Allogeneic and Matched Unrelated Donor Stem Cell Transplantation for Congenital Immunodeficiencies: Busulfan-based Conditioning with Campath- 1H, Radiation, and Sirolimus

National Institute of Allergy and Infectious Diseases (NIAID) / National Institutes of Health (NIH)

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Précis

Congenital immunodeficiencies – including chronic granulomatous disease, leukocyte adhesion deficiency and others – comprise a group of disorders in which the immune system fails to develop normally due to a genetic defect. As a result, affected patients suffer from recurrent infections and have a significantly shortened life expectancy. The current management of these patients is limited to close surveillance for infections, administration of prophylactic antimicrobials, and rapid and aggressive treatment of suspected and documented infections with broad-spectrum antibiotics. Although often effective, these treatments can require long hospitalizations, impacting on the overall quality of life significantly, and lead to significant morbidity, such as renal failure and deafness.

Currently, the only available cure for these disorders is bone marrow transplantation, which most commonly uses an HLA-matched related sibling as the donor (Allogeneic Stem Cell Transplantation). However, as only 30% of patients in the general population have an HLA-matched related sibling, allogeneic related transplantation is often not an option, resulting in the need for matched unrelated donor transplantation. The National Marrow Donor Program serves as both a national registry of volunteers who are willing to donate progenitor cells to eligible recipients as well as a repository of cord blood products. Despite continued improvement in the use of transplantation schemas – including the development of nonmyeloablative regimens – there remain significant morbidity and mortality associated with transplantation, in particular, graft versus host disease (GvHD).

GvHD is a result of the graft recognizing host antigens as foreign, typically in the presence of inflammation, and results in a type of iatrogenic autoimmune disease. For patients with non-malignant diseases, the aim of the transplant is solely to replace the defective or deficient cell population. Furthermore, as a graft versus tumor effect is not required, regimens designed to establish tolerance induction and/or stable mixed chimerism may be preferable for cure in this patient population; therefore, alternate transplant strategies can and should be used to further suppress the development of any GvHD effects. To reduce the morbidity and mortality associated with transplantation, we propose to use a combination of uniquely designed conditioning regimens to achieve adequate engraftment in congenitally immunodeficient patients, using either alloPBSC transplantation for patients with an HLA-matched related sibling donor, or MUD transplantation for those without an appropriate HLA-matched related sibling donor. For the alloPBSC transplantation (Group 1), we propose using a novel busulfan-based, nonmyeloablative conditioning regimen combined with Campath-1H, an immunosuppressive monoclonal antibody, and sirolimus, a tolerance inducing immunosuppressant used for GvHD prophylaxis. For the MUD transplantation (Group 2), we will also use a similar conditioning regimen, with a few modifications (due to the increased risk of graft rejection with HLA-matched but unrelated cells) to perform matched unrelated and cord blood transplantation in patients with immunodeficiencies. Given its novelty, this combination will be tested in a pilot trial and will be compared to historical controls.

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ABBREVIATION LIST

Abbreviation	Text
Ab	Antibody
ADA-SCID	Adenosine deaminase deficient severe combined immunodeficiency
AI	Associate Investigator
AlloPBSC	Allogeneic peripheral blood stem cell (transplantation)
ANC	Absolute neutrophil count
ARDS	Acute respiratory distress syndrome
ATG	Anti-thymocyte globulin
AUC	Area under the curve
BMT	Bone marrow transplantation
BUN	Blood urea nitrogen
CBC	Complete blood count
CC	Clinical Center
CGD	Chronic Granulomatous Disease
cGY	Centigray
CLAD	Canine leukocyte adhesion deficiency
CMV	Cytomegalovirus
CRF	Case report form
DHR	Dihydrorhodamine 123 (assay)
DLCO	Diffusing capacity for the lungs measured using carbon monoxide
DSMB	Data and Safety Monitoring Board
DTM	Department of Transfusion Medicine
EBV	Epstein Barr virus
ECOG	Eastern Cooperative Oncology Group
ECP	Extracorporeal photopheresis
ESR	Erythrocyte sedimentation rate
FEV-1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor
GvHD	Graft versus host disease
HB	Hematology Branch
Hb	Hemoglobin
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HSC	Hematopoietic stem cell
HTLV-I/II	Human T cell lymphotrophic virus type I, type II
HVOD	Hepatic veno-occlusive disease

Abbreviation	Text
ICH	International Conference on Harmonisation
IgM, IgG, IgA	Immunoglobulin M, G, A
IL-2	Interleukin 2
IRB	Institutional Review Board
IV	Intravenous
IVIG	Intravenous immunoglobulin
LAD	Leukocyte Adhesion Deficiency
LHD	Laboratory of Host Defenses
MUGA	Multi-acquisition gated
MUD	Matched unrelated donor
NCI-CTC	National Cancer Institute-Common Toxicity Criteria
NMDP	National Marrow Donor Program
NHLBI	National Heart, Lung, and Blood Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
PBPC	Peripheral blood progenitor cells
PBSC	Peripheral blood stem cells
PCP	*Pneumocystis carinii* pneumonia
PCR	Polymerase chain reaction
PI	Principal Investigator
PO	Latin *per os* (by mouth)
PPD	Purified protein derivative
PUVA	Combination of psoralen and long-wave ultraviolet radiation
QD	Latin *quaque die* (every day)
RBC	Red blood cells
RCHSPB	Regulatory Compliance and Human Subjects Protection Branch
RPR	Rapid plasma reagin
SAE	Serious adverse event
SCID	Severe combined immunodeficiency
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
SOP	Standard operating procedure
ß-hCG	Beta-human chorionic gonadotropin
TBI	Total body irradiation
TNF-α	Tumor necrosis factor-alpha
UCB	Umbilical cord blood

1.0 INTRODUCTION

1.1 Design

This is an open-label pilot study designed to evaluate whether the use of uniquely designed conditioning regimens reduces the occurrence of graft versus host disease (GvHD) after a bone marrow transplantation (BMT) for congenitally immunodeficient patients using a matched sibling donor (allogeneic) or matched unrelated donor (peripheral blood stem cells [PBSC] or cord blood), while still achieving adequate engraftment for normal immune function post transplant. For this study there will be two groups: Group 1 will be congenitally immunodeficient patients with a matched sibling donor and Group 2 will be congenitally immunodeficient patients who do not have a matched sibling donor. For patients in Group 1, we will use a novel busulfan-based nonmyeloablative-conditioning regimen combined with Campath-1H (a humanized immunosuppressive monoclonal antibody) and graft versus host disease (GvHD) prophylaxis with sirolimus (a tolerance inducing immunosuppressant). For patients in Group 2, we will use also use a busulfan-based nonmyeloablative-conditioning regimen with Campath-1H, but combine it with total body irradiation (TBI) and GvHD prophylaxis with sirolimus. These drugs are FDA approved and all are well-known drugs used in various transplant regimens; however, they have neither been used in this combination nor at these doses (busulfan) as specified in Section 4.4. As part of this study, we will also assess the level and kinetics of immune reconstitution in patients receiving these drugs.

1.2 Study Objectives

1.2.1 Primary Objectives

The primary objective for this study is to evaluate the use of Campath-1H and sirolimus in conjunction with a novel busulfan-based conditioning regimen with or without the addition of radiation to decrease or eliminate the occurrence of graft versus host disease after either allogeneic or matched unrelated (including cord blood) transplantation in patients with congenital immunodeficiencies. The goal is to attain an engraftment rate of 100% with no occurrence of Grade 3 or higher GvHD.

1.2.2 Secondary Objectives

- To further elucidate the required dose levels of busulfan as compared to historical controls in conditioning regimens for transplantation in general
- To measure the engraftment rate and the engraftment kinetics using such a regimen
- To assess the level and kinetics of immune reconstitution when using these conditioning regimens.
- To further elucidate the factors involved in the development of graft versus host disease.

1.3 Background

1.3.1 Chronic Granulomatous Disease

Chronic Granulomatous Disease (CGD) is one of a group of inherited disorders affecting neutrophil function. Due to a mutation in one of the genes encoding the phagocyte NADPH oxidase, patients with CGD are susceptible to recurrent bacterial and fungal infections. Besides life threatening infections, these patients develop granulomas that can result in genitourinary or gastrointestinal tract obstruction. Iatrogenic or infection related end organ damage has also been seen in long-term survivors of CGD. Despite improvements in infection prevention and control, 2% of patients with CGD die each year, and few patients survive to age 50 [1].

Treatment of CGD - Prophylactic Antibiotics and Interferon gamma

All CGD patients receive prophylactic antibiotic coverage with agents such as trimethoprim/sulfamethoxazole. Interferon gamma therapy has been shown to decrease the incidence of infection, and is therefore also part of the prophylactic regimen. It is, however, not well tolerated, and long-term compliance is rare. When infections do arise, emphasis is placed on identifying the pathogen and treating with the appropriate antibiotic for a prolonged period of time. When granuloma formation complicates an infection, a course of steroids is often effective [2].

Gene Therapy for CGD

The genes responsible for the four forms of CGD have been cloned. A number of small animal studies including xenogeneic models have shown proof of principal using vectors corrective for both the p47 mutation and the gp91 mutation. [3-5] Results of a Phase I clinical trial of gene therapy for patients with the p47-deficient form of CGD have also been reported as well as a more recent study treating patients with the gp91 mutations [6]. The authors demonstrated that autologous hematopoietic progenitors can be transduced with a retroviral vector containing the missing gene. These progenitor cells were then safely infused back into the patient, and small numbers of gene-corrected neutrophils were detectable in the peripheral blood for up to 6 months. However, without a selective advantage conferred upon the transduced cells, there was insufficient engraftment and/or proliferation to sustain a long-term cure. More recently, a German-Swiss collaborative study has resulted in two patients achieving clinical benefit after receiving genetically transduced cells with the use of busulfan preconditioning. Both patients maintained a level of 20% of corrected cells in the peripheral blood [7]. Most recently, the first patient succumbed to a CGD related infection felt to be secondary to poor expression of the gp91 protein on a per cell level. We are now in the process of establishing a new clinical gene therapy trial for patients with X-linked CGD; however, only those patients with an ongoing infection, without an HLA-matched sibling donor, and without evidence of bone marrow dysfunction will be eligible as it is still experimental in nature and, unlike allogeneic transplantation, does not have a proven track record of benefit. For patients without an underlying infection, and without a matched sibling donor, transplantation with cells obtained from an unrelated donor remains the only other treatment option.

Allogeneic Transplantation:
Allogeneic transplantation has been used successfully to cure patients with CGD. The vast majority of successful transplants have used myeloablative conditioning regimens. The toxicity and associated morbidity and mortality of these regimens, however, have limited the widespread use of allo transplants as a therapeutic option for many patients and their referring physicians. More recent efforts using nonmyeloablative regimens have reduced mortality and morbidity, but these regimens have simultaneously incurred an increased rate of graft rejection. In addition, despite an overall reduction in toxicity, patients still experienced significant GvHD as in the study performed here, where 2 of 15 patients died within one year of transplantation. [20] A third patient died suddenly from sepsis 2 years post transplant, although there was no obvious evidence of GvHD and the patient had had a successful engraftment. However of the remaining patients, including those who did not engraft, all are alive. A number of these survivors have successfully undergone second transplants with good results. Thus, alternative regimens are needed to improve initial engraftment and further reduce the GvHD incidence.

Matched Unrelated Donor Transplantation:
To date, 5 patients have undergone matched unrelated donor transplantation using either bone marrow or cord blood; 2 patients rejected the graft, and the other 3 have had successful engraftment with follow up more than 1 year post transplant. Of those engrafted, only 1 had GvHD. [8, 9]

1.3.2 Leukocyte Adhesion Deficiency

Leukocyte Adhesion Deficiency (LAD) is an autosomal recessive illness resulting from a genetic mutation of the CD18 gene that encodes the ß2 subunit of the leukocyte integrin family. As a result, the neutrophils of these patients are unable to migrate and adhere to sites of infection. Children are then prone to recurrent bacterial infections and, in fact, are typically diagnosed at birth with an umbilical cord stump infection. Patients are managed with antibiotics, and occasionally granulocyte infusions. The severity of the defect, (i.e., the level of CD18 production) correlates with the severity of disease, so even a modest increase in production is helpful.

