D)

7.7 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 as a standard procedure under a separate NHLBI protocol (94H0010 or 20H0099) and cryo-preserved at DTM until the day of transplant.

7.8 GVHD prophylaxis

Sirolimus (Rapamune®) will be started on day -1 with a loading dose of 5mg PO q4h for three doses in patients $\geq$16 years of age and 1mg/m2/dose PO q4h for three doses in patients <16 years of age (maximum 5mg per dose), then continued at 5 mg/day in patients $\geq$ 16 years of age and 1mg/m2/dose daily in patients <16 years of age (maximum 5mg per dose) with goal trough levels being 5-15 ng/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.

If at least one year post-transplant the recipient displays >50% lymphoid donor chimerism, the sirolimus dose can be tapered. Donor/recipient chimerism levels will be checked periodically during and after the taper. If lymphoid and/or myeloid donor chimerism levels decrease by $\geq$ 20%, the sirolimus and/or other immunosuppression may be restarted clinically indicated.

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

7.9 Transfusion support (See Appendix B)

Filtered and irradiated blood products will be used in all patients, regardless of CMV status.

7.10 Nutrition

Parenteral nutrition will be instituted as clinically indicated.

7.11 Hospital discharge

Patient 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

7.12 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 either the principal investigator, lead associate investigator or the attending protocol investigator.

8.0 DONOR CELLS FOR RESEARCH

Evaluation of related donor suitability and eligibility will be done in accordance with existing Standard Policies and Procedures under a separate NHLBI protocol as follows:

8.1 Pre-study consult and evaluation

1. HLA-A, -B, -DR typing of as many family members as necessary
2. Confirm HLA identity of donor with patient
3. History and physical examination
4. Hemoglobin electrophoresis
5. Hepatitis B, C, HIV, HTLV-I/II, CMV antibodies, RPR, West Nile virus, T. cruzi
6. CBC with differential, coagulation screen, comprehensive metabolic panel
7. Extended red cell phenotyping,
8. HLA antibody screening if indicated
9. Fit to donate: Orientation - visit to Department of Transfusion Medicine - inspection of veins to determine the need for a central line for apheresis.
10. Donors less than 18 years of age will be evaluated by a mental health specialist with pediatric expertise or a social worker about their participation as a donor
11. Consent/assent to undergo filgrastim (G-CSF) mobilization.
12. Donors with sickle cell trait will be evaluated with assessment of oxygen saturation by pulse oximetry on days 3, 4, and 5 of the mobilization and have a follow-up visit with physician within 2 weeks after PBPC collection.
13. If a central venous line is required, blood counts and coagulation studies will be performed before line placement

8.2 Blood donation for research

Up to 50 mL of donor blood may be collected prior to cell donation (and prior to GCSF mobilization) for research. The amount of blood that may be drawn from adult donors (i.e., those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period. For pediatric donors, 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.

Donors will undergo standard cell collections under a separate NHLBI protocol (94H0010 or 20H0099).

8.3 Neuropsychological and quality of life testing

Donors will undergo neuropsychologic testing (please see Addendum A)

9.0 MANAGEMENT OF RECIPIENT COMPLICATIONS

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

9.1 CMV reactivation: See BMT Consortium Guidelines

9.2 Acute GVHD: See BMT Consortium Guidelines

9.3 Chronic GVHD:

Sirolimus will be continued with the duration dependent on the presence or absence of GVHD. Prednisone will be dosed according to severity. Change to alternate day steroid along with sirolimus therapy when response is established.

Non-responding patients may be treated with other standard of care therapies (such as cyclosporine, psoralen + ultraviolet-A (PUVA), photopheresis, azathioprine, thalidomide, daclizumab, infliximab, mycophenolate or tacrolimus) at the discretion of the attending physician.

9.4 Graft rejection and falling chimerism (poor engraftment):

This transplant protocol uses a nonmyeloablative preparative regimen. Therefore, autologous recovery is anticipated in patients who fail to engraft. For falling chimerism in the myeloid and/or CD3 compartment, unselected stem cell product ('boost') from the original donor may be used after preconditioning, with Busulfan IV 3.2 mg/kg on days -5, -4, and -3 and Campath IV 0.03mg/kg on day -7, 0.1mg/kg on day -6, and 0.2mg/kg on days -5 and -4 followed by sirolimus or equivalent immunosuppressant as in protocols 14-H-0111 and 15-H-0098. Busulfan dosing may be reduced or substituted with melphalan 100-140mg/m^2 in those with renal or liver dysfunction to minimize organ injury. These medications are widely used at the Clinical Center and other transplant centers, and their side effects are well known. Recipient bone marrow harvest or plerixafor mobilization before stem cell boost is likely not needed, but may be considered if one or more risk factors for pancytopenia post infusion are present (e.g. age >35, low stem cell number of the boost, or low blood counts before the start of the pre-conditioning regimen). Marrow harvest procedure is a common practice among transplant community to ensure a back-up source of cells is available to avoid prolonged period of pancytopenia.

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

9.5 Relapse of disease

Patients with relapse may be treated with standard of care treatment options under a separate NHLBI protocol (94H0010 or 20H0099), with or without stem cell rescue (2nd transplant) or referred back to their primary physician depending on what is considered to be in the best interest of the patient (also see section 11.5.2).

10.0 RESEARCH STUDIES:

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.

Specimen management

Storage: Research specimens 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 investigator, which will serve as a link to the patient's clinical information collected as part of this research protocol. No samples will be provided to investigators outside the branch without IRB notification and an executed MTA. Therefore, confidentiality is protected.

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

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

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

Sharing: Samples and/or medical data may be provided to other investigators at the NIH or at other institutions for studies related to sickle cell disease and immune system function, without any personal identifying information. These samples cannot be traced back to individual subjects by collaborating investigators. These de-identified samples and/or data may be shared with other investigators involved with CIBMTR in collaborative studies to compare HSCT outcomes, such as mixed chimerism, GVHD, infection, changes in organ function, and mortality.

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

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

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

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

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

10.1 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 at 6, 12, 24 months. We will also use gel electrophoresis and/or flow cytometry to assess the amount of normal hemoglobin within the erythroid cells.

10.2 Bone marrow samples

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

10.3 Transthoracic echocardiography

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

10.4 Immune reconstitution

Lymphocyte subpopulations (absolute number of CD3, CD4, CD8, CD16, CD56, and CD19 cells) and immunoglobulin levels (IgG, IgA, and IgM) will be quantified at 6 and 12 months post-transplant (and yearly thereafter until results have normalized). Splenic function will be measured in appropriate patients by Tc99m-labeled radiocolloid scan of the liver and spleen 2 years' post-transplant. This will be compared to the scan from pre-transplant. Liver and spleen scans have only been shown to be reversible in the children with SCD after transplant, thus it is not performed in those 16 and older. The spleen of older adolescents and adults with SCD are atrophic, and likely not to regain function after transplant, thus the liver and spleen scans are not performed in those age 16 and older.

10.5 Assessment of cytokines and lymphocyte functions

Donor serum and lymphocytes will be collected pre-transplant. Patient serum and lymphocytes will be collected pre-transplant and at 6, 12, and 18 months post-transplant. Serum tumor necrosis factor, interferon-gamma, and interleukin-17 may be quantified. Levels may continue to be followed every 6 to 12 months thereafter based on the results during the first 18 months.

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

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

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

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

All of these research tests may be run at the laboratory of Dr. Jonathan Powell at Johns Hopkins Hospital. Samples will be assigned a unique code known only to the principal and associate investigators.

Transplant recipient blood samples may be sent to the Immune Tolerance Network (ITN) run by NIAID for the study of immune function and tolerance in the setting of stem cell transplantation. Samples will be assigned a unique code know only to the principal and associate investigators.

10.6 Dual X-ray Absorptiometry (DEXA) Scan

For adult subjects (age 18 and older), up to 2 DEXA scans (to allow for repeat measurement if there are areas of the bone that need clarification) will be performed at baseline and every 1-2 years post-transplant to evaluate patients for osteopenia and osteoporosis. The frequency will be determined by the severity of their bone disease.