Gene Therapy
As with those affected by CGD, patients with LAD have been treated with gene therapy. Unfortunately, to date, there have been no clinical successes, again in part due to the fact that the corrective gene does not confer a survival advantage to the neutrophils. Experiments in a dog model of LAD known as CLAD (canine leukocyte adhesion deficiency), however, have shown proof of principal, and gene therapy remains a target for the cure of this disease.

Allogeneic Transplantation
Transplantation has also been used to cure a number of patients with LAD. However, in the largest study of this rare disease, of the 5 patients transplanted using an HLA-matched sibling donor, 2 patients died from transplant complications. Overall, of the 14 patients transplanted using a parental haplotype or a sibling HLA-matched donor, 5 required a second transplant due to graft failure, and the overall mortality was 28%. Of note, 3

patients did develop mixed chimerism and remained phenotypically cured. Additionally, it has been shown in the dog model that even very low levels of CD18 positive neutrophils are sufficient to cure the animals. Hence nonmyeloablative-conditioning regimens may prove less toxic, yet still effective for this patient population.

Matched Unrelated Donor Transplantation

To date, there are 4 patients reported as having undergone matched unrelated donor transplantation for LAD. Of these, 1 patient died from infection after a second transplant for graft failure. Among the 3 survivors, 1 patient had Grade II acute GvHD limited to the skin, another had chronic GvHD of the skin (both of these exhibited 100% engraftment), while the third patient exhibited no GvHD, but had only 60% donor chimerism at 3 years after transplantation [10, 11].

1.3.3 CD40 Ligand Deficiency

CD40 ligand deficiency is an X-linked disorder of B and T cell function due to lack of CD40 ligand expression on T cells, which is necessary for CD40 signaling on B cells. This lack of cross binding between B cells and T cells leads to, among other things, a failure of B cell immunoglobulin class switching, resulting in elevated IgM levels and low to absent IgA and IgG levels. Hence this disease is also known as Hyper IgM syndrome and patients are prone to unusual viral and parasitic infections due to this lack of humoral immunity. In addition, the defective T cells cannot interact properly with antigen presenting cells, leading to an increased susceptibility to opportunistic infections, autoimmune disorders, and malignancies. Treatment is limited, but includes intravenous immunoglobulin (IVIG) and, of course, prophylactic antibiotics, – in particular, sulfonamide/trimethoprim combinations for *Pneumocystis carinii* pneumonia (PCP) prophylaxis. As a result, the median life expectancy as noted from a French study of 38 patients, is about 25 years. [12]

Allogeneic Transplantation
Although allogeneic transplantation is the only cure available to date, using traditional methods the overall success rate is only 68%.

Matched Unrelated Donor Transplantation
There is 1 report detailing the results from 4 patients undergoing unrelated donor transplantation. All patients are doing well except for 1 who had initial neutrophil engraftment, but continued to require platelet transfusion support. This patient ultimately developed secondary graft failure and died after developing acute respiratory distress syndrome (ARDS) from progressive pulmonary aspergillosis. Three of the patients developed acute GvHD and 1 also had chronic GvHD. [13]

1.3.4 National Marrow Donor Program

The National Marrow Donor Program (NMDP) was established in 1986 as the result of a Federal contract that was awarded to create and maintain a registry of volunteer hematopoietic stem cell (HSC) donors. Physicians search the NMDP Registry on behalf of patients in need of an HSC transplant who have no suitable matching related donor. In

1999 the NMDP added a Cord Blood Registry to provide more donor source options for patients in need of an unrelated HSC transplant. At the end of July 2003, the NMDP Registry listed more than 5 million volunteer donors, 28,000 cord blood units (CBU), and had facilitated over 16,000 unrelated HSC transplants.

1.4 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 (i.e., the graft versus leukemia effect). [14] 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. [15-18] 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 lower 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 (AlloPBSC) transplantation for malignant indications, patients with non-malignant disorders, such as those with immunodeficiencies, do not require full and/or rapid donor engraftment for cure of the disease. While it is generally accepted that GvHD is less severe in patients conditioned with low intensity preparative regimens, graft rejection is preferable to the development of lethal GvHD in the setting of immunodeficiencies.

Several groups have begun to investigate this approach to improve the applicability and outcome following allogeneic BMT, and preliminary results have been encouraging. 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, and extension to patients previously excluded from allogeneic transplant trials such as those infected with the human immunodeficiency virus has been proven feasible. [19] However, GvHD remains a significant problem, since it occurs at a rate not demonstrably different from that observed with conventional AlloPBSC transplantation, and continues to be the principal cause of death. This has also been seen in other centers developing other regimens. The Laboratory of Host Defenses (LHD) has also used nonmyeloablative techniques in patients with CGD and has shown success, although there was still significant GvHD in some patients. [20] The regimen used here, as with the majority of other popular nonmyeloablative regimens, was based on the same types of drug combinations used previously, with only an alteration in dosing. Newer agents and different combinations may prove to be more successful at truly reducing the development of graft versus host disease associated with transplantation.

For immunodeficient patients who lack an HLA-matched sibling donor, matched unrelated donor (MUD) transplantation has also been used. In a retrospective study that reviewed patients with various types of severe combined immunodeficiency (SCID), the survival of patients was 80% at 168 months post transplantation. Most deaths were related to either GvHD or infection, and 3 of 41 patients experienced graft failures. There was a 73% incidence of GvHD, but only 22% of patients had Grade 3 or higher. As this study was retrospective, the conditioning regimens and GvHD prophylaxis differed amongst the centers and patients, so the effects of conditioning cannot be determined from this study. [21]

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Umbilical cord blood has been an attractive option as there is data to suggest that there is less risk of GvHD induction, given the immunologic immaturity of these cells. It is also possible to transplant across more HLA mismatches with less risk of GvHD or rejection. However, the disadvantages to using cord blood have related to the smaller number of grafted cells and the resultant delayed immune recovery post transplant. In a 1997 analysis, better survival was associated with: age less than 6 years, weight less than 20 kg, an infusion of greater than 37 million nucleated cells per kg, and a CMV (cytomegalovirus) negative donor. [22] Hence, cord blood transplant has been traditionally limited to recipients of young age and low body mass. In an effort to increase the cell dose, the University of Minnesota in particular has been using more than one cord blood product per patient, with very promising results. In these patients with high-risk hematologic malignancies, the disease free survival was 57% at 1 year, and 72% overall survival for patients transplanted while in remission. [23]

In addition, transplantation centers have been developing reduced intensity (or nonmyeloablative conditioning regimens) for use in MUD and umbilical cord blood transplantation, and have applied these methods to both adults and children. With a median nucleated cell dose of 3.7 x 10^7/kg recipient body weight, the overall engraftment was between 76% and 95% depending on the conditioning regimen used in a single center trial. The incidence of GvHD was 9% and survival was 39% in this high-risk group of patients. [24]

Matched unrelated donor and umbilical cord transplantations have also been used in patients with immunodeficiencies, although the number of patients is limited (See Background). Cord blood from an HLA matched sibling was used in 1 patient with CGD after a busulfan based conditioning regimen. Oneyear post transplant, the patient appeared to have a Dihydrorhodamine 123 (assay) DHR of 92% and normal T and B cell numbers. As the risk of graft rejection is higher when using unrelated donor cells, the conditioning regimen for patients without a related donor will be modified to include low dose irradiation. This will provide additional immunosuppression along with some myelosuppression. In order to continue to minimize any toxicity, the busulfan dose will be reduced from 10mg/kg to 5mg/kg.

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1.5 Study Agent

The study agents are Total Body Irradiation, Busulfan, Campath-1H, and sirolimus.

These drugs are well-known, FDA-approved drugs used in various transplant regimens; however, they have not been used in this combination at these doses.

1.5.1 Total Body Irradiation:

Preclinical and Clinical Data: In attempts to decrease transplant related 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 Sculman et al. where 200 centigray (cGy) was adequate for inducing remission, albeit short lived, in refractory patients ineligible for other standard treatments. [25] Doses of 100 to 500 cGy have been shown to have very little toxicity in both murine and rhesus transplant models. [26-29] Even patients with Fanconi anemia, who would be considered high risk for radiation induced toxicities, tolerate moderate dose (500 cGy) radiation based regimens. [30] 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 fundamental treatment modality used to establish immunosuppression in both BMT and solid organ transplantation in animal models. [31, 32] There are also data obtained from canine experiments demonstrating stable mixed chimerism with the use of 200cGy total body irradiation alone, followed by GvHD prophylaxis with cyclosporin A (CysA) and mycophenolate mofetil (MMF). [33] Initial human trials using this regimen of 200 cGy with MMF and CysA were encouraging with approximately 20% graft failure. More recent data using fludarabine, an immunosuppressive but nonmyeloablative agent, together with 200cGy have shown similar results in 44 patients transplanted to date ([34] and personal communication, B. Sandmaier). 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 needed for an unrelated transplant without significantly altering the adverse effect profile.

1.5.2 Busulfan

Busulfan is an alkylating chemotherapeutic agent originally designed for the treatment of chronic myelogenous leukemia. It was subsequently determined to have broad myelosuppressive effects, and was combined with cyclophosphamide as part of a conditioning regimen for allogeneic transplantation. However, it also has erratic absorption and pharmacokinetics when used in its oral formulation and, therefore, requires careful monitoring and frequent dosing. More recently, an intravenous formulation has been developed with more predictable pharmacokinetics. It is known that busulfan does not require cell cycling (mitosis) and, therefore, can specifically target the quiescent hematopoietic progenitor cells, making it an even more effective myelosuppressive agent that is particularly well suited for transplantation conditioning. The toxicities are for the most part dose related and well managed with anti-emetics and careful monitoring.

Preclinical Data

Murine experiments: A series of experiments have been performed using busulfan as a conditioning regimen in mice. Dose-escalation studies show that between 10 and 20 mg/kg given as a single intraperitoneal injection consistently resulted in significant levels of engraftment with very little to no toxicity. In fact, all mice survived, even when treated with 20 mg/kg without subsequent transplantation of progenitor cells (Kang, data not published).

Rhesus experiments: A series of transplants have also been performed using autologous genetically modified cells in non-human primates. [35] While reduced dosages of 4 mg/kg and 6 mg/kg resulted in reduced toxicity, they also resulted in lower levels of successful engraftment. Preliminary data using divided dosing of 4 mg/kg per day on 2 days suggest that there may be improved engraftment but little to no increased toxicity at these low levels. This would also correlate with the clinical data below.

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Clinical Data

Autologous Transplantation: Busulfan has been used in significantly reduced doses for autologous transplantation of genetically modified cells. Aiuti et al., using a 4mg/kg total dose in the setting of adenosine deaminase deficient severe combined immunodeficiency (ADA-SCID), have achieved phenotypic correction in 4 of 5 patients treated to date. The patient who failed treatment received a very low cell dose number. [36]

Busulfan as a single agent was used to perform a second transplant in a patient with CGD who achieved only T cell engraftment despite conditioning with melphalan, Campath-1H, and fludarabine and multiple donor lymphocyte infusions. As the patient continued to have sequelae from his underlying CGD, we decided to condition him with 10mg/kg of busulfan followed by a $CD34^+$ selected graft from the original donor (NIAID Institutional Review Board [IRB] Protocol 04-I-0289). Now, more than 1 year after transplantation, he has normal neutrophil function with 100% chimerism as measured in the peripheral blood and by bone marrow $CD34^+$ cell analysis. Thus, in this case, clinical safety and efficacy of this conditioning agent was successfully demonstrated. A different protocol (NIAID IRB Protocol 06-I-0289) proposes to use busulfan as a single agent for autologous transplantation of genetically modified cells in patients without an HLA-matched sibling. However, in the allogeneic setting, busulfan alone would be inadequate, given the risk of graft rejection and also the need for some graft versus host disease prophylaxis. Results from allogeneic transplant studies using lower doses of the intravenous formulation of busulfan have also been encouraging (Dr. S. Solomon, Hematology Branch (HB)/National Heart, Lung, and Blood Institute (NHLBI), personal communication).

1.5.3 Alemtuzumab (Campath-1H):

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 [37-40], T and B cell malignancies [41], treatment of solid organ rejection [42-44], and has recently been approved for use in chronic lymphocytic leukemia, a B cell malignancy, as a result of its profound immunosuppressive properties. [45-47]

Preclinical and Clinical Data: Alemtuzumab

More recently, alemtuzumab has been used prospectively to prevent graft rejection in human renal allotransplantation. A total of 31 patients have been transplanted using 20 mg of alemtuzumab on Day 0 and Day 1 of transplantation in combination with half-dose

cyclosporine, which has been shown to be ineffective when used alone. At the 3-year follow up, there were no grafts lost to rejection [44]. For allogeneic bone marrow transplantation, the results have been equally encouraging with data suggesting that the use of alemtuzumab, as compared to fludarabine, reduces the risk of GvHD even in the unrelated donor setting [48-50]. In one study of 44 patients, including 8 patients receiving unmanipulated marrow from matched unrelated donors and who would therefore be at very high risk for developing GvHD, only 2 patients had acute GvHD, both of which were Grade II. Only 1 patient developed chronic GvHD. Follow up to this study has included an additional 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 3 patients developing Grade III GvHD and none developing Grade IV. [51] From another study of 30 high risk patients undergoing unrelated matched bone marrow transplantation, only 5 patients developed GvHD, none of which was greater than Grade II. 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 anti-thymocyte globulin (ATG), which is a nonspecific antibody directed against lymphocytes and is also used in conditioning regimen, alemtuzumab is better tolerated and has no risk of causing serum sickness.

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Additionally, a protocol has been initiated within the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) using Campath-1H in combination with sirolimus and low-dose radiation. Although patient accrual has been slow, results have been promising with 2 patients engrafting without any evidence of GvHD, with 1 patient already 1 year post transplantation. A third patient has lost the graft but this may be partly due to difficulties in maintaining his sirolimus, as well as due to receiving an inadequate dose of radiation due to an error in protocol implementation.

Commented [JPK5]: Level, dosage; compliance issue?

1.5.4 Sirolimus (Rapamune®/rapamycin):

Sirolimus is an immunophilin drug similar to cyclosporine; however, unlike cyclosporine, which inhibits the phosphatase calcineurin and thereby prevents the production of interleukin 2 (IL-2), sirolimus prevents translation of mRNAs encoding cell-cycle regulators. As a result, sirolimus only inhibits the ability of lymphocytes to proliferate in response to IL-2. Powell et al. demonstrated that cells cultured and stimulated in the presence of sirolimus became anergic, while cells cultured in the presence of cyclosporine did not. [52]

Animal Studies

To confirm the *in vitro* data in an *in vivo* model, we sought to compare sirolimus to the standard post-transplant immunosuppressant, cyclosporine, in a nonmyeloablative setting. Splenocytes from F1 hybrid mice were transplanted into a 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 cyclosporine, beginning 1 day before cell infusion. On Day 0, they were given 300 cGy total body irradiation (TBI) followed by $10x10^6$ splenocytes obtained from granulocyte colony-stimulating factor (G-CSF) mobilized BalbC/C57Bl6 hybrid donors.

Commented [JPK6]: 10^7?

The immunosuppressants were continued for 4 weeks beyond cell infusion, and chimerism was monitored using flow cytometric analysis. The mice who were given 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 cyclosporine lost their engraftment even while receiving the cyclosporine. Application of this regimen to transgenic mice expressing human sickle hemoglobin (a disease that is also a non-malignant hematologic disorder curable by transplantation) exclusively produced correction of anemia and red cell sickling even at very low levels of donor chimerism.

In a series of experiments performed by Hale et al., sirolimus also proved superior to cyclosporine 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 cyclosporine when added to anti-lymphocyte globulin and bone marrow in a murine model. Further, mice receiving sirolimus accepted a second same-donor skin graft but rejected third-party grafts, demonstrating the development of tolerance.

Clinical Data:

Sirolimus has less renal toxicity than cyclosporine. A randomized trial comparing the addition of sirolimus at either 2 mg or 5 mg vs. azathioprine to a cyclosporine and prednisone regimen 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%). [53] In a similar study comparing sirolimus vs. cyclosporine 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 levels in the sirolimus group. [54] 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 cyclosporine.

Commented [JPK7]: Do you mean mg/kg?

1.6 Qualifications of Investigators

This clinical study will be conducted by a multidisciplinary team encompassing expertise in immunology, infectious diseases, and hematology required for the care of patients undergoing bone marrow transplantation. The care teams also are knowledgeable in the conduct of Good Clinical Practice (GCP) principles of clinical research and the regulatory requirements for the protection of human subjects. All investigators collaborating in this study have met the training requirements of the Office of Human Subjects Research. Copies of the curricula vitae to demonstrate the experience and qualification of all of the investigators (Principal Investigator [PI] and Associate Investigators [AI]) will be kept updated and on file.

1.7 Conflict of Interest

No reportable conflicts of interest have been identified at the present time for any of the investigators conducting this study. If such conflicts of interest should develop in the future, the PI will take immediate corrective action and the Institutional Review Board (IRB) will be notified.

1.8 Conduct of the Study

This study will be conducted in accordance with all applicable laws and regulations, policies of the National Institute of Allergy and Infectious Diseases (NIAID) IRB as well as the policies of NIAID and NIH. The Principal Investigator will assure that no deviation from or changes to the protocol will take place without prior documented approval from the IRB, except where necessary to eliminate an immediate hazard(s) to the study participants. The PI will promptly report to the IRB any changes in research activity and all unanticipated problems involving risk to human subjects or others.

2.0 STUDY DESIGN

2.1 ENDPOINTS:

2.1.1 Primary Endpoint

Engraftment of allogeneic or matched unrelated (including cord blood) hematopoietic progenitor cells using moderate-dose busulfan and Campath-1H with or without whole body irradiation so as to attain phenotypic correction of congenital immunodeficiencies.

2.1.2 Secondary Endpoints

Achieve engraftment without development of graft versus host disease.

Establish stable mixed chimerism.

Improve rapidity of immune reconstitution.

Determine days to neutrophil recovery.

Measure incidence of Cytomegalovirus (CMV) reactivation.

Measure transfusion requirements.

2.2 Sample Size Justification

2.2.1 Rationale for Subject Selection

Over the next 5 years, a total of 30 subjects (10 sibling donors and 20 patients with congenital immunodeficiencies), each with a 6 out of 6 HLA-matched sibling donor or a suitable matched unrelated donor or cord blood product, will be enrolled in this study. Gender, ethnic background, and/or race will not be criteria for including or excluding patients during the study.

2.2.2 Donors of Allogeneic Stem Cells

Transplantation of immunologically competent progenitor cells derived from either bone marrow or peripheral blood is currently the only available cure for patients with congenital immunodeficiencies and is considered an accepted standard clinical intervention for these diseases. Such progenitor cells will be provided from any of 3

sources: sibling donors having a 6 out of 6 HLA-match to the recipient, or a suitably matched unrelated donor, or umbilical cord blood.

Children who are < 2 years of age or who weigh ≤18 kg will be excluded as donors. The risks of the apheresis procedure are related to the weight of the child, more precisely his/her extracorporeal volume, which is weight-dependent. The risks have to do with (1) need for a central line, (2) need for an allogeneic red cell prime, and (3) need for systemic heparinization because the subject is too small to get citrate. Weight considerations will be:

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

19 – 25 kg: A central line is required. Donors may or may not need red cell priming (at the discretion of the Apheresis department). Although there may be a need for heparinization, generally donors are anticoagulated with citrate.

≤ 18 kg: All donors in this weight range are excluded from participation, as they would require a central line, red cell priming, and systemic heparinization.

3.0 SUBJECT ENROLLMENT

3.1 Patient Recruitment

Patients and their family members who may already be participating/have participated in existing NIH studies and are being referred by their NIH physician into the screening for inclusion in this study will be recruited.

Participants will also be recruited from outside the existing NIH patient population. The Clinical Center (CC) Patient Recruitment and Public Liaison Office serves to provide clinical study advertisement and contact information for both self-referring patients and physician referrals from outside NIH. We expect to enroll about 3-4 patients per year into this study. The participants and their relatives selected for this study will reflect gender and ethnic diversity that is representative of the population.

3.2 Inclusion/Eligibility Criteria

3.2.1 Patients (Recipient):

- Must have a confirmed congenital immunodeficiency
- Must have sufficient complications from underlying disease to warrant undergoing transplantation.
- Ages 3 years – 65 years
- HLA-matched family donor available or an HLA matched unrelated PBSC graft available, or a minimum of 4/6 HLA matched cord blood product. (If the size of the cord blood graft is less than 3.0 x 10^7 cells, a second appropriate 4/6 or greater match cord blood product must be available.)

Commented [ck8]: Recommend that this be defined a priori.

- Ability to comprehend and willingness to sign the informed consent or have a parent/guardian consent if the donor is a minor; assent being obtained from minors as appropriate
- Negative serum beta-human chorionic gonadotropin (ß-hCG) for women of child-bearing potential
- Must be HIV negative
- Must not be pregnant or breastfeeding
- Must be able to stay within one hour's travel of the NIH for the first 3 months after transplantation and have a family member or other designated companion to stay with during the post transplant period.
- Must provide a durable power of attorney for health care decisions to an appropriate adult relative or guardian in accordance to NIH -200 "NIH Durable Power of Attorney for Health Care Decision Making".
- If of child-bearing potential, must agree to consistently use contraception throughout study participation and for 3 months post-study. Acceptable forms of contraception are:
    - Condoms, male or female, with or without a spermicide
    - Diaphragm or cervical cap with spermicide
    - Intrauterine device
    - Contraceptive pills or patch, Norplant®, Depo-Provera®, or other FDA-approved contraceptive method
    - Male partner has previously undergone a vasectomy.

3.2.2 Allogeneic Donor (Sibling donor only where the collection is done at NIH)

- HLA-matched (i.e., 6 of 6 alleles identical) family donor
- Ages $\geq$ 2 years and weight $\geq$ 18 kg (in so far that the weight difference between recipient and donor does not exceed a reasonable likelihood of being able to obtain an adequate cell dose from the donor with no more than 2 aphereses)
- Fit to receive G-CSF and give peripheral blood stem cells (normal blood counts, normotensive, and no history of stroke)
- Ability to comprehend and willing to sign informed consent or have parent/guardian consent if donor is a minor; assent obtained from minors as appropriate
- Must be HIV, hepatitis and syphilis negative
- Must not be pregnant or breastfeeding

* Inclusion criteria for donors for matched unrelated products from the National Marrow Donor Program is done per NMDP protocol.

3.3 Exclusion Criteria

3.3.1 Patient (Recipient)

- Age <3 years or > 65 years
- Eastern Cooperative Oncology Group (ECOG) performance status of 3 or more (See Supportive Care guidelines, available at http://intranettst2.cc.nih.gov/bmt/clinicalcare)
- Diffusion capacity of carbon monoxide (DLCO) < 60% predicted
- Left ventricular ejection fraction < 40%
- Transaminases > 5x upper limit of normal based on the patient's clinical situation and at the discretion of the investigator
- Psychiatric disorder or mental deficiency severe enough as to make compliance with the BMT treatment unlikely, and/or making informed consent impossible
- Major anticipated illness or organ failure incompatible with survival from AlloPBSC transplant
- Pregnant or lactating
- HIV positive
- Uncontrolled seizure disorder

3.3.2 Allogeneic Donor (Sibling donor only where the collection is done at NIH)

- Pregnant or lactating
- Donor unfit to receive G-CSF and undergo apheresis. (e.g., uncontrolled hypertension, history of congestive heart failure or unstable angina, thrombocytopenia, signs and symptoms of acute mononucleosis)
- HIV positive
- ≤18 kg: All donors in this weight range are excluded from participation as they would require a central line, red cell priming, and systemic heparinization.
- Recent exposure to infectious diseases such as chickenpox.
- Age <2 years

* Exclusion criteria for donors for matched unrelated products for the National Marrow Donor Program is done per NMDP protocol.