10.7 Neuropsychologic testing: see Addendum B

10.8 Quality of life testing: see Addendum B

11.0 BIOSTATISTICAL CONSIDERATIONS

11.1 Sample size

This study will accrue a range of 60-75 recipients and 60-75 donors, to allow for at least 50 successfully transplant patients and at least 15 donors who complete the 1 year post-transplant neuropsychological testing.

11.2 The parameters to be monitored are:

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 0.5×10^9/l and 1.0×10^9/l).
4) Platelet recovery (days to platelet count of 50×10^9, days to transfusion independence)
5) Red cell recovery (days to transfusion independence)
6) Incidence and severity of acute GVHD
7) Incidence and severity of chronic GVHD
8) Incidence of Graft Rejection
9) Non-hematologic effects attributable to the preparative regimen
10) Transplant related mortality by day 200
11) Disease-free survival and overall survival
12) Hemoglobin F and S, levels in appropriate patients
13) Development of further neurologic disease and assessment of neurocognitive status in appropriate patients
14) Gonadal organ function as reflected by hormone levels and normal menstrual cycles
15) Assessment of immune reconstitution
16) Assessment of cytokines and lymphocyte function
17) Quality of life
18) Neuropsychologic function

11.3 Study endpoints

Primary endpoint
The primary endpoint is treatment success at one year, defined as full donor type hemoglobin on hemoglobin electrophoresis for patients with SCD and transfusion-independence for patients with β-thalassemia.

This trial is designed to estimate treatment success, which is anticipated to be about 80%. The study started with a sample size of 25 and this will allow us to estimate the success of engraftment. For example, if the estimated rate is .80, the 95% confidence interval would be approximately (.64, .96). This would allow us to rule out rate of treatment success of less than .64. If the estimated rate is .70, the 95% confidence interval would be approximately (.52, .88) and we could rule out rate of treatment success below .50. If the lower bound of the 95% confidence interval is raised to 0.7, the number of subjects needed to accrue with respect to success rate is listed below.

Success rate	0.90	0.89	0.88	0.87	0.86	0.85	0.84	0.83	0.82	0.81	0.80	0.79
Number of subjects needed	9	11	13	16	19	22	27	33	40	49	62	79

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, +30, , +60, and +100 by microsatellite analysis of the peripheral blood. More frequent monitoring may be required.
2) Incidence of acute and chronic GVHD or relapse rate. GvHD or relapse together count together toward the combined endpoint for regimen failure (see section 11.4 below).
3) Disease-free survival and overall survival
4) Transplant-related mortality

Exploratory endpoints:
1) Immunologic function post-transplant
2) Quality of life and neuropsychologic function post-transplant (both donors and recipients) (see Addendum B)
3) Effect of transplant on end-organ function (e.g. renal function, see Addendum A)

The accrual ceiling range would allow for about 50 successfully transplanted recipients, assuming a graft rejection rate of about 10-15%. Among the transplanted patients, about 10% of the patients have β-thalassemia, sickle β-thalassemia (+), or sickle β-thalassemia (0) – collectively sickle/thal group, and another 10% have sickle SC disease. These expected accrual figures in the subgroups are small and statistical power for detecting differences in engraftment rates are low for moderate differences, e.g. power is less than 20% for engraftment rates of 90% and 70% in the larger and smaller subgroups, respectively. Thus, when the subgroups' engraftment rates are reported the discussion will explicitly acknowledge the small sample size supporting the observations so interpretations are appropriately cautious.

Additionally, the accrual ceiling range would also allow for at least 15 donors who return for their 1 year post-transplant testing to repeat neuropsychologic testing.

11.4 Stopping rules

For regimen failures: Because this regimen is designed to decrease the risk of severe acute and chronic GVHD in patients with non-life threatening conditions, the study will be stopped and the

design re-evaluated after any death. We will also monitor the study for excessive graft rejection and excessive severe (acute Grade III-IV and chronic extensive) GVHD which we would consider a failure of the regimen. So regimen failure is a composite endpoint, which means graft rejection, acute Grade III-IV GVHD, or chronic extensive GVHD. As adult patients have already been enrolled on our protocol, regimen failures will be monitored separately for pediatric patients.

Regimen failure will be evaluated for each individual during the 12 month period following the start of transplant. We will stop the study if posterior probability that the regimen failure rate is greater than 30% exceeds 0.90. Stopping means that serious consideration will be given to modifying or terminating the protocol. Stopping boundaries are given in the table below. Beta prior distributions are used for these calculations with parameters alpha, beta = 2.1, 4.9 for regimen failure.

Note that one need not evaluate the number of patients in the first column in order to stop. For example, if 4 of the first 4 patients fail to engraft, we will have crossed the boundary given in the first row. For 50 patients, stopping rule would be met if 29 patients failed.

Group Sequential Monitoring Plan

# of patients with 12 month transplant anniversary	Regimen Failures
5	4
10	6
15	8
20	10
25	12
30	13
35	15
40	17
45	18
50	20
55	22
60	24

The monitoring plan was evaluated by simulation. If the true regimen failure rate is .3, we will stop about 10% of the time. True regimen failure rates of .2, .4, and .6 entail stopping about 2%, 34% and 90% of the time respectively. This is acceptable performance for a stopping rule.

For neuropsychologic testing: We propose to stop the study if accrual is such that we cannot meet the target of 15 donor-recipient paired neuropsychology responses within our sample size limit of 75 transplants. For instance, if after the 70th transplant, we have only 9 donor-recipient pairs, we will be unable to obtain data on 15 donor-recipient pairs and further enrollment will be stopped. Our stopping rule is to halt enrollment if:

we reach 56 transplants but data from only 7 donor-recipient pairs are available, or
we reach 66 transplants but data from only 8 donor-recipient pairs are available, or
we reach 70 transplants but data from only 9 donor-recipient pairs are available, or
we reach 71 transplants but data from only 10 donor-recipient pairs are available, or
we reach 72 transplants but data from only 11 donor-recipient pairs are available, or
we reach 73 transplants but data from only 12 donor-recipient pairs are available, or
we reach 74 transplants but data from only 13 donor-recipient pairs are available.

11.5 Off study criteria

11.5.1 *Withdrawal by the patient from the transplant procedure*

Patients and their donors will be given ample opportunity to withdraw from the study prior to admission for transplant. Thereafter, the nature of the procedure does not permit safe withdrawal from the protocol.

The patient and donor have the right at any time to elect not to participate in the research aspects of the protocol (donation of blood and bone marrow for non-routine tests).

11.5.2 *Withdrawal by the physician from experimental protocol*
Patients with disease relapse may be taken off protocol but will continue to be monitored by our institution for a minimum of 6 months post-transplant for possible infectious complications related to the conditioning regimen. The patient will then be consented to the Hematology Branch evaluation and treatment protocol (94-H-0010) or to the Sickle Cell Branch natural history protocol (04-H-0161) for alternative treatments or referred back to his/her referring physician depending on what is considered to be in the best interest of the patient.

12.0 DATA AND SAFETY MONITORING

12.1 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.

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

12.2 Grading of adverse events:

Definitions: Definitions will be in accordance to HRPP Policy 801 "Reporting Research Events" The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTCAE version 4.0. A copy of the criteria can be down-loaded from the CTEP home page at http://ctep.cancer.gov/reporting/ctc.html.

Grading of adverse events:

Grade	Category	Description
1	Mild	Mild; asymptomatic; clinical or diagnostic observations only; intervention not indicated
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL

3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
4	Life threatening	Life-threatening consequences; urgent intervention indicated
5	Death	Death related to AE

Attribution of Adverse Events:

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

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

12.3 NIH IRB and CD reporting

Expedited Reporting

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

Reports to the IRB at the time of Continuing Review:

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

Reports to the CD:

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

DSMB:

Serious unanticipated problem reports will be forwarded immediately to the Data and Safety Monitoring Board (DSMB). All SAEs and a summary of the adverse events that are determined to be possibly, probably or definitely related to this investigational treatment will be included for review by the DSMB.