Commented [g9]: It may be useful to provide a link to this protocol or include criteria as an appendix to this protocol.

3.4 Study Enrollment Procedures

3.4.1 Consent Obtainment: Allogeneic Recipients and Donors

Informed consent will be obtained from all subjects during the first visit to the Clinical Center and before any screening procedures are conducted. The Principal and/or Associate Investigators (PI/AI) will obtain the consent during a face-to-face interview.

The PI and/or AI will counsel all participants. The process of obtaining consent will be done with the presence of the PI, other study members i.e. Study Coordinator and/or Research Nurse, and a Case Manager. The Case Manager will serve as the impartial third part witness who attests to the information given to the patients as well as the validity of the signature obtained. The consent document for this study will be thoroughly reviewed, questions will be answered, and the consent form will then be signed in the presence of a witness.

If a subject is a minor, the parent or legal guardian who signs the consent for the minor must be legally recognized as such. Where deemed appropriate by the PI/AI and the child's parent or legal guardian, the child will also be included in all discussions about the study and minor's assent will be obtained. The parent or legal guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent.

During this study, all new information relating to risks and/or adverse events will be provided orally and/or in writing to all enrolled or prospective patient and donor participants and their parent/guardian. Documentation will be provided to the IRB and if necessary, the informed consent document will be amended to reflect relevant information.

3.4.2 Consent for Unrelated Donors

Informed consent for donors in the National Marrow Donor Program is obtained per NMDP protocol.

3.4.3 Participation of Children

Congenital immunodeficient patients of all ages, especially children, suffer from debilitating and often lethal diseases that are incurable with conventional treatments. Currently, the only cure available for these disorders is bone marrow transplantation. This study aims to decrease the risk of transplantation related mortality, thus making more patients candidates for potentially curative therapy. Bone marrow transplantation, however, poses greater than minimal risk with the prospect of direct benefit to pediatric participants (45CFR 46.102). As such, adequate provisions are made for soliciting the assent of the children and the permission of their parents or guardians (see Sections 3.4.2 and 9.4). Children less than the age of 3 are excluded from participation as recipients on this protocol as the NIH does not have sufficient support to deal with possible complications related to transplant in pediatric patients less than 3. Patients older than 65 are excluded as well as it is known from standard transplantation that these patients have a higher risk of morbidity and mortality related to transplantation. Given the experimental nature of this protocol, the risk benefit ratio is not warranted to include these patients at this time. Further, the number of patients who would be eligible otherwise is exceedingly small.

4.0 STUDY IMPLEMENTATION

4.1 Criteria for Withdrawal

4.1.1 Patient's (Recipient's) Voluntary Withdrawal

The patient (recipient) will be allowed to withdraw at any time, even after receiving the conditioning agents (as autologous recovery is expected) or after receiving the allograft, although withdrawal at these stages would be highly discouraged. Once given the conditioning agents and/or allograft, if the patient (recipient) wishes to withdraw, he or she will have to remain within the care of the study physicians until either autologous or graft-mediated recovery is attained.

Post – Infusion Obligations

The patient (recipient) will be required to report to the clinic for follow-up assessments as specified in the study guidelines and Section 4.4, that is, weekly for the first 100 days, at 4 and 6 months post-transplant, then every 6 months for the next year, then every 12 months for the next 5 years post-transplant.

4.1.2 Allogeneic Donor's Voluntary Withdrawal

Donors will be given ample opportunity to withdraw from the study prior to peripheral blood progenitor cells (PBPC)-collection by apheresis. Donors also have the right, at any time, to elect not to participate in the research aspects of the study (donation of blood and bone marrow for non-routine tests).

4.1.3 Involuntary Withdrawal of a Patient (Recipient)

- If, after 2 G-CSF mobilization attempts on the donor at least 2 weeks apart, an inadequate cell number has been collected, the patient and donor will be withdrawn from the study, unless another donor is available.
- Patients who fail to demonstrate donor T-cell engraftment will be taken off study treatment but will continue to be monitored by the study staff for 6 months post transplant for possible infectious complications related to the conditioning regimen. After this period of follow up, patients will be referred back to their primary physician for further therapy.
- Patients with disease relapse will be taken off study treatment but will continue to be monitored by the study staff for a minimum of 6 months post transplant for possible infectious complications related to the conditioning regimen. The patient will then be approached to be consented for NIAID IRB Protocol 05-I-0213 (Screening and Baseline Assessment of Patients with Abnormalities of Immune Function) if not already enrolled 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.
- If this clinical trial is officially terminated. This applies to the situation where the investigator, the sponsor or any regulatory agency for any reason terminates the entire study.

- If a subject becomes pregnant. Patients will be taken off study treatment and referred back to their primary physician for further therapy but will have contact follow-up by the study staff to document the outcome of the pregnancy.
- If a patient develops a medical condition or circumstance where, in the opinion of the investigator, it is in the subject's best interest to discontinue participation in the study.

4.1.4 Involuntary Withdrawal of an Allogeneic Donor

- If, after 2 G-CSF mobilization attempts at least 2 weeks apart on the donor, an inadequate cell number has been collected, the patient and donor will be withdrawn from the study, unless another donor is available.
- If this clinical trial is officially terminated. This applies to the situation where the investigator, the sponsor or any regulatory agency for any reason, terminates the entire study.
- If a donor becomes pregnant.
- If a donor develops a medical condition or circumstance where, in the opinion of the investigator, it is in the subject's best interest to discontinue participation in the study.

4.2 Study Method/Parameters

4.2.1 Patient (Recipient) – Initial Screening

The following will be done as part of the initial screen for all patients (recipient):

- High resolution molecular HLA typing of patient and as many family members as possible to confirm complete matching of the donor
- Dihydrorhodamine 123 (DHR) assay or other disease specific analysis
- Serum ß-HCG for women of child-bearing potential
- Complete medical history and physical examination, including weight.

4.2.2 Patient (Recipient) – Baseline Testing and Evaluation

- Coagulation screen, complete blood count (CBC) with differential
- Chem 20 panel
- Erythrocyte sedimentation rate (ESR)
- Antibody screen for hepatitis B and C virus (HBV, HCV), human immuno-deficiency virus (HIV), human T cell lymphotrophic virus type I and II (HTLV-I/II), CMV, Epstein Barr virus (EBV), toxoplasma, and syphilis. Consider purified protein derivative (PPD) test for patients from areas where tuberculosis is prevalent.
- HLA antibody screen

- Lymphocyte phenotype
- T cell receptor Vß spectra type
- Serum troponin-I
- Complete lipid profile with triglycerides
- Bone marrow aspirate
- Collection of 24-hour urine for assessment of creatinine clearance
- Chest radiograph, pulmonary function testing: forced vital capacity (FVC), forced expiratory volume in 1 second (FEV-1), diffusing capacity for the lungs measured using carbon monoxide (DLCO)
- Sinus CT scans
- Cardiac function: EKG, multi-acquisition gated (MUGA) scan and/or ECHO, 24-hour Holter monitor
- Nutritional assessment
- Dental exam and clinical review
- Social worker interview
- Ophthalmology consultation
- Interview with members of primary care team and visit to unit
- Consent form signed
- Durable power of attorney form completed

4.2.3 Allogeneic Donor – Initial Screening

- High resolution molecular HLA-typing of as many family members as necessary both to find a possible donor and to confirm HLA matching

4.2.4 Allogeneic Donor Baseline – Testing and Evaluation

- Confirm HLA identity of donor with patient
- History and physical examination
- CBC with differential, coagulation screen, Chem 20 panel
- Lymphocyte phenotype
- Hepatitis B, C, HIV, HTLV-I/II, CMV, EBV antibodies, rapid plasma reagin (RPR)
- HLA antibody (Ab) screening
- Fit to donate: Orientation, visit to Department of Transfusion Medicine (DTM), inspection of veins to determine the need for a central line for apheresis
- Consent to undergo G-CSF mobilization (see below)

- Serum ß-HCG for women of child-bearing potential.

4.2.5 NMDP Protocol

Unrelated donors will be screened and evaluated according to NMDP guidelines.

4.2.6 Cord Blood Tests

For Cord Blood products, confirmatory HLA testing will be performed at the appropriate contract lab according to NMDP standard operating procedure (SOP).

Samples will be collected and stored according to the NMDP follow up protocol. A donor blood sample will be obtained from the NMDP for lymphocyte phenotyping testing, which will be done here at NIH.

4.3 Allogeneic Donor Intervention

4.3.1 Mobilization and Collection of Allogeneic Peripheral Blood Progenitor Cells (see Appendix C)

Starting at least 2 months before the anticipated transplant date, the eligible donor will receive granulocyte colony stimulating factor (G-CSF) at 10μg/kg/day subcutaneously for 5-6 days.

On the 5th day, the donor will be given a dose of G-CSF in the morning and then will be escorted down to the apheresis center in the DTM where a standard peripheral blood apheresis will be performed according to the DTM SOP. The blood 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 G-CSF and the $CD34^+$ cell dose needed, based on the kilogram weight of the recipient. This will range up to 35 liters processed per day over 2 to 3 days, not to exceed a total of 75 liters over 3 days unless reviewed and approved by the medical director of the apheresis clinic. In pediatric subjects, defined as less than 40 kg, a maximum of 8 blood volumes will be processed per day, for up to 2-3 days.

The collections of peripheral blood progenitor cells (PBPC) will then be cryopreserved. Although the intent is to use the product within one year of collection, products may be used up to 10 years after collection and storage in accord with the Red Cross guidelines for maintenance and use of cryopreserved products. If the two collections from the first mobilization combined are still inadequate, a repeat G-CSF mobilization (a minimum of 2 weeks later at a higher dose of G-CSF will be done with the pooled products of the two mobilizations being given to the patient at the time of transplant.

4.3.2 Allogeneic Peripheral Blood Progenitor Cell Transplant

The target collection number for progenitor cells is $\geq 5 \times 10^6$ $CD34^+$ cells/kg. This product will be collected in advance and cryopreserved. Donors will undergo apheresis for one or two days. The minimum dose for a PBSC transplant will be $\geq 3.0 \times 10^6$ $CD34^+$ cells/kg. If this is not achieved in one apheresis procedure, then the donor will undergo a

second apheresis the following day. If the combined product is still inadequate, the donor will be brought back after a minimum of two weeks in between collection and administration of G-CSF to be remobilized again using G-CSF at a dose of 16μg/kg. This will be added to the original collection. If after two such attempts, an inadequate cell number has been collected, the patient and donor will be withdrawn from the study, unless another donor is available.

4.3.3 Unrelated Donor Product Selection

The product to be chosen for unrelated transplantation will follow the algorithm below:

A)10/10 HLA match (Based on molecular high resolution typing) PBSCT

If not available

B) Best match cord blood product, minimum 4/6 HLA match with cell dose of 3 x 10^7 nucleated cells/kg recipient weight.

If the cell dose is insufficient, a second cord blood product will be used in combination (double cord) to ensure the minimum cell dose. Again HLA matching will be a minimum of 4/6 HLA in relation to both the recipient and the first product.

4.3.4 MUD Recipient Apheresis

For those patients undergoing matched unrelated donor transplantation either with PBSC or cord blood, the patient will provide consent to undergoing NIAID IRB Protocol 94-I-0073 for mobilization and apheresis. Although the regimen is considered nonmyeloablative, given the higher risk of rejection of unrelated donor cells, $CD34^+$ cells will be collected from the recipient. This product will be stored as a back up in the case of graft failure or rejection and lack of autologous recovery. Again, although the primary purpose of the collection is to use it within the peri-transplant period in the event of graft failure, products may be used up to 10 years after collection and storage as according to the Red Cross guidelines for maintenance and use of cryopreserved products.

4.4 Patient (Recipient) Intervention

4.4.1 Central Venous Line Placement

A triple lumen Hickman catheter will be placed by an interventional radiologist or a surgeon if the patient does not already have a catheter in place. Management of this access will be according to the SOP of the CC and nursing unit.