13.0 HUMAN SUBJECT PROTECTIONS

13.1 Rationale for subject selection

All patients with confirmed β-thalassemia and/or SCD as defined in section 5.0 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

To date, all of the patients are living, none of the patients experienced GVHD, and most patients are free from their SCD. Therefore, we feel that this nonmyeloablative and potentially curative approach is reasonable in children. Four years of age will be the minimum age allowed for recipients on the protocol due to safety limits at the NIH.

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 14.6).

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 "mini" 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 total body irradiation as the basis for such an approach. A modest increase in the dosage of TBI from the 200 cGy used in prior studies to 300 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, and disease relapse. The major discomforts are those of nausea, anorexia, diarrhea, fever and malaise, and intolerance of the isolation period. The initial ten patients with SCD that were treated according to this protocol at the NIH did not experience significant toxicity related to the transplant regimen or GVHD. In less than 10% of our transplanted patients, there have been episodes of bleeding from development of an inhibitor to coagulation factor(s). The amount of bleeding can range from minor to severe, and patients typically need hospitalization for treatment and monitoring. Other groups have also reported this complication [99,100,101,102]. Severe hemolytic anemia from antibodies detected post-transplant. Treating this form of hemolysis include corticosteroids, IVIg, and/or other adjunct therapy. There may be an increased risk for hematologic malignancy, which may be related to severe sickle cell disease, inflammatory marrow from frequent vaso-occlusive crises, iron overload with increased reactive oxygen species, prior hydroxyurea, radiation, immunosuppression, infection, and other unknown factors. Lastly, organ injury and/or dysfunction in the brain, heart, kidney, thyroid, muscles, liver, and lung can result from one or more portions of the transplant process (conditioning regimen, medications, and infections).

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 multicentre, 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 last dose. In addition, one or more episodes of febrile neutropenia (ANC 500 cells/μL were reported in 10% of patients). The following types of infections were reported: Grade 3 or 4 sepsis in 12% of patients with one fatality, Grade 3 or 4 pneumonia in 15% with five fatalities, and opportunistic infections in 17% with four fatalities. Candida infections were reported in 5% of patients; CMV infections in 8% (4% of Grade 3 or 4 severity); Aspergillosis in 2% with fatal Aspergillosis in 1%; fatal Mucormycosis in 2%; fatal Cryptococcal pneumonia in 1%; *Listeria monocytogenes* meningitis in 1%; disseminated Herpes zoster in 1%; Grade 3 Herpes simplex in 2%; and *Torulopsis* pneumonia in 1%. PCP pneumonia occurred in one (1%) patient who discontinued PCP prophylaxis. In one of the earlier studies where anti-herpes and anti-PCP prophylaxis was optional, 37 (66%) patients had 47 infections while or after receiving Campath therapy.

Immunosuppression/Opportunistic Infections: Alemtuzumab induces profound lymphopenia. Anti-infective prophylaxis is recommended upon initiation of therapy and for a minimum of 2 months following the last dose of Alemtuzumab or until $CD4^+$ counts are 200 cells/μL. The median time to recovery of $CD4^+$ counts to 200/μL was 2 months, however, full recovery (to baseline) of $CD4^+$ and $CD8^+$ counts may take more than 12 months. Because of the potential for Graft versus Host Disease (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)/μL, at 2 months after discontinuation of Alemtuzumab therapy, 207/μL, and 6 months after discontinuation, 470/μL. The pattern of change in median $CD8^+$ lymphocyte counts was similar to that of $CD4^+$ cells. In some patients treated with Alemtuzumab, $CD4^+$ and $CD8^+$ lymphocyte counts had not returned to baseline levels at longer than 1-year post therapy.

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

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 mucous ulcers. Other possible toxicities are listed here and include those reported with >/=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, delayed/impaired wound healing, 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, pericarditis;
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, hyperesthesia, hypotonia, insomnia, neuropathy, paresthesia, somnolence;
Respiratory System: asthma, atelectasis, bronchitis, cough increased, epistaxis, hypoxia, lung edema, pleural effusion, pneumonia, rhinitis, sinusitis;
Skin and Appendages: fungal dermatitis, hirsutism, pruritus, skin hypertrophy, skin ulcer, sweating;
Special Senses: abnormal vision, cataract, conjunctivitis, deafness, ear pain, otitis media, tinnitus;
Urogenital System: albuminuria, or proteinuria, bladder pain, dysuria, hematuria, hydronephrosis, impotence, kidney pain, kidney tubular necrosis, kidney injury, 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, and pancreatitis.

13.3.4 Related to radiation

The side effects of radiation have been well described.[101] 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.[103] Importantly, the majority of the non-neoplastic effects were sub clinical and/or reversible.[104] 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 10years, and 6.7% at 15 years, with higher doses of TBI associated with a higher risk of solid cancers.[105] 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,[106,107] but the highest risk factor was felt to be the presence of chronic graft versus host disease, and long term treatment with cyclosporine.[108,109] Therefore the actual risk cannot be quantified for the low dose of 300cGy to be used in this trial; however is presumed to be minimal.

The additional radiation exposure from DEXA or liver/spleen scans is small and is not expected to significantly increase the risk presented by TBI.

13.3.5 Related to antimicrobials in general: Allergic reactions, renal impairment (gentamicin, vancomycin, amphotericin, acyclovir), "red man" syndrome - vancomycin, hepatic damage (acyclovir, rifampicin)

13.3.6 Related to bone marrow aspirate and biopsy: No major risks are involved with bone marrow aspirate and biopsy. However, a small risk of infections, pain, bleeding, and hematoma formation at the site of the aspiration exists with the procedure.

13.3.7 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 or infections may rarely occur.

13.3.8 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.

13.3.9 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 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.

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

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

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

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

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

13.3.13 Related to Plerixafor, if required prior to stem cell boost
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 appetite. Injection reactions also occurred including erythema, burning, bruising, pain, pruritus, 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.

13.4 Hazards and discomforts - donor

13.4.1 Related to neuropsychiatric testing
Neuropsychiatric testing will take approximately 1.5 to 2 hours to perform. During this time, the donor may experience frustration, fatigue, or distress. If these symptoms occur, the donor will be allowed to take a break. He or she will not have to answer every question, and testing will be discontinued if the donor asks. The clinical social worker and/or study investigators will be available if the donor would like to discuss his/her concerns.

13.4.1 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 or infections may rarely occur.

13.4.5 Consenting to pregnancy testing in minors of childbearing age
We will inform the minor during the assent process that for safety, we need to do a pregnancy test. She will also be told that if it is positive, we will counsel her and help her tell her parents. If the minor does not want to proceed, she will be advised not to sign the assent, and her enrollment on this screening protocol will end.

13.5 Risks in relation to benefit

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.

13.5.2 For pediatric transplant subjects (ages 4-18)

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

(a) *the risk is justified by the anticipated benefit to the subjects*: We are offering pediatric subjects with a probably lethal hematological disease, incurable with conventional treatments other than allogeneic BMT, an alternative to symptomatic therapy. This regimen, with its nonmyeloablative intent, has substantially lower risk for chemotherapy related toxicities, less injury to organs, and based on data in our current patients, lower risk for transplant related mortality and GvHD. On the other hand, there is a higher risk of graft rejection, but the magnitude of increase is unknown.

(b) *the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches.* The protocol aims to decrease the risk of transplant-related mortality, thus making more patients candidates for potentially curative therapy. Recently a reduced intensity study in pediatric patients with SCD showed that there is a chance for fertility preservation.[15] Thus the use of low dose TBI and alemtuzumab would be expected to have higher potential for fertility preservation; and

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

13.5.3 For adults and pediatric donors participating in blood collection for research and neuropsychological testing

These tests are considered to be no greater than minimal risk to the subject.

13.6 Informed consent

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

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

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

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

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

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

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

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

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

Assent Procedures:

Participants 14 years of age or older will review and discuss the adult consent with the parents and research team, and sign the standard adult consent in the assent line. If a minor is between the ages of 7 and 13 years of age, then the minor will sign the minor assent form (donor or recipient). Minors under 7 years of age will provide verbal assent for participation in this study.

Adult consent for the minor participation will be obtained from one parent or guardian given that the study involves no more than minimal risk to individual minor subjects who are donors, and greater than minimal risk for recipients, but with the prospect of direct benefits . In cases where parents share joint legal custody in making medical decisions of their child (e.g. by a custody agreement or court order) both parents must give their parental permissions regardless of level of risk of the research. Exceptions may be made if one parent is deceased, becomes incompetent or is not reasonably available (e.g. in prison). The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent.

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.

Consent for Minors when they reach the age of majority:

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

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

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

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

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

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

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

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

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

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

14.0 CONFLICT OF INTEREST

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

15.0 PHARMACEUTICALS

15.1 Alemtuzumab (Campath-1H, Campath®)

Supply: Available through the Campath Distribution Program (The Sanofi Foundation for North America 1-877-422-6728). Vials are provided through this program upon completion of a patient specific request form. Prior to submission of a drug request the patient must provide authorization for the release of medical information (NIH-527). Refer to the Pharmacy Department or Clinical Pharmacy Specialist for additional details on drug procurement.
Product description: Alemtuzumab is available in single use vials. Each single use, clear glass vial of Campath contains 30 mg alemtuzumab in 1 mL of solution (8.0 mg sodium chloride, 1.44 mg dibasic sodium phosphate, 0.2 mg potassium chloride, 0.2 mg monobasic potassium phosphate, 0.1 mg polysorbate 80, and .0187 mg disodium edetate dihydrate). No preservatives are added. Each carton contains three Campath vials (NDC 50419-357-03) or one Campath vial (NDC 50419-357-01).
Storage and stability: Vials of alemtuzumab should be stored at 2-8°C (36-46°F) and protected from sunlight. The vial should not be frozen; if the vial has been frozen it should be discarded. Alemtuzumab contains no antimicrobial preservative. Alemtuzumab should be used within 8 hours after dilution. Alemtuzumab solutions may be stored at room temperature (15-30°C) or refrigerated. Alemtuzumab solutions should be protected from light.
Preparation: The vial should be inspected for visible particulate matter and discoloration prior to administration. If particulate matter is present or the solution is discolored, the vial should not be used. The vial should not be shaken prior to use. The necessary amount of alemtuzumab should be withdrawn from the vial into a syringe. The vial contains no preservatives and is intended for single use only; the vial should be discarded with any unused portion after withdrawal of desired dose. The desired dose is then injected into 100 mL sterile 0.9% Sodium Chloride USP or 5% Dextrose in Water USP. The bag should be gently inverted to mix the solution. The syringe is discarded.

Administration: Alemtuzumab should be administered intravenously only. The infusion should be administered over a 2-hour period.

15.2 Filgrastim (G-CSF, Neupogen)

Supply: Commercially available.
Product description: Filgrastim injection is available in a concentration of 300mcg/ml in 1ml (300mcg) and 1.6ml (480mcg) vials.
Preparation: For subcutaneous administration, the appropriate prescribed dose is drawn up from the vial with no further dilution prior to administration. For intravenous administration, the commercial solution for injection should be diluted prior to administration. It is recommended that the prescribed dose be diluted with dextrose 5% in water (DO NOT DILUTE WITH NORMAL SALINE) to a concentration greater than 5mcg/ml. Filgrastim diluted to concentrations between 5 and 15mcg/ml should be protected from absorption to plastic materials by the addition of Albumin (Human) to a final concentration of 2mg/ml. When diluted in 5% dextrose or 5% dextrose plus Albumin (Human), filgrastim is compatible with glass bottles, PVC and polyolefin IV bags, and polypropylene syringes.
Storage and Stability: Filgrastim for injection should be stored in the refrigerator at 2º to 8ºC (36º to 46ºF). Avoid shaking.
Route of administration: Subcutaneous injection or intravenous infusion over 15-30 minutes.

15.3 Sirolimus (Rapamune®)

Supply: Commercially available.
Product description: Sirolimus is available in 1 mg and 2 mg tablets and an oral solution (1 mg/mL concentration) in a 60 mL amber glass bottle.
Preparation: For administration of the oral liquid, the measured dose may be diluted in water or orange juice prior to administration. Empty the correct amount of sirolimus oral liquid into only a glass or plastic container holding at least two ounces of water or orange juice. No other liquids, including grapefruit juice, should be used for dilution. Stir vigorously and drink at once. Refill the container with an additional volume (minimum 4 ounces or 120 mL) of water or orange juice, stir vigorously, and drink at once.
Storage and Stability: Oral tablets should be stored at room temperature: 20-25 °C (68-77 °F). Oral solution should be refrigerated (2-8 °C or 36-46 °F). Once the oral solution bottle is opened, the contents should be used within one month. If necessary, the patient may store the bottles at room temperatures up to 250 °C for a short period of time (e.g. not more than 15 days for the bottles).
Route of administration: Oral.

15.4 Busulfan

Busulfan is a bifunctional alkylating agent approved for use as a conditioning agent prior to allogeneic hematopoietic stem cell transplantation. The IV formulation is commercially available as busulfan (Otsuka America Pharmaceutical, Inc.)
Generic: busulfan
Classification: alkylating agent
Action: Alkylates and crosslinks DNA. It has a more marked effect on myeloid cells than on lymphoid cells.
Source: For patient administration, IV busulfan is purchased by the NIH Clinical Center Pharmacy Department from commercial sources. The drug is supplied as a clear, colorless sterile solution in 10mL single use vials. Each vial of busulfan contains 60mg (6mg/mL) of busulfan.
Stability and Storage: Unopened vials of parenteral busulfan are stable until the date indicated on the package when stored under refrigeration at 2-6 degrees C (36-46 degrees F).
Product description: Injection
Preparation: Parenteral busulfan must be diluted prior to use with either 0.9% sodium chloride or 5% dextrose injection. The diluent quantity should be 10 times the volume of busulfan so that the final

concentration of busulfan is approximately 0.5mg/mL. Busulfan should be administered intravenously via a central venous catheter as a 3-hour infusion every 24 hours for a total of 3 doses over 3 days.
Route: Intravenous

15.5 **Plerixafor (AMD3100, Mozobil®)**

Generic*:* plerixafor
Classification*:* hematopoietic stem cell mobilizer
Action*:* Reversibly inhibits binding of stromal cell-derived factor-1-alpha (SDF-1α), 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 25oC (77oF); excursions permitted to 15o-30oC (59o-86oF). [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 240µg 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.

15.6 Regulatory Considerations for Off Label Usage

Sirolimus, alemtuzumab, and plerixafor will be used in this study beyond what is indicated in the package inserts. Sirolimus is approved by the FDA as an immunosuppressive agent for the prophylaxis of organ rejection in patients aged ≥ 13 years receiving renal transplants, in renal patients at high immunologic risk, and in lymphangioleiomyomatosis. Alemtuzumab is approved for the treatment of relapsing forms of multiple sclerosis. Plerixafor is indicated in combination with granulocyte-colony stimulating factor (G-CSF) to mobilize hematopoietic stem cells (HSCs) to the peripheral blood for collection and subsequent autologous transplantation in patients with non-Hodgkin's lymphoma and multiple myeloma. The use of these drugs meet the requirements for an exemption from the IND regulations, 21 CFR 312, specifically:

1. The investigational drug is lawfully marketed in the United States
2. The investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use of the drug product
3. The investigation is not intended to support a significant change in advertising to an existing lawfully marketed prescription drug product
4. The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.
5. The investigation will be conducted in compliance with the requirements for institutional review set forth in FDA regulations 21 CFR 56, and requirements for informed consent as set forth in FDA regulations 21 CFR 50
6. The investigation will be conducted in compliance with FDA regulations 21 CFR 312.7: Promotion and charging for investigational drugs.