4.4.2 Treatment Plan

- All patients will be admitted to an inpatient unit for a minimum of 10 days prior to PBSC infusion. The total anticipated number of inpatient days is 40.
- Baseline studies will be performed as medically necessary to ensure no occult infection, which could necessitate delay of the transplant procedure. These will consist of Chest CT, EKG, and erythrocyte sedimentation rate (ESR).

4.4.3 Group 1 -Preparative Regimen Schema (Daily Management) for Allogeneic Recipients

Day -10 to Day -9 (10 to 9 days prior to cell infusion):

- Patient will have serum troponin-I, ECHO, 24-hour Holter, Chest CT, EKG and daily ESR and CBC with differential.
- The patient will be monitored with vital signs with weight taken approximately every 8 hours while awake.

Day -8 (8 days prior to cell infusion):

- ESR and CBC with differential
- Diphenhydramine (1-1.25mg/kg with maximum dose of 12.5 mg intravenously), acetaminophen 10-15mg/kg orally (PO) followed by **alemtuzumab 0.03 mg/kg** in 100cc normal saline infused over 2 hours.
- The patient will be monitored with vital signs with weight taken approximately every 8 hours while awake.

Day -7 (7 days prior to cell infusion):

- ESR and CBC with differential
- Diphenhydramine 1-1.25 mg/kg with maximum dose of 12.5 mg intravenously), acetaminophen 10-15 mg/kg PO followed by **alemtuzumab 0.1 mg/kg** in 100cc normal saline infused over 2 hours.
- The patient will be monitored with vital signs with weight taken approximately every 8 hours while awake.

Day -6,-5,-4 (6,5,4 days prior to cell infusion):

- ESR and CBC with differential
- Diphenhydramine 1-1.25 mg/kg with maximum dose of 12.5 mg intravenously), acetaminophen 10-15 mg/kg PO followed by **alemtuzumab 0.3 mg/kg** in 100cc normal saline infused over 2 hours.
- The patient will be monitored with vital signs with weight taken approximately every 8 hours while awake.

Day -3 (3 days prior to cell infusion):

- The patient will be administered **clonazepam 0.6 mg/kg** IV or equivalent medication (i.e., phenytoin).
- 30 minutes afterwards, the patient will receive IV **busulfan at 5 mg/kg** prepared in normal saline infused over 2 hours.

- Laboratory tests to be obtained are: ESR and CBC with differential. Drug (busulfan) levels will be obtained at the end of infusion, approximately 135, 150, 180, 240, 300, and 360 minutes after starting the infusion of the drug.
- The patient will be monitored with vital signs taken every 15 minutes for one hour, then every hour (q1h) until the completion of busulfan infusion, followed by approximately every 4 hours (q4h) while awake. (See also Section 11.6, administration of busulfan). Weight.

Day -2 (2 day prior to cell infusion):

- The patient will be administered **clonazepam 0.6 mg/kg** IV.
- Thirty minutes afterwards, the patient will receive IV **busulfan at 5 mg/kg** prepared in normal saline given over 2 hours.
- The patient will be monitored with vital signs taken every 15 minutes for one hour, then q1h until the completion of busulfan infusion, followed by approximately q4h while awake. (See also 11.7, administration of busulfan). Weight.
- Laboratory tests to be obtained are: ESR and CBC with differential.

Day -1 (1 day prior to cell infusion):

- No treatment
- Laboratory tests to be obtained are: ESR and CBC with differential. Weight
- **For adult patients**, begin **sirolimus 5 mg** PO q4h for 3 doses, then **5 mg** once a day (QD) to maintain trough levels between 10-20 ng/mL. Patients will take sirolimus from Day -1 to Day 100 (minimum).
- **For pediatric patients**, begin **sirolimus 3 mg/m^2** PO q4h for 3 doses, then **1 mg/m^2** once a day (QD) to maintain trough levels between 10-20 ng/mL. Pediatric patients will take sirolimus from Day -1 to Day 100 (minimum).

Day 0 (cell infusion day):

- The patient will be given the allogeneic peripheral blood allograft, which has been thawed and prepared following the SOP established in DTM.
- The cells will be infused through patient's central line as per CC policy and SOP on administration of blood products.
- Vital signs will be taken within 15 minutes prior to the transfusion and then at approximately 15 minute intervals until the (last) bag is infused, followed twice by q1h vital signs, then approximately q4h while awake. Weight
- Laboratory tests to be obtained are: ESR and CBC with differential.

4.4.4 Group 2- Preparative Regimen Schema (Daily Management) for Matched

Unrelated and Cord Blood Recipients

Day -11 to Day -9 (11 to 9 days prior to cell infusion):

- Patient will have serum troponin-I, ECHO, 24-hour Holter, Chest CT, EKG and daily ESR and CBC with differential.
- The patient will be monitored with vital signs with weight taken approximately every 8 hours while awake.

Day -8 (8 days prior to cell infusion):

- ESR and CBC with differential
- Diphenhydramine 1-1.25 mg/kg with maximum dose of 12.5 mg intravenously), acetaminophen 10-15 mg/kg orally (PO) followed by **alemtuzumab 0.03 mg/kg** in 100cc normal saline infused over 2 hours.
- The patient will be monitored with vital signs with weight taken approximately every 8 hours while awake.

Day -7 (7 days prior to cell infusion):

- ESR and CBC with differential
- Diphenhydramine1-1.25 mg/kg with maximum dose of 12.5 mg intravenously), acetaminophen 10-15 mg/kg PO followed by **alemtuzumab 0.1 mg/kg** in 100cc normal saline infused over 2 hours.
- The patient will be monitored with vital signs with weight taken approximately every 8 hours while awake.

Day -6,-5,-4 (6,5,4 days prior to cell infusion):

- ESR and CBC with differential
- Diphenhydramine 1-1.25 mg/kg with maximum dose of 12.5 mg intravenously), acetaminophen 10-15 mg/kg PO followed by **alemtuzumab 0.3 mg/kg** in 100cc normal saline infused over 2 hours.
- The patient will be monitored with vital signs with weight taken approximately every 8 hours while awake.

Day -3 (3 days prior to cell infusion):

- The patient will be administered **clonazepam 0.6 mg/kg** IV or equivalent medication (i.e., phenytoin).
- 30 minutes afterwards, the patient will receive IV **busulfan at 2.5 mg/kg** prepared in normal saline infused over 2 hours.

- Laboratory tests to be obtained are: ESR and CBC with differential. Drug (busulfan) levels will be obtained at the end of infusion, approximately 135, 150, 180, 240, 300, and 360 minutes after starting the infusion of the drug.
- The patient will be monitored with vital signs taken every 15 minutes for one hour, then every hour (q1h) until the completion of busulfan infusion, followed by approximately every 4 hours (q4h) while awake. (See also Section 11.7, administration of busulfan). Weight

Day -2 (2 days prior to cell infusion):

- The patient will be administered **clonazepam 0.6 mg/kg** IV.
- Thirty minutes afterwards, the patient will receive IV **busulfan at 2.5 mg/kg** prepared in normal saline given over 2 hours.
- The patient will be monitored with vital signs taken every 15 minutes for one hour, then q1h until the completion of busulfan infusion, followed by approximately q4h while awake. (See also Section 11.7, administration of busulfan). Weight
- Laboratory tests to be obtained are: ESR and CBC with differential.

Day -1 (1 day prior to cell infusion):

- The patient will be administered **total body irradiation (TBI) of 300 cGy in two fractions of 1.5 cGy each in the Radiation Oncology Branch of the National Cancer Institute** as follows:

  All patients will be treated with a linear accelerator using energies higher than 4MV. TBI will be delivered with lateral fields using extended SSD/SAD values of 200-500cm (depending on machine/vault size) and partial transmission lung blocks. Patients will then receive AP/PA Mediastinal Boost fields using full blocks in the lung area. Patients will be treated with TBI to a total dose of 300 cGy delivered in 150 cGy fractions bid, at least 6 hours apart (two total treatments in one day). The mediastinal boost fields will be delivered at a dose of 75cGy per fraction for a total dose of 150cGy, also at least six hours apart. Treatment planning will be performed to provide a mean lung dose of 150 cGy. Occasionally, the total dose/technique of TBI may require modifications due to patient factors (unexpected or severe (grade 4-5) adverse events, serious medical illnesses not conducive to stable patient transfer, patient refusal, etc) or treatment factors (linear accelerator machine offline, etc.) Modifications to the radiation treatment will be at the discretion of the treating radiation oncologist and will be discussed with the Principal Investigator or Study Chairperson.
- Laboratory tests to be obtained are: ESR and CBC with differential.

- **For adult patients**, begin **sirolimus 5 mg** PO q4h for 3 doses, then **5 mg** once a day (QD) to maintain trough levels between 10-20 ng/mL. Patients will take sirolimus from Day -1 to Day 100 (minimum).
- **For pediatric patients**, begin **sirolimus 3mg/m2** PO q4h for 3 doses, then **1 mg/m^2** once a day (QD) to maintain trough levels between 10-20 ng/mL. Pediatric patients will take sirolimus from Day -1 to Day 100 (minimum).

Day 0 (cell infusion day):

- The patient will be given the unrelated donor peripheral blood allograft, which has been thawed (if cord blood) and prepared following the SOP established in DTM. Adult unrelated donor products will not be frozen and therefore will not require thawing.
- The cells will be infused through patient's central line as per CC policy and SOP on administration of blood products.
- Vital signs will be taken within 15 minutes prior to the transfusion and then at approximately 15 minutes intervals until the (last) bag is infused, followed twice by q1h vital signs, then approximately q4h while awake. Weight
- Laboratory tests to be obtained are: ESR and CBC with differential.

4.4.5 Post Transplant Monitoring (Day +1 to Discharge)

The following will be obtained while the patient is an in-patient until discharged:

- Once daily: CBC with differential, Chem 20 panel, direct bilirubin, weight
- Every 8 hours while awake: temperature, pulse, blood pressure, respiratory rate
- Twice weekly: reticulocytes, pre-albumin, and coagulation screen
- Weekly: CMV surveillance, serum cholesterol, triglycerides, and sirolimus levels
- On Days +14, +30: peripheral blood will be drawn to assess for donor-host chimerism in the lymphoid and myeloid lineages. A sample of peripheral blood will also be obtained for chimerism analysis at the time of neutrophil recovery (i.e., absolute neutrophil count [ANC] > 500 cells/μL). On day +14 a lymphocyte phenotype will also be obtained.

Commented [ck10]: What will this consist of (i.e., PCR assay, antigenemia assay, cultures, etc?)

4.4.6 Hospital Discharge Criteria

Patient will be discharged when the following criteria are fulfilled:

- Patient afebrile, positive weight balance, no parenteral feeding required.
- Neutrophil count greater than 1000 on 3 consecutive days.
- Platelet transfusion requirement absent or manageable as an outpatient.
- Patient or family able to care for Hickman catheter.

4.4.7 Discharge to Day 100 (Out-Patient)

- At least weekly: A complete physical exam, CBC with differential, DHR in appropriate patients, coagulation screen, Chem 20 panel, temperature, pulse, blood pressure, respiratory rate, weight, CMV antigenemia, and sirolimus levels to guide dosing, serum Immunoglobulin levels.

- Monthly: Serum cholesterol, triglycerides, pregnancy test will be done on all females of child-bearing potential monthly until 12 weeks after sirolimus is discontinued.

- On Day +60 and Day +100: Peripheral blood will be drawn to assess for donor-recipient chimerism in the lymphoid and myeloid lineages. At day 100 a lymphocyte phenotype will also be determined.
  Ninety days (+/- 1 week) after the last dose Campath-1H: ECHO, serum troponin-I, and 24 hour Holter monitor

4.4.8 Beyond Day 100

- At 6, 12, 18, 24, 36, 48 and 60 months: CBC with differential, DHR in appropriate patients, Chem 20 panel, chest CT, chimerism analysis by PCR, and karyotype, lymphocyte phenotype, Vß spectra type, pulmonary function (12, 24 months). Pregnancy test for females of child-bearing potential, serum cholesterol, triglycerides will be done at 6 months, Serum troponin levels and 24 hour Holter monitor will done at the 6 month follow up visit.

- At the 12 month evaluation: Bone marrow aspirate samples will be obtained

- Patients will be screened for CMV beyond Day 100 post transplant for a minimum of 6 months.