16.0 Compensation

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

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

APPENDIX A: PERFORMANCE STATUS GRADING

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

LANSKY PERFORMANCE STATUS SCALE

100 Fully active, normal
90 Minor restrictions in strenuous physical activity
80 Active, but tired more quickly
70 Greater restriction of play *and* less time spent in play activity
60 Up and around, but active play minimal; keeps busy by being involved in quieter activities
50 Lying around much of the day, but gets dressed; no active playing participates in all quiet play and activities
40 Mainly in bed; participates in quiet activities
30 Bedbound; needing assistance even for quiet play
20 Sleeping often; play entirely limited to very passive activities
10 Doesn't play; does not get out of bed
0 Unresponsive

KARNOFSKY PERFORMANCE STATUS SCALE

100 Normal; no complaints; no evidence of disease
90 Able to carry on normal activity; minor signs or symptoms of disease
80 Normal activity with effort; some sign or symptoms of disease
70 Cares for self; unable to carry on normal activity or do active work
60 Requires occasional assistance, but is able to care for most personal needs
50 Requires considerable assistance and frequent medical care
40 Disabled; requires special care and assistance
30 Severely disabled; hospitalization is indicated, though death not imminent
20 Very sick; hospitalization necessary; active support treatment is necessary
10 Moribund; fatal processes progressing rapidly

0 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 or provide product as per transfusion medicine standard practice.

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 peri-transplant maneuvers, such as transfusion or red cell exchanges with allogeneic red cells, may be performed to reduce the risk of ischemic events by reducing the levels of S hemoglobin. Pre-transplant coordination with the Department of Transfusion Medicine 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, 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. Regarding patients with minor ABO incompatibility, immediate complications at the time of infusion due to ABO compatibility can be managed by standard DTM policies for manipulation of the graft to remove plasma. The appropriate transfusion policy in the peri-transplant period for the ABO group of red cell, plasma and platelet transfusions in patients with ABO incompatible donors is also managed according to standard DTM transplant policies. In addition, the DTM will use red cell serologic testing to carefully monitor those patients who have minor ABO incompatibility with the donor for evidence of hemolysis in the peri-transplant period using a standard operating procedure. 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 target fraction of hemoglobin S to less than 30% to reduce the incidence of post-transplant stroke and other events that may be associated with high hemoglobin S levels. ADSOL leuko-reduced packed red cells will be used for the exchange.

These patients will have a hemoglobin electrophoresis performed to determine their initial fraction of S hemoglobin (%S). 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 hemoglobin S content and the desired end hematocrit and hemoglobin S 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 hematocrit (hct) = Patient's Total Packed Red Cell Volume (PRCV)
>
> (PRCV) x %S = Patient Total Packed RBC Volume of S Hemoglobin (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 hemoglobin S 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 %S should be 30% after the final transfusions bring the hematocrit up to 35%.

The target for the %S after the exchange (before additional transfusions) is 35/Hct x 30%, where Hct is the patient hematocrit before the exchange. After the exchange the patient will then receive a volume of red cells equal to approximately (0.35-Hct)(wt)(70 ml/kg).

Patients undergoing red cell exchange may experience citrate toxicity from the anticoagulant used in the apheresis procedure and contained in the ADSOL red cells. A citrate infusion rate will be calculated by the DTM fellow 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 x 10^6/kg, and the minimum is 5 x 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 G-CSF Dose (range)
20-30kg	300 mcg (10.0 to 15 mcg/kg)
31-37kg	480 mcg (13.0 to 15.5 mcg/kg)
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. Plerixafor administration

Plerixafor may be used alone or as described in the prescribing information (http://products.sanofi.us/Mozobil/mozobil.html). On day 5 of G-CSF and the donor is undergoing apheresis, stem cell mobilization is considered suboptimal (CD34+ count <30), and total projected stem cell number collected after the planned 2 days of apheresis is unlikely to meet the minimum of 5 x 10e6 CD34+ cells/kg of recipient weight, plerixafor may be given at 240 ug/kg subcutaneously in the evening of day 5, with another dose of G-CSF, and followed by apheresis the next day. If the minimum CD34 count is not reached after 2 apheresis, the patient can return about 1 month later to be treated again with G-CSF with or without plerixafor, based on the patient's previous response. The GCSF and plerixafor dosing will be discussed between the PI and DTM. G-CSF will be given subcutaneously for five consecutive days. On the evening of the day 4, plerixafor at 240 µg/kg subcutaneously will be given followed by apheresis on day 5. If the goal CD34 yield is not met, the patient can receive a 6th dose of G-CSF on the evening of day 5 and a second dose of plerixafor, followed by second apheresis the next day. The patient will return within one week of apheresis (+/- 2 days) for history and physical, complete blood count, and chemistry/liver panel if needed. Alternative dosing regimen may be used based on published literature (Micallef IN, Sinha S et al, BBMT 2013) and discussion with DTM.

V. 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 cryo-preserved 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, bone marrow products, and lymphocyte products will undergo plasma removal, with resuspension in an infusible isotonic solution, according to SOPs in 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.

APPENDIX D: PREPARATIVE REGIMEN

Patients will receive the preparative regimen as described below. 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 transplant protocols/procedures per current institutional practice.

Admission, exchange transfusion if necessary

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

Day –7 Diphenhydramine 1mg/kg (maximum 50mg) I.V., Acetaminophen 10-15mg/kg (maximum 650mg) PO then followed 30 minutes later by Alemtuzumab 0.03mg/kg in 100mL normal saline infused over 2 hours

Day –6 Diphenhydramine 1mg/kg (maximum 50mg) IV, Acetaminophen 10-15mg/kg (maximum 650mg) PO, then followed 30 minutes later by Alemtuzumab 0.1 mg/kg in 100mL normal saline infused over 2 hours

Day -5 to Day -3 Diphenhydramine 1mg/kg (maximum 50mg) IV, Acetaminophen 10-15mg/kg (maximum 650mg) PO, then followed 30 minutes later by Alemtuzumab 0.3 mg/kg in 100mL normal saline infused over 2 hours

Day –2 Total Body Irradiation (TBI), 300 cGy radiation given as per the Department of Radiology standard of practice

Day –1 Begin sirolimus (Rapamune®) 5 mg PO q4h x three doses in patients ≥ 16 years of age and 1mg/m2/dose PO q4h x three doses in patients less than 16 years of age with a maximum of 5mg per dose.

Day 0 Continue sirolimus (Rapamune®) 5mg PO daily in patients ≥ 16 years of age and 1mg/m2/dose PO daily in patients <16 years of age with a maximum of 5mg per dose. Trough levels will be maintained between 5-15 ng/ml. Infusion of unmanipulated Granulocyte colony-stimulating factor (Neupogen®) - mobilized peripheral blood stem cells

APPENDIX E: Administration Scheme for Busulfan

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

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

Busulfan dosing will be based on ideal body weight or actual body weight, whichever is lower. For recipients who are greater than 120% of ideal body weight, busulfan will be dosed on an adjusted ideal body weight (ideal body weight plus 25% of the difference between ideal and actual weight). Four

busulfan blood samples will be drawn following the administration of the test dose in a green top (sodium heparin) collection tube (1 mL specimen volume). Optimally, samples should be obtained from a peripheral vein in the arm opposite to the central line where busulfan is infused. If a peripheral vein sample is not feasible, the samples should be obtained from a different central catheter lumen than that used for the busulfan infusion. The first specimen should be drawn immediately after termination of the 2-hour intravenous infusion of 0.8 mg/kg busulfan. Additional specimens should also be drawn at 1 hour, 2 hours and 4 hours after termination of infusion. Each sample should be placed on wet ice immediately after collection. All samples will be sent to the NIH Clinical Center laboratory for send out to the NIH contract laboratory (Mayo Medical Laboratories).