Commented [mp11]: Consider including visit windows.

Commented [mp12]: How often? Will this be weekly for 6 months?

4.4.9 Post Transplant Medical Management

- **Infection Prophylaxis** (See also Supportive Care Guidelines, available at http://intranettst2.cc.nih.gov/bmt/clinicalcare)

  Includes: PCP prophylaxis, HSV for appropriate patients, IVIG

  Itraconazole or voriconazole fungal prophylaxis

- **Fever Regimen** (See Supportive Care Guidelines)

- **GvHD Prophylaxis (**see Appendix A**)**

- Sirolimus (Rapamune®) will be started on Day -1 with adult loading dose of 5mg q4h for 3 doses, and then continued at 5 mg/day with a goal of trough levels being 10-20 ng/mL. **For pediatric patients**, sirolimus will be started on day-1 with the loading dose of **3 mg/m^2** PO q4h for 3 doses, and then continued at **1 mg/m^2** once a day (QD) to maintain trough levels between 10-20 ng/mL.

The sirolimus will be given for a minimum of 3 months to all patients; however, the total duration of administration will be determined by the presence or absence of GvHD and the level and kinetics of donor-recipient chimerism.

- **Transfusion Support (**See Appendix B**)**
- **Nutrition**

  Parenteral nutrition will be instituted if the patient's daily caloric intake drops below 1000 Kcal/day.

4.4.10 Management of Patient Complications

The major complications are cytomegalovirus reactivation, acute and chronic GvHD, and relapse of the original disease. Patients with these complications will be treated as follows:

- **CMV Reactivation:** (See Supportive Care Guidelines)
- **Acute GvHD** (See also Supportive Care Guidelines)

  Begin with standard dose cyclosporine (12 mg/kg given in 2 doses to maintain a level between 200-400 μg/L).

  Consider institution of extracorporeal photopheresis (ECP) in those patients willing to be so treated.

- **Chronic GvHD**

  Sirolimus will be continued at 5 mg/day with a goal of trough levels being 10-20 ng/mL (See Appendix A) with the duration dependent on the presence or absence of GvHD.

  Prednisone 20-60 mg or 1-5 mg/kg in pediatric patients daily 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, combination of psoralen and long-wave ultraviolet radiation [PUVA], thalidomide, mycophenolate, ECP, azathioprine, daclizumab, or infliximab) at the discretion of the attending physician.

- **Graft Rejection**

  This transplant study uses a nonmyeloablative preparative regimen. Therefore, autologous recovery is anticipated in patients who fail to engraft. Patients who fail to demonstrate donor T-cell engraftment will be taken off study treatment, but will continue to be monitored by study staff for 6 months post-transplant, for possible infectious complications related to the conditioning regimen. Afterwards, they will be referred back to their primary physician for further therapy.

- **Relapse of Original Disease**

  Patients will be off study treatment and will be referred back to their primary physician to return to their standard treatment, after 6 months of monitoring for infectious complications.

5.0 STUDY MODIFICATIONS

Graft failures will be monitored throughout the study. Specific need for preparative regimen modifications based on the incidence of graft failures will be made as discussed in Section 4.4.10.

6.0 ANALYSIS OF THE RESEARCH STUDY

Specimens collected strictly for research purposes will not be read by a pathologist. 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.

6.1 Chimerism Studies

We will use PCR analysis of microsatellites to identify the contribution of the donor PBPC to post-transplant hematopoiesis and to detect donor lymphocytes in the circulation (i.e., donor-recipient chimerism).

6.2 Bone Marrow Samples

A volume (up to 25 mL) of bone marrow aspirate will be collected for research studies at the pre-transplant, Day 100, and 6-month evaluation. These will be used to help elucidate the contribution of the progenitor cells to the circulating component.

6.3 Transthoracic Echocardiography

Transthoracic echocardiography (ECHO) will be performed to assess baseline ejection fraction prior to first (i.e., Day -10 to Day -8) and at 90 days (+/- 1 week) after the last Campath-1H (alemtuzumab) infusion (i.e., Day -3).

7.0 DATA AND SAFETY MONITORING

Principal Investigator: The safety of interventions and treatments associated with this study will be under continuous review by the investigative team. Accrual, efficacy, and safety data will be monitored by the PI. The data generated during this study will be monitored by the Principal Investigators for safety and compliance with protocol-specified requirements. Close cooperation between the designated members of the Protocol Team will occur to evaluate and respond to individual adverse events in a timely manner.

NIAID IRB: Accrual and safety data will be reviewed annually by the IRB. A grade 4 SAE occurrence in any particular patient will necessitate the study being placed on voluntary hold by

the Principal Investigator until the safety data has undergone a review by the DSMB. The IRB will also be notified of the event per reporting requirements. In the event of an emergency outside the facilities of NIH, the patient's personal physician will assess the situation and liaise with the PI accordingly, and transfer of care to NIH can be organized as clinically indicated. All privacy and confidentiality of all participants will be maintained according to NIH guidelines and policy. Prior to implementation of this study, the protocol and the proposed patient consent forms will be reviewed and approved by the properly constituted IRB operating according to the Title 45 of the Code of Federal Regulations, Part 46 (45 CFR 46) and 21 CFR 56. The IRB will also approve all amendments to the protocol or informed consent document, and conduct continuing annual review so long as the study is open to accrual or follow up of subjects.

NIAID Intramural Data and Safety Monitoring Board (DSMB): While the NIAID Intramural Data and Safety Monitoring Board (DSMB) is required to monitor studies that use gene therapy methodology or involve multi-center studies presenting more than minimal risk to subjects or that generate randomized blinded data, there is provision that allows DSMB to monitor studies that pose more than minimal risk to its subjects.

This study involves pediatric patients as young as 3 years who will undergo either an AlloBMT or MUD transplantation. Bone marrow transplantation carries a mortality rate as high as 40%. While this study aims to decrease the risk of transplant related mortality, 2 of the conditioning agents (Siromilus and Campath 1-H) are not approved by the FDA to be used in children <13 years of age. Because this study poses more than a minimal risk to subjects, it falls under the NIAID DSMB policy on studies that may require DSMB monitoring.

The NIAID DSMB will review the data and analysis plans of all intramural NIAID clinical studies that require DSMB oversight. The DSMB consists of experts in transplant related infectious diseases, biostatistics, and clinical trials. After the initial review, which occurs prior to opening the study to enrollment, the DSMB will review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial on a semi-annual basis. The DSMB members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review is sufficient for evaluation of the safety and welfare of study participants. All cumulative safety data reports from the trial will be submitted to the Board within 14 business days prior to the review. The DSMB will also assess the performance of overall study operations and any other relevant issues, as necessary.

All serious adverse events (SAEs) will be included in these interim reviews. SAEs that are unexpected and thought to be related to the experimental portion of the study will also be forwarded immediately to the DSMB via the DSMB Executive Secretary. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

8.0 ADVERSE EVENTS

8.1 Adverse Events Reporting Plan

The NCI Common Terminology Criteria for Adverse Events (CTC version 3.0, http://ctep.cancer.gov/forms/CTCAEv3.pdf) will be use for scoring of adverse events from any of the research procedures.

An adverse event is defined as an untoward, undesired, unplanned, clinical event in the form of a sign, symptom, disease, or laboratory or physiological observation occurring in a subject participating in this clinical study regardless of causal relationship. A medical condition that is present when the subject enters the study is not defined as an adverse event unless this medical condition worsens after the subject has been entered into the study. Clinically significant laboratory abnormalities which require medical intervention (for example, abnormal X-rays, EKGs, etc.) that occur or worsen during the clinical study also are adverse events.

A serious adverse event is any event occurring within the confines of the study that results in any of the following outcomes:

- *Death*
- *A life-threatening adverse event*-for the purpose of this study, this type of event is defined as any adverse event that places the subject at immediate risk of death from the event as it occurred. A life-threatening event does not include an event that, had it occurred in a more severe form, might have caused death, but as it actually occurred, did not create an immediate risk of death.
- *New hospitalization or prolongation of existing hospitalization* for the purpose of the study is for overnight admissions only. Emergency room and day or night visits are not considered hospitalizations. Any elective hospitalization for a preexisting condition that has not worsened does not constitute a serious adverse event.
- *A persistent disability/abnormality* is defined as a substantial disruption in a person's ability to conduct normal life functions.
- *Congenital anomaly/birth defect*

Additionally, important medical events that do not meet the above criteria may be considered serious adverse events when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. If there is any doubt whether the information constitutes a serious adverse event, the information will be treated as a serious adverse event for the purposes of this study.

The relationship between an adverse event and the study procedures will be determined by the PI on the basis of his or her clinical judgment and the following definitions:

- Definitely related: An adverse event that follows a reasonable temporal sequence from the study procedures; follows a known response pattern to the study procedures; and, when appropriate to the protocol, is confirmed by improvement after stopping the study procedures and by reappearance of the reaction after repeat exposure to the study procedures; and cannot be reasonably explained by known characteristics of the subject's clinical state or by other therapies.
- Probably related: An adverse event that follows a reasonable temporal sequence from the study procedures; follows a known response pattern to the study procedures; and, when appropriate to the protocol, is confirmed by improvement after stopping the study procedures; and cannot be reasonably explained by the known characteristics of the patient's clinical state or by other exposures.

- Possibly related: An adverse event that follows a reasonable temporal sequence from the study procedures and follows a known response pattern to the study procedures but could have been produced by the subject's clinical state or by other therapies.
- Unlikely Related: A potential relationship between study agent and the adverse event could exist (i.e., the possibility cannot be excluded), but the adverse event is most likely explained by causes other than the study agent (e.g., could readily have been produced by the subject's clinical state or could have been due to environmental or other interventions)
- Not related: An adverse event for which sufficient information exists to indicate that the etiology is unrelated to the study procedures. Two or more of the following variables apply:
    a. The adverse event does not follow a reasonable temporal sequence after the study procedures.
    b. The adverse event is readily explained by the patient's clinical state or other therapies.
    c. Negative dechallenge—the adverse event does not abate upon cessation of the study procedures (assuming that it is reasonable to expect abatement of the adverse event within the observed interval).

Serious adverse events that are not related to the study procedures may be considered to be related to the conduct of the protocol.

8.1.1 The following are examples of situations that may be deemed significant and for which the Principal Investigator may institute a voluntary clinical hold:

- A grade 4 SAE in any particular patient will necessitate placing the study on a voluntary hold by the Principal Investigator until the safety data has undergone a review by the DSMB.
- A significant rate of development of GVHD in treated patients or failure to engraft in more than one patient. The Principal Investigator may decide to place the study on a clinical hold for consideration of study amendment even if these events do not fulfill the criteria for stopping the study as defined in Section 10.0.
- Any other criteria as determined by the Principal Investigator to warrant a clinical hold.

8.2 Toxicity Criteria

The severity of adverse events will be assessed according to the National Cancer Institute (NCI) Common Terminology Criteria Scale for Adverse Events (CTC version 3.0, http://ctep.info.nih.gov/reporting/ctc.html) The following definitions will be used for toxicities that are not defined in the Common Toxicity Criteria Scale (please note that dose adjustment of the study drug is not applicable to this study):

1. Mild (Grade 1): The adverse event is noticeable to the patient but does not interfere with routine activity. The adverse event does not require discontinuing the study procedures.
2. Moderate (Grade 2): The adverse event interferes with routine activity but responds to symptomatic therapy or rest. The adverse event may require modifying the study procedures but not discontinuing the study procedures.
3. Severe (Grade 3): The adverse event significantly limits the subject's ability to perform routine activities despite symptomatic therapy. In addition, the adverse event leads to discontinuing the study procedures.
4. Life-threatening (Grade 4): The adverse event requires discontinuing the study procedures. The patient is at immediate risk of death.
5. Death related to SAE (Grade 5)

Abnormal biological or vital sign values that are considered clinically relevant by the investigator will be reported as an adverse event or as a serious adverse event. Certain information, while not necessarily meeting the definition of an adverse event, may nonetheless be of value for reporting.

The severity of the AE will be recorded in the appropriate section of the AE Case Report form (CRF) and entered into a database for Institutional Review Board (IRB), Data and Safety Monitoring Board (DSMB) review.

8.3 Recipients' Adverse Events

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

8.3.1 AE for the Screening Period

As part of the screening for enrollment into this study, we will conduct diagnostic tests that may lead to the discovery of conditions that may necessitate the stopping of enrollment into the trial. If any of the following events occur or are discovered that have no relation to the screening interventions, we will document but will not be reporting to the IRB unless they meet the criteria of an SAE:

- Eastern Cooperative Oncology Group (ECOG) performance status of 3 or more. (See Supportive Care guidelines, available at http://intranettst2.cc.nih.gov/bmt/clinicalcare)
- Diffusion capacity of carbon monoxide (DLCO) < 60% predicted.
- Left ventricular ejection fraction: < 40%.

- Transaminases > 5x upper limit of normal based on the patient's clinical situation and at the discretion of the investigator.
- Psychiatric disorder or mental deficiency severe enough as to make compliance with the BMT treatment unlikely, and/or making informed consent impossible.
- Major anticipated illness or organ failure incompatible with survival from AlloPBSC transplant.
- Patient becomes pregnant or lactating
- HIV positive
- Uncontrolled seizure disorder

8.3.2 Adverse Events for Enrolled Subjects

The following expected outcomes will be documented but will not be reported to the IRB unless they meet the criteria of an SAE.

- Transient cardiac arrhythmias
- Transient cardiac insufficiency
- Pulmonary insufficiency
- Neutropenia and its complications
- Thrombocytopenias and its complications
- Anemia and its complications
- Transfusion reactions
- Treatable infections from bacteria, viruses, protozoa, and fungi
- Late effects of transplant regimens including: cataracts, infertility, growth impairment, hypothyroidism, and dental caries.
- Headache, insomnia, psychosis, mood changes, disorientation, seizures from metabolic imbalance.
- Nausea, vomiting, diarrhea, mucositis, weight loss, dry mouth, hiccoughs, constipation.
- Well characterized drug reactions – allergic manifestation, red man syndrome
- Well characterized drug adverse effects from drugs routinely used in transplant recipients (e.g., preparative regimen, immunosuppressive drugs, and antimicrobials).
- Common adverse effects of antiemetics, analgesics, anti-inflammatory agents, and known complications of steroid therapy.
- Complications from intravenous catheters, thrombotic occlusion, infection, local reactions, cardiac arrhythmia.

The following <u>expected outcomes</u> will not be reported to the IRB at each occurrence unless they meet the criteria of an SAE. The PI will incorporate these events into the protocol and consent document as appropriate. They will be reported in summary form at the time of continuing review and at termination of the clinical study.

- Acute GvHD
- Chronic GvHD
- Graft failure/graft rejection
- Veno-occlusive disease
- Hemorrhagic cystitis
- Regimen-related toxicity
- CMV reactivation and disease

8.4 Donors

The following are expected outcomes for the Group 1 donors that will be listed in the protocol and informed consent but will not be reported to the IRB unless they meet the criteria of an SAE:

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

The following expected outcomes for the Group 1 donor would not be reported to the IRB at each occurrence unless they meet the criteria of an SAE. The PI will incorporate these events into the protocol and consent document as appropriate. They will be reported in summary form at the time of continuing review and at termination of the study.

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

8.5 Serious Adverse Events

A serious adverse event (SAE) is defined as one of the following outcomes (in accordance with FDA 21 CFR 312.32 and the NIH intramural guidance for principal investigators on reporting adverse events):

- Death from any cause

- Life threatening event, i.e., an event that places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred
- Any event that requires or prolongs in-patient hospitalization
- Any event that results in persistent or significant disability/incapacity
- Any congenital anomaly/birth defect diagnosed in a child of a subject who participated in this study
- Other medically important events that in the opinion of the investigator may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above

8.6 Reporting of Serious Adverse Events to the Principal Investigator

All SAEs will be reported to:

Elizabeth Kang, M.D.
Building 10-CRC, 6W- Room 63752
Phone: 301-402-7567
Email: Ekang@niaid.nih.gov

8.7 Adverse Event Reporting Requirements to the NIAID IRB

Because risk in this study is primarily due to the experimental treatment defined in the research protocol for which efficacy and potential harms are untested and uncertain, the Principal Investigators will perform close monitoring of patient safety with prompt reporting of serious adverse events to the IRB. A grade 4 SAE in any particular patient will necessitate the study being placed on voluntary hold by the Principal Investigator until the safety data has undergone a review by the DSMB. The IRB will be notified of the event and the DSMB action. All reporting of such events will be done via email notification to the DSMB and IRB within 24 hours of our having knowledge of the details. A written report will be sent within 7 days of the email notification.

Reporting of adverse events are outlined as follows:

- All deaths will be reported in writing to the IRB within 7 days.

Serious and unexpected adverse events:

- Life-threatening events will be reported in writing to the IRB within 7 days of our having knowledge of the details
- Non-life-threatening events will be reported in writing to the IRB within 15 days of our having a working knowledge of the details.
- In-patient admission to the NIH or other hospital for infection or other inflammatory process likely related to transplantation will not be considered a serious adverse event for expedited reporting purposes, but will be noted in information provided at the time of the continuing review. Exception will be admission to an intensive care unit or transfer to an intensive care unit which will

be reported as a serious adverse event to the IRB within 15 days of our having a working knowledge of the details.

The following types of adverse events will be reported to the IRB as part of the continuing review of the study:

- Severe or medically significant adverse events that are possibly/ probably/definitely related to study procedures and require medical intervention. An example of such events may include mild to moderate allergic reaction (hives, headaches, muscle or joint pains), shortness of breath, fevers, visual changes, numbness or tingling in any part of the body. Mild expected adverse events are recorded in the medical record but are not reported in the continuing reviews.
- Any other event or condition that in the judgment of the investigators represents a reportable event in this category.

8.8 Reporting Serious Adverse Events to DSMB

Reports of SAEs that are unexpected and thought to be related to the experimental portion of the study will be forwarded within 24 hours to the DSMB. All SAEs will be included for review semiannually by the DSMB. If the serious adverse event is thought to be related to the experimental component of the study, accession to the protocol will be stopped until full discussions with the DSMB have been held.

9.0 HUMAN SUBJECT PROTECTIONS

9.1 Recruitment Plans and Procedures

Recruitment will be made from patients or their family members who may already be participating or have participated in existing NIH studies and are being referred by their NIH physician for inclusion in this study.

Participants will also be recruited outside the existing NIH patient population. The Clinical Center Patient Recruitment and Public Liaison Office serves to provide protocol advertisement and contact information for both self-referring patients and physician referrals from outside NIH. The Clinical Center Volunteer Program website will provide the participants selected for this protocol will reflect the gender and ethnic diversity that is representative of the population.

9.2 Institutional Review Board

A copy of the protocol, proposed informed consent form, other written subject information, and any proposed advertising material will be submitted to the IRB for written approval. In addition, the study will not proceed without prior approval by the IRB.

The investigator must submit and, where necessary, obtain approval from the IRB, for all subsequent protocol amendments and changes to the informed consent document. The investigator will notify the IRB of deviations from the protocol and serious adverse events. The investigator will be responsible for obtaining IRB approval of the annual Continuing Review throughout the duration of the study.

9.3 Protocol Amendments and Study Termination

No revisions to this protocol will be permitted without documented approval from the IRB that granted the original approval for the study. In the event of a medical emergency, the investigators shall perform any medical procedures that are deemed medically appropriate.

The Principal Investigators and NIAID-IRB reserve the right to terminate the study. The investigator will notify the IRB in writing of the study's completion or early termination.

9.4 Informed Consent

Before a subject may be enrolled in the study, it is the investigator's responsibility to obtain written informed consent/assent from the subject, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study, and before any protocol-specific procedures or study medications are administered. All written information and other material to be used by subjects and investigative staff must use vocabulary and language that are clearly understood. If the subject is a minor, the parent or legal guardian must sign the consent (See Section 3.4.1).

The acquisition of informed consent will be documented in the subject's medical records, as required by 21 CFR 312.62, and the informed consent form will be personally signed and dated by the subject and by the person who conducted the informed consent discussion. The original signed informed consent form will be retained in the medical chart and a copy of the consent form will be provided to the subject.

9.5 Privacy and Confidentiality Provisions

The privacy and confidentiality of the participating subjects will be protected to the extent provided by federal, state, and local law. Subject identifiers will be attached to clinical and laboratory data. Data will be kept in laboratory or clinical sites and locked or password protected. Medical records will be made available for review when required by the Food and Drug Administration, NIAID, or other authorized users, only under the guidelines set by the Federal Privacy Act. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The investigator is obligated to inform the subjects that the above named representatives will review their study-related records without violating the confidentiality of the subjects. Subjects will not be identified in any reports on this study.

9.6 Policy Regarding Research-Related Injuries

The Clinical Center will provide short-term medical care for any injury resulting from participation in this research. In general, the National Institutes of Health, the Clinical Center, or the Federal Government will provide no long-term medical care or financial compensation for research-related injuries.

9.7 Remuneration

No remuneration will be provided to patients. All medical treatments associated or necessitated by the protocol will be provided without charge to the patient, including medications taken as an outpatient.

9.8 Hazards and Discomforts – Recipient

9.8.1 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. 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. Adverse effects of those drugs novel to nonmyeloablative transplantation are described in detail here.

9.8.2 Related to Radiation

Adverse effects of radiation have been well described. The most common include nausea and mucositis. Toxicities associated with TBI also include infections, cataracts, pulmonary insufficiency, liver toxicity and secondary late malignancies. [55, 56] Treatment-related mortality in series receiving comparable doses are rare. The risk of interstitial pneumonitis is of particular relevance given the use of busulfan with radiation; however, in order to reduce this risk, the lungs will be shielded and additionally, there will be one day of rest between the busulfan infusion and the administration of radiation. Importantly, the majority of the nonneoplastic effects were sub clinical and/or reversible. [57] Studies attempting to evaluate the risk induced by radiation alone suggest that there is a higher rate of solid tumors after radiation-based regimens. Curtis et al. reported on 19,229 patients and found a cumulative incidence rate of 2.2% at 10 years, and 6.7% at 15 years, with higher doses of TBI associated with a higher risk of solid cancers. [58] 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, in a study of 1036 patients, no relation could be found to radiation but the highest risk factor was felt to be the presence of chronic graft versus host disease, and long term treatment with cyclosporine. [59] Therefore the actual risk cannot be quantified for the low dose of 300cGy to be used in this trial; however, it is presumed to be minimal. [60-62]

On this protocol, the total radiation dose will be 300cGy. In order to further reduce the adverse effects on nonlymphoid tissue, the radiation will be divided and given in two fractions. Additionally, we will use lung shielding to minimize the pulmonary toxicity and in males use testicular shielding to minimize the risks of sterility secondary to radiation. Should there be any graft rejection with this low dose of radiation, the IRB will be notified and the dose will be increased to 200cGy x2 with the same shielding used.

9.8.3 Related to Alemtuzumab (Campath-1H, Campath®)

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The safety and efficacy of alemtuzumab were evaluated in a multicenter, open-label, non-comparative study in 93 patients with refractory B-cell chronic lymphocytic leukemia (B-CLL) who had been previously treated with alkylating agents and had failed treatment with fludarabine. Adverse events are detailed below. Previous treatment with alkylating agents and fludarabine may have contributed to both the range and severity of the adverse events 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, corticosteroids, and 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 infections (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 $\leq$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 1 fatality, Grade 3 or 4 pneumonia in 15% with 5 fatalities, and opportunistic infections in 17% with 4 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 an earlier study, where anti-herpes and anti-PCP prophylaxis was optional, 37 (66%) patients had 47 infections while or after receiving Campath-1H 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 $\geq$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 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%) of which were fatal.
- Anemia: Forty-four (47%) patients had one or more episodes of new onset National Cancer Institute-Common Toxicity Criteria (NCI-CTC) Grade 3 or 4 anemia. Sixty-two (67%) patients required red blood cell (RBC) transfusions. In addition, erythropoietin use was reported in 19 (20%) patients. Autoimmune hemolytic anemia secondary to alemtuzumab therapy was reported in 1% of patients. A positive Coombs test without hemolysis was reported in 2% of patients.
- 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 cells/μL, at 2 months after discontinuation of alemtuzumab therapy, 207 cells/μL, and 6 months after discontinuation, 470 cells/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 for ≥1 year post therapy.