17.0 References

1. Rodgers GP, Noguchi CT, Schechter AN (1994) Sickle Cell Anemia. Scientific American: 48-57.
2. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, et al. (1994) Mortality in sickle cell disease. Life expectancy and risk factors for early death [see comments]. N Engl J Med 330: 1639-1644.
3. Feld JJ, Kato GJ, Koh C, Shields T, Hildesheim M, et al. (2015) Liver injury is associated with mortality in sickle cell disease. Aliment Pharmacol Ther 42: 912-921.
4. Wayne AS, Schoenike SE, Pegelow CH (2000) Financial analysis of chronic transfusion for stroke prevention in sickle cell disease. Blood 96: 2369-2372.
5. Adams RJ, McKie VC, Hsu L, Files B, Vichinsky E, et al. (1998) Prevention of a First Stroke by Transfusions in Children with Sickle Cell Anemia and Abnormal Results on Transcranial Doppler Ultrasonography. N Engl J Med 339: 5-11.
6. Lee MT, Piomelli S, Granger S, Miller ST, Harkness S, et al. (2006) Stroke Prevention Trial in Sickle Cell Anemia (STOP): extended follow-up and final results. Blood 108: 847-852.
7. Ware RE, Helms RW (2012) Stroke With Transfusions Changing to Hydroxyurea (SWiTCH). Blood 119: 3925-3932.
8. DeBaun MR, Casella JF (2014) Transfusions for silent cerebral infarcts in sickle cell anemia. The New England journal of medicine 371: 1841-1842.
9. Steinberg MH, McCarthy WF, Castro O, Ballas SK, Armstrong FD, et al. (2010) The risks and benefits of long-term use of hydroxyurea in sickle cell anemia: A 17.5 year follow-up. American Journal of Hematology 85: 403-408.
10. Fitzhugh CD, Lauder N, Jonassaint JC, Telen MJ, Zhao X, et al. (2010) Cardiopulmonary complications leading to premature deaths in adult patients with sickle cell disease. Am J Hematol 85: 36-40.
11. Steinberg MH, Barton F, Castro O, Pegelow CH, Ballas SK, et al. (2003) Effect of hydroxyurea on mortality and morbidity in adult sickle cell anemia: risks and benefits up to 9 years of treatment. JAMA : the journal of the American Medical Association 289: 1645-1651.
12. Walters MC, Patience M, Leisenring W, Eckman JR, Scott JP, et al. (1996) Bone Marrow Transplantation for Sickle Cell Disease. N Engl J Med 335: 369-376.
13. Vermylen C, Cornu G, Ferster A, Brichard B, Ninane J, et al. (1998) Haematopoietic stem cell transplantation for sickle cell anaemia: the first 50 patients transplanted in Belgium. Bone Marrow Transplant 22: 1-6.
14. Bernaudin F, Socie G, Kuentz M, Chevret S, Duval M, et al. (2007) Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease. Blood 110: 2749-2756.
15. King AA, Kamani N, Bunin N, Sahdev I, Brochstein J, et al. (2015) Successful matched sibling donor marrow transplantation following reduced intensity conditioning in children with hemoglobinopathies. American journal of hematology.
16. Platt OS, Guinan EC (1996) Bone marrow transplantation in sickle cell anemia--the dilemma of choice [editorial; comment]. N Engl J Med 335: 426-428.
17. Kalinyak KA, Morris C, Ball WS, Ris MD, Harris R, et al. (1995) Bone marrow transplantation in a young child with sickle cell anemia. Am J Hematol 48: 256-261.

18. Gladwin MT, Barst RJ, Gibbs JS, Hildesheim M, Sachdev V, et al. (2014) Risk factors for death in 632 patients with sickle cell disease in the United States and United kingdom. PloS one 9: e99489.
19. Rotz SJ, O'Riordan MA, Kim C, de Lima M, Gladwin MT, et al. (2015) Traffic Light: prognosis-based eligibility for clinical trials of hematopoietic SCT in adults with sickle cell anemia. Bone marrow transplantation 50: 918-923.
20. Weatherall DJ, Clegg JB (1996) Thalassemia--a global public health problem. Nat Med 2: 847-849.
21. Gabutti V, Piga A, Sacchetti L, Sandri A, Biginelli M, et al. (1989) Quality of life and life expectancy in thalassemic patients with complications. Prog Clin Biol Res 309: 35-41.
22. Zurlo MG, De Stefano P, Borgna-Pignatti C, Di Palma A, Piga A, et al. (1989) Survival and causes of death in thalassaemia major. Lancet 2: 27-30.
23. Olivieri NF, Buncic JR, Chew E, Gallant T, Harrison RV, et al. (1986) Visual and auditory neurotoxicity in patients receiving subcutaneous deferoxamine infusions. N Engl J Med 314: 869-873.
24. Porter JB, Huehns ER (1989) The toxic effects of desferrioxamine. Baillieres Clin Haematol 2: 459-474.
25. Olivieri NF, Koren G, Harris J, Khattak S, Freedman MH, et al. (1992) Growth failure and bony changes induced by deferoxamine. Am J Pediatr Hematol Oncol 14: 48-56.
26. Cassinerio E, Roghi A, Orofino N, Pedrotti P, Zanaboni L, et al. (2015) A 5-year follow-up in deferasirox treatment: improvement of cardiac and hepatic iron overload and amelioration in cardiac function in thalassemia major patients. Annals of hematology 94: 939-945.
27. Pennell DJ, Porter JB, Piga A, Lai Y, El-Beshlawy A, et al. (2014) A 1-year randomized controlled trial of deferasirox vs deferoxamine for myocardial iron removal in beta-thalassemia major (CORDELIA). Blood 123: 1447-1454.
28. Maggio A, Vitrano A, Capra M, Cuccia L, Gagliardotto F, et al. (2009) Long-term sequential deferiprone-deferoxamine versus deferiprone alone for thalassaemia major patients: a randomized clinical trial. British journal of haematology 145: 245-254.
29. Viprakasit V, Nuchprayoon I, Chuansumrit A, Torcharus K, Pongtanakul B, et al. (2013) Deferiprone (GPO-L-ONE((R))) monotherapy reduces iron overload in transfusion-dependent thalassemias: 1-year results from a multicenter prospective, single arm, open label, dose escalating phase III pediatric study (GPO-L-ONE; A001) from Thailand. American journal of hematology 88: 251-260.
30. Piga A, Longo F, Musallam KM, Cappellini MD, Forni GL, et al. (2013) Assessment and management of iron overload in beta-thalassaemia major patients during the 21st century: a real-life experience from the Italian WEBTHAL project. British journal of haematology 161: 872-883.
31. Lucarelli G, Galimberti M, Polchi P, Angelucci E, Baronciani D, et al. (1991) Bone marrow transplantation in thalassemia. Hematol Oncol Clin North Am 5: 549-556.
32. Lucarelli G, Clift RA, Galimberti M, Angelucci E, Giardini C, et al. (1999) Bone marrow transplantation in adult thalassemic patients. Blood 93: 1164-1167.
33. Lucarelli G, Galimberti M, Giardini C, Polchi P, Angelucci E, et al. (1998) Bone marrow transplantation in thalassemia. The experience of Pesaro. Ann N Y Acad Sci 850: 270-275.

34. Gaziev D, Polchi P, Galimberti M, Angelucci E, Giardini C, et al. (1997) Graft-versus-host disease after bone marrow transplantation for thalassemia: an analysis of incidence and risk factors. Transplantation 63: 854-860.
35. Bernardo ME, Piras E, Vacca A, Giorgiani G, Zecca M, et al. (2012) Allogeneic hematopoietic stem cell transplantation in thalassemia major: results of a reduced-toxicity conditioning regimen based on the use of treosulfan. Blood 120: 473-476.
36. Kharbanda S, Smith AR, Hutchinson SK, McKenna DH, Ball JB, et al. (2014) Unrelated donor allogeneic hematopoietic stem cell transplantation for patients with hemoglobinopathies using a reduced-intensity conditioning regimen and third-party mesenchymal stromal cells. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 20: 581-586.
37. Gaziev J, Marziali M, Isgro A, Sodani P, Paciaroni K, et al. (2013) Bone marrow transplantation for thalassemia from alternative related donors: improved outcomes with a new approach. Blood 122: 2751-2756.
38. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED (1981) Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. N Engl J Med 304: 1529-1533.
39. Kollet O, Peled A, Byk T, Ben-Hur H, Greiner D, et al. (2000) beta 2 microglobulin-deficient (B2m(null)) NOD/SCID mice are excellent recipients for studying human stem cell function. Blood 95: 3102-3105.
40. Porter DL, Roth MS, McGarigle C, Ferrara JL, Antin JH (1994) Induction of graft-versus-host disease as immunotherapy for relapsed chronic myeloid leukemia. N Engl J Med 330: 100-106.
41. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, et al. (1995) Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. Blood 86: 2041-2050.
42. van den Bos C, Kieboom D, Wagemaker G (1993) Correction of murine beta-thalassemia by partial bone marrow chimerism: selective advantage of normal erythropoiesis. Bone Marrow Transplant 12: 9-13.
43. Andreani M, Manna M, Lucarelli G, Tonucci P, Agostinelli F, et al. (1996) Persistence of mixed chimerism in patients transplanted for the treatment of thalassemia. Blood 87: 3494-3499.
44. Walters MC, Patience M, Leisenring W, Rogers ZR, Aquino VM, et al. (2001) Stable mixed hematopoietic chimerism after bone marrow transplantation for sickle cell anemia. Biol Blood Marrow Transplant 7: 665-673.
45. Huss R, Deeg HJ, Gooley T, Bryant E, Leisenring W, et al. (1996) Effect of mixed chimerism on graft-versus-host disease, disease recurrence and survival after HLA-identical marrow transplantation for aplastic anemia or chronic myelogenous leukemia. Bone Marrow Transplant 18: 767-776.
46. Giralt S, Estey E, Albitar M, van Besien K, Rondon G, et al. (1997) Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. Blood 89: 4531-4536.
47. Slavin S, Nagler A, Naparstek E, Kapelushnik Y, Aker M, et al. (1998) Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow

transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood 91: 756-763.
48. Khouri IF, Keating M, Korbling M, Przepiorka D, Anderlini P, et al. (1998) Transplant-lite: induction of graft-versus-malignancy using fludarabine- based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. J Clin Oncol 16: 2817-2824.
49. Childs R, Clave E, Contentin N, Jayasekera D, Hensel N, et al. (1999) Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. Blood 94: 3234-3241.
50. Childs R, Chernoff A, Contentin N, Bahceci E, Schrump D, et al. (2000) Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. N Engl J Med 343: 750-758.
51. Kang EM, de Witte M, Malech H, Morgan RA, Phang S, et al. (2002) Nonmyeloablative conditioning followed by transplantation of genetically modified HLA-matched peripheral blood progenitor cells for hematologic malignancies in patients with acquired immunodeficiency syndrome. Blood 99: 698-701.
52. Shulman LN, Tarbell NJ, Storen E, Marcus K, Mauch PM (1998) Low-dose total body irradiation and G-CSF without hematopoietic stem cell support in the treatment of relapsed or refractory acute myelogenous leukemia (AML), or AML in second or subsequent remission. International Journal of Radiation Oncology*Biology*Physics 42: 1113-1117.
53. Giri N, Kaushiva A, Wu T, Sellers SE, Tisdale JF (2001) The effects of SCF/G-CSF prestimulation on radiation sensitivity and engraftment in nonmyeloablated murine hosts. Exp Hematol 29: 779-785.
54. Kang E, Giri N, Wu T, Sellers S, Kirby M, et al. (2001) In vivo persistence of retrovirally transduced murine long-term repopulating cells is not limited by expression of foreign gene products in the fully or minimally myeloablated setting. Hum Gene Ther 12: 1663-1672.
55. Huhn RD, Tisdale JF, Agricola B, Metzger ME, Donahue RE, et al. (1999) Retroviral marking and transplantation of rhesus hematopoietic cells by nonmyeloablative conditioning. Hum Gene Ther 10: 1783-1790.
56. Kang EM, Hanazano Y, Frare P, Vanin EF, De Witte M, et al. (2001) Persistent low-level engraftment of rhesus peripheral blood progenitor cells transduced with the fanconi anemia C gene after conditioning with low-dose irradiation. Mol Ther 3: 911-919.
57. Dufour C, Rondelli R, Locatelli F, Miano M, Di Girolamo G, et al. (2001) Stem cell transplantation from HLA-matched related donor for Fanconi's anaemia: a retrospective review of the multicentric Italian experience on behalf of AIEOP-GITMO. Br J Haematol 112: 796-805.
58. Myburgh JA, Smit JA, Browde S (1981) Transplantation tolerance in the primate following total lymphoid irradiation (TLI) and bone marrow (BM) injection. Transplant Proc 13: 434-438.
59. Myburgh JA, Smit JA, Hill RR, Browde S (1980) Transplantation tolerance in primates following total lymphoid irradiation and allogeneic bone marrow injection. II. Renal allografts. Transplantation 29: 405-408.

60. Storb R, Yu C, Sandmaier BM, McSweeney PA, Georges G, et al. (1999) Mixed hematopoietic chimerism after marrow allografts. Transplantation in the ambulatory care setting. Ann N Y Acad Sci 872: 372-375; discussion 375-376.
61. Feinstein L, Sandmaier B, Maloney D, McSweeney PA, Maris M, et al. (2001) Nonmyeloablative hematopoietic cell transplantation. Replacing high-dose cytotoxic therapy by the graft-versus-tumor effect. Ann N Y Acad Sci 938: 328-337; discussion 337-329.
62. Dick AD, Meyer P, James T, Forrester JV, Hale G, et al. (2000) Campath-1H therapy in refractory ocular inflammatory disease. Br J Ophthalmol 84: 107-109.
63. Coles AJ, Wing M, Smith S, Coraddu F, Greer S, et al. (1999) Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. Lancet 354: 1691-1695.
64. Isaacs JD, Burrows N, Wing M, Keogan MT, Rebello PR, et al. (1997) Humanized anti-CD4 monoclonal antibody therapy of autoimmune and inflammatory disease. Clin Exp Immunol 110: 158-166.
65. Killick SB, Marsh JC, Hale G, Waldmann H, Kelly SJ, et al. (1997) Sustained remission of severe resistant autoimmune neutropenia with Campath-1H. Br J Haematol 97: 306-308.
66. Pawson R, Dyer MJ, Barge R, Matutes E, Thornton PD, et al. (1997) Treatment of T-cell prolymphocytic leukemia with human CD52 antibody. J Clin Oncol 15: 2667-2672.
67. Newman DK, Isaacs JD, Watson PG, Meyer PA, Hale G, et al. (1995) Prevention of immune-mediated corneal graft destruction with the anti- lymphocyte monoclonal antibody, CAMPATH-1H. Eye 9: 564-569.
68. Friend PJ, Waldmann H, Hale G, Cobbold S, Rebello P, et al. (1991) Reversal of allograft rejection using the monoclonal antibody, Campath- 1G. Transplant Proc 23: 2253-2254.
69. Calne R, Moffatt SD, Friend PJ, Jamieson NV, Bradley JA, et al. (1999) Campath IH allows low-dose cyclosporine monotherapy in 31 cadaveric renal allograft recipients. Transplantation 68: 1613-1616.
70. Osterborg A, Fassas AS, Anagnostopoulos A, Dyer MJ, Catovsky D, et al. (1996) Humanized CD52 monoclonal antibody Campath-1H as first-line treatment in chronic lymphocytic leukaemia. Br J Haematol 93: 151-153.
71. Bowen AL, Zomas A, Emmett E, Matutes E, Dyer MJ, et al. (1997) Subcutaneous CAMPATH-1H in fludarabine-resistant/relapsed chronic lymphocytic and B-prolymphocytic leukaemia. Br J Haematol 96: 617-619.
72. Kottaridis PD, Milligan DW, Chopra R, Chakraverty RK, Chakrabarti S, et al. (2000) In vivo CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. Blood 96: 2419-2425.
73. Hale G, Waldmann H (1996) Recent results using CAMPATH-1 antibodies to control GVHD and graft rejection. Bone Marrow Transplant 17: 305-308.
74. Hale G, Jacobs P, Wood L, Fibbe WE, Barge R, et al. (2000) CD52 antibodies for prevention of graft-versus-host disease and graft rejection following transplantation of allogeneic peripheral blood stem cells. Bone Marrow Transplant 26: 69-76.
75. Kanda Y, Oshima K, Asano-Mori Y, Kandabashi K, Nakagawa M, et al. (2005) In vivo alemtuzumab enables haploidentical human leukocyte antigen-mismatched hematopoietic stem-cell transplantation without ex vivo graft manipulation. Transplantation 79: 1351-1357.

76. Chakraverty R, Peggs K, Chopra R, Milligan DW, Kottaridis PD, et al. (2002) Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. Blood 99: 1071-1078.
77. Powell JD, Lerner CG, Schwartz RH (1999) Inhibition of cell cycle progression by rapamycin induces T cell clonal anergy even in the presence of costimulation. J Immunol 162: 2775-2784.
78. Powell JD, Fitzhugh C, Kang EM, Hsieh M, Schwartz RH, et al. (2005) Low-dose radiation plus rapamycin promotes long-term bone marrow chimerism. Transplantation 80: 1541-1545.
79. Hale DA, Gottschalk R, Fukuzaki T, Wood ML, Maki T, et al. (1996) Extended skin allo- and xenograft survival in mice treated with rapamycin, antilymphocyte serum, and donor-specific bone marrow transfusion. Transplant Proc 28: 3269.
80. Hale DA, Gottschalk R, Fukuzaki T, Wood ML, Maki T, et al. (1997) Superiority of sirolimus (rapamycin) over cyclosporine in augmenting allograft and xenograft survival in mice treated with antilymphocyte serum and donor-specific bone marrow. Transplantation 63: 359-364.
81. Hale DA, Gottschalk R, Umemura A, Maki T, Monaco AP (2000) Establishment of stable multilineage hematopoietic chimerism and donor- specific tolerance without irradiation. Transplantation 69: 1242-1251.
82. Antin JH, Kim HT, Cutler C, Ho VT, Lee SJ, et al. (2003) Sirolimus, tacrolimus, and low-dose methotrexate for graft-versus-host disease prophylaxis in mismatched related donor or unrelated donor transplantation. Blood 102: 1601-1605.
83. Schleuning M, Judith D, Jedlickova Z, Stubig T, Heshmat M, et al. (2009) Calcineurin inhibitor-free GVHD prophylaxis with sirolimus, mycophenolate mofetil and ATG in Allo-SCT for leukemia patients with high relapse risk: an observational cohort study. Bone Marrow Transplant 43: 717-723.
84. Kahan BD (2000) Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. The Rapamune US Study Group. Lancet 356: 194-202.
85. Groth CG, Backman L, Morales JM, Calne R, Kreis H, et al. (1999) Sirolimus (rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Sirolimus European Renal Transplant Study Group. Transplantation 67: 1036-1042.
86. Kang EM, Areman EM, David-Ocampo V, Fitzhugh C, Link ME, et al. (2002) Mobilization, collection, and processing of peripheral blood stem cells in individuals with sickle cell trait. Blood 99: 850-855.
87. Abboud MR, Jackson SM, Barredo J, Holden KR, Cure J, et al. (1996) Neurologic complications following bone marrow transplantation for sickle cell disease. Bone Marrow Transplant 17: 405-407.
88. Walters MC, Patience M, Leisenring W, Eckman JR, Buchanan GR, et al. (1996) Barriers to bone marrow transplantation for sickle cell anemia. Biol Blood Marrow Transplant 2: 100-104.
89. Walters MC, Sullivan KM, Bernaudin F, Souillet G, Vannier JP, et al. (1995) Neurologic complications after allogeneic marrow transplantation for sickle cell anemia [see comments]. Blood 85: 879-884.

90. Saraf SL, Oh AL, Patel PR, Jalundhwala Y, Sweiss K, et al. (2016) Nonmyeloablative Stem Cell Transplantation with Alemtuzumab/Low-Dose Irradiation to Cure and Improve the Quality of Life of Adults with Sickle Cell Disease. Biol Blood Marrow Transplant 22: 441-448.
91. Powars DR (1990) Sickle cell anemia and major organ failure. Hemoglobin 14: 573-598.
92. Bakir AA, Hathiwala SC, Ainis H, Hryhorczuk DO, Rhee HL, et al. (1987) Prognosis of the nephrotic syndrome in sickle glomerulopathy. A retrospective study. Am J Nephrol 7: 110-115.
93. Abbott KC, Hypolite IO, Agodoa LY (2002) Sickle cell nephropathy at end-stage renal disease in the United States: patient characteristics and survival. Clin Nephrol 58: 9-15.
94. Castro O, Hoque M, Brown BD (2003) Pulmonary hypertension in sickle cell disease: cardiac catheterization results and survival. Blood 101: 1257-1261.
95. Gladwin MT, Sachdev V, Jison ML, Shizukuda Y, Plehn JF, et al. (2004) Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. N Engl J Med 350: 886-895.
96. Sharpsteen JR, Jr., Powars D, Johnson C, Rogers ZR, Williams WD, et al. (1993) Multisystem damage associated with tricorporal priapism in sickle cell disease. Am J Med 94: 289-295.
97. Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, et al. (1995) Effect of Hydroxyurea on the Frequency of Painful Crises in Sickle Cell Anemia. N Engl J Med 332: 1317-1322.
98. Castro O, Brambilla DJ, Thorington B, Reindorf CA, Scott RB, et al. (1994) The acute chest syndrome in sickle cell disease: incidence and risk factors. The Cooperative Study of Sickle Cell Disease. Blood 84: 643-649.
99. Brentjens RJ, Smith L, Reich L, Jakubowski AA (2001) Development of spontaneous factor VIII inhibitor in association with acute graft-versus-host disease. Bone marrow transplantation 27: 887-889.
100. Seidler CW, Mills LE, Flowers ME, Sullivan KM (1994) Spontaneous factor VIII inhibitor occurring in association with chronic graft-versus-host disease. American journal of hematology 45: 240-243.
101. Wiestner A, Cho HJ, Asch AS, Michelis MA, Zeller JA, et al. (2002) Rituximab in the treatment of acquired factor VIII inhibitors. Blood 100: 3426-3428.
102. Loh Y, Oyama Y, Statkute L, Quigley K, Yaung K, et al. (2007) Development of a secondary autoimmune disorder after hematopoietic stem cell transplantation for autoimmune diseases: role of conditioning regimen used. Blood 109: 2643-2548.
103. Deeg HJ (1983) Acute and delayed toxicities of total body irradiation. Seattle Marrow Transplant Team. Int J Radiat Oncol Biol Phys 9: 1933-1939.
104. Thomas O, Mahe M, Campion L, Bourdin S, Milpied N, et al. (2001) Long-term complications of total body irradiation in adults. Int J Radiat Oncol Biol Phys 49: 125-131.
105. Curtis RE, Rowlings PA, Deeg HJ, Shriner DA, Socie G, et al. (1997) Solid cancers after bone marrow transplantation. N Engl J Med 336: 897-904.
106. Gerrard GE, Vail A, Taylor RE, Pitchford WG, Gilson D, et al. (1998) Toxicity and dosimetry of fractionated total body irradiation prior to allogeneic bone marrow transplantation using a straightforward radiotherapy technique. Clin Oncol 10: 379-383.

107. Bhatia S, Louie AD, Bhatia R, O'Donnell MR, Fung H, et al. (2001) Solid cancers after bone marrow transplantation. J Clin Oncol 19: 464-471.
108. Kolb HJ, Socie G, Duell T, Van Lint MT, Tichelli A, et al. (1999) Malignant neoplasms in long-term survivors of bone marrow transplantation. Late Effects Working Party of the European Cooperative Group for Blood and Marrow Transplantation and the European Late Effect Project Group. Ann Intern Med 131: 738-744.
109. Deeg HJ, Socie G, Schoch G, Henry-Amar M, Witherspoon RP, et al. (1996) Malignancies after marrow transplantation for aplastic anemia and fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients. Blood 87: 386-392.
110. Lemery SJ, Hsieh MM, Smith A, Rao S, Khuu HM, et al. (2011) A pilot study evaluating the safety and CD34+ cell mobilizing activity of escalating doses of plerixafor in healthy volunteers. Br J Haematol 153: 66-75.
111. Gattillo S, Marktel S, Rizzo L, Malato S, Malabarba L, et al. (2015) Plerixafor on demand in ten healthy family donors as a rescue strategy to achieve an adequate graft for stem cell transplantation. Transfusion 55: 1993-2000.