Cardiac: The following events were reported in at least one patient treated on studies where Campath-1H was used as a single agent: cardiac failure, cyanosis, atrial fibrillation, cardiac arrest, ventricular arrhythmia, ventricular tachycardia, angina pectoris, coronary artery disorder, myocardial infarction, and pericarditis. Some of these cardiac abnormalities may be irreversible. For this reason, we will monitor subjects with an echocardiogram, a 24 hour Holter monitor and serum troponin levels before treatment begins, after the last dose of Campath-1H, and at the 6 month follow up visit. We will also closely monitor subjects for cardiac symptomatology and ask them to immediately report any cardiac symptoms (palpitations, irregular pulse, difficulty in breathing, dizziness, swelling in the ankles, chest discomfort, or pain).

9.8.4 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:

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Body as a Whole: abdomen enlarged, abscess, ascites, cellulitis, chills, face edema, flu syndrome, generalized edema, hernia, Herpes zoster infection, lymphocele, malaise, pelvic pain, peritonitis, sepsis;

Cardiovascular System: atrial fibrillation, congestive heart failure, hemorrhage, hypervolemia, hypotension, palpitation, peripheral vascular disorder, postural hypotension, syncope, tachycardia, thrombophlebitis, thrombosis, vasodilatation;

Digestive System: anorexia, dysphagia, eructation, esophagitis, flatulence, gastritis, gastroenteritis, gingivitis, gum hyperplasia, ileus, liver function tests abnormal, mouth ulceration, oral moniliasis, stomatitis;

Endocrine System: Cushing's syndrome, diabetes mellitus, glycosuria, hypercholesterolemia, hyperlipidemia;

Hemic and Lymphatic System: ecchymosis, leukocytosis, lymphadenopathy, polycythemia, thrombotic thrombocytopenic purpura (hemolytic-uremic syndrome);

Metabolic and Nutritional: acidosis, alkaline phosphatase increased, blood urea nitrogen (BUN) increased, creatine phosphokinase increased, dehydration, healing abnormal, hypercalcemia, hyperglycemia, hyperphosphatemia, hypocalcemia, hypoglycemia, hypomagnesemia, hyponatremia, lactic dehydrogenase increased, serum glutamic oxaloacetic transaminase (SGOT) increased, serum glutamic pyruvic transaminase (SGPT) increased, weight loss;

Musculoskeletal System: arthrosis, bone necrosis, leg cramps, myalgia, osteoporosis, tetany;

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

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, bladder pain, dysuria, hematuria, hydronephrosis, impotence, kidney pain, kidney tubular necrosis, nocturia, oliguria, pyelonephritis, pyuria, scrotal edema, testis disorder, toxic nephropathy, urinary frequency, urinary incontinence, urinary retention.

Less frequently occurring adverse events included: mycobacterial infections, Epstein-Barr virus infections, and pancreatitis.

9.8.5 Related to Busulfan

Commonly listed adverse events include nausea and vomiting, hair loss, and seizure. The risk of seizure will be minimized by the administration of a prophylactic anticonvulsant medication 30 minutes prior to busulfan treatment.

Hematological Effects: The most frequent serious consequence of treatment with busulfan at the recommended dose and schedule is profound myelosuppression occurring in all patients. Severe granulocytopenia, thrombocytopenia, anemia, or any combination thereof may develop. Frequent complete blood counts, including white blood cell differentials, and quantitative platelet counts should be monitored during treatment and until recovery is achieved. Absolute neutrophil counts dropped below 500 cells/μL (i.e., $<0.5x10^9$/L) at a median of 4 days post transplant in 100% of patients treated in the busulfan clinical trial. The absolute neutrophil count recovered at a median of 13 days following allogeneic transplantation when prophylactic G-CSF was used in the majority of patients. Thrombocytopenia (< 25,000 cells/μL or requiring platelet transfusion) occurred at a median of 5-6 days in 98% of patients. Anemia (hemoglobin <8.0g/dL) occurred in 69% of patients. Antibiotic therapy and platelet and red blood cell support should be used when medically indicated.

Pulmonary: Bronchopulmonary dysplasia with pulmonary fibrosis is a rare but serious complication following chronic busulfan therapy. The average onset of symptoms is 4 years after therapy (range 4-10 years).

Cardiac: Cardiac tamponade has been reported in pediatric patients with thalassemia (8/400 or 2% in one series) who received high doses of oral busulfan and cyclophosphamide as the preparatory regimen for hematopoietic progenitor cell transplantation. Of these 8 children, 6 died and 2 were saved by rapid pericardiocentesis. Abdominal pain and vomiting preceded the tamponade in most patients. No patients treated in the busulfan injection clinical trials experienced cardiac tamponade.

Neurological: Seizures have been reported in patients receiving high dose oral busulfan at doses producing plasma drug levels similar to those achieved following the recommended dosage of busulfan. Despite prophylactic therapy with phenytoin, one seizure was reported during an autologous transplantation clinical trial of Busulfex®. This episode occurred during the cyclophosphamide portion of the conditioning regimen, 36 hours after the last busulfan dose. Anti-convulsant prophylactic therapy should be initiated prior to busulfan treatment.

Hepatic Effects: Current literature suggests that high busulfan area under the plasma concentration versus time curve (AUC) values (>1500μM/min) may be associated with an increased risk of developing hepatic veno-occlusive disease (HVOD). Patients who have received prior radiation therapy, greater than or equal to 3 cycles of chemotherapy, or a prior progenitor cell transplant may be at an increased risk of developing HVOD with the recommended busulfan dose and regimen. Based on clinical examination and laboratory findings, hepatic veno-occlusive disease was diagnosed in 8% (5/61) of patients treated with busulfan in the setting of allogeneic transplantation, was fatal in 2/5

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cases (40%), and yielded an overall mortality from HVOD in the entire study population of 2/61 (3%). Of the 5 patients diagnosed with HVOD, 3 were retrospectively found to meet the Jones' criteria. The incidence of HVOD reported in the literature from the randomized, controlled trials were 7.7% to 12%.

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Others: Other reported adverse reactions include: headache (mild or moderate 64%, severe 5%), abdominal pain (mild or moderate 69%, severe 3%) asthenia (mild or moderate 49%, severe 2%), allergic reaction (mild or moderate 24%, severe 2%), injection site inflammation (mild or moderate 25%), injection site pain (mild or moderate 15%), chest pain (mild or moderate 26%), back pain (mild or moderate 23%), myalgia (mild or moderate 16%), arthralgia (mild or moderate 13%), and ear disorder in 3%.

Over dosage: There is no known antidote to busulfan. The principal toxic effects are bone marrow depression and pancytopenia but the central nervous system, liver, lungs, and gastrointestinal tract may be affected. The hematologic status should be closely monitored and vigorous supportive measures instituted, if necessary. Dialysis may be considered in the management of overdose, as there is 1 report of successful dialysis of busulfan. Busulfan is metabolized by conjugation with glutathione; thus, administration of glutathione may be considered.

9.9 Hazards and Discomforts – Donor

9.9.1 Related to G-CSF

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

G-CSF has been given to large numbers of normal donors without major adverse effects or long-term consequences. The immediate adverse effects of G-CSF in 50-75% of recipients are bone pain, fatigue, insomnia, myalgia and headache. These are usually mild and are self-limiting. Reversible thrombocytopenia, with platelet counts falling to the region of 100,000/mm^3 is frequent and to less than 60,000/mm^3 after a third apheresis. Two patients have been reported to experience non-fatal splenic rupture after more prolonged treatment with higher doses of G-CSF. Of these 2 patients, 1 had concurrent mononucleosis, a second cause for splenic rupture. Patients with ongoing ischemic heart disease have been reported to have angina seemingly temporally related to G-CSF administration and apheresis.

9.9.2 Related to Central Line Placement

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

9.9.3 Related to Apheresis

Adverse reactions related to apheresis include hypotension resulting from transient blood volume loss and cutaneous paresthesia from the use of anticoagulant. The former toxicity can be corrected by postural changes and volume replacement. The latter is manageable with slowing the rate of anticoagulant infusion and/or providing calcium supplement. In exceptional instances, the donor may be required to donate PBPC a third time or to give bone marrow. Donation of PBPC on 3 successive days significantly increases the risk of thrombocytopenia (<100,000/μL). However, thrombocytopenia is transient and unlikely to cause clinical sequelae. There is no additional risk to the donor giving marrow after PBPC donation other than normally would be associated with bone marrow harvesting.

9.10 Risks in Relation to Benefit

9.10.1 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.

The research, therefore, involves more than a minor increase over minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102)

9.10.2 For Pediatric Donors

The stem cell collection procedure is considered part of the research for the donors, namely the use of G-CSF in young children for mobilizing peripheral blood progenitor cells. The risks associated with the stem cell collection procedure would be considered as risks of research for the pediatric donors. Therefore participation as a stem cell donor in this study is subject to the criteria set forth in 45 CFR 46, Subpart D.

9.10.3 For Pediatric Donors Involved in Laboratory Research Studies

The inclusion of children satisfies the criteria set forth in 45 CFR 46.404 as follows:

(a) *The research does not involve greater than minimal risk*. On this study, we will obtain blood specimens for research from pediatric donors that will be concurrent with clinically indicated sampling. Therefore, there is no risk associated with sample collection for research because research will only be performed on material obtained during standard clinical intervention.

Research specimens will be stored in Dr. Malech's laboratory (LHD labs, Bldg 10 CRC room 5-3750). Samples will never be labeled with the child's name. Samples will be assigned a unique code known only to the principal investigator, which will serve as a link to the child's clinical information collected as part of this research protocol. No samples will be provided to investigators outside the branch. Therefore, confidentiality is protected.

Only those laboratory tests involving no greater than minimal risk will be conducted. Research will include genetic testing; therefore, there is genetic testing-associated risk.

(b) Adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians, as set forth in 45 CFR 46.408.

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

9.11 Informed consent

The investigational nature and research objectives of this trial, as well as the procedures and their attendant risks and discomforts will all be carefully explained to the subject, and a signed informed consent document will be obtained prior to entry onto this study. Drs. Kang or Malech will lead this discussion.

If the subject is a minor, the parent who signs the consent for the minor must be a legally recognized parent or guardian. Where deemed appropriate by the clinician, and the child's parent or guardian, the child will also be included in all discussions about the trial and a minor's assent will be obtained. The parent or guardian will sign on the designated line on the informed consent document attesting to the fact that the child had given assent.

If at any time during participation in the protocol 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 or parent/legal guardian. Documentation will be provided to the IRB and if necessary the informed consent will be amended to reflect relevant information.

10.0 BIOSTATISTICAL CONSIDERATIONS

This trial is designed to estimate the engraftment rate, which is anticipated to be about 80%. The study has a sample size of 10 per group and this will allow us to estimate the engraftment rate with reasonable precision. See table below:

N	expected # engraftment assuming true rate is 80%	95 % binomial exact CI	
10	8	.4439045	.9747893
15	12	.5191089	.956688
25	20	.5929626	.9316885

N	expected # engraftment assuming true rate is 70%	95 % binomial exact CI
10	7	.3475471 .9332605
15	11	.4489968 .9221285
25	18	.5061232 .8792833

10.1 Stopping Rules

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 (acute and chronic*)* Grade III-IV GvHD, which we would consider a failure of the regimen. Therefore, regimen failure is a composite endpoint that means graft rejection, acute Grade III-IV GvHD, or chronic Grade III-IV GvHD. Regimen failure will be evaluated for each patient during the 6 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.

11.0 PHARMACEUTICALS

11.1 Alemtuzumab (Campath-1H, Campath®)

Generic name: alemtuzumab

Classification: monoclonal antibody

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

Availability: Commercial: Berlex Laboratories

Dose: 30 mg IV 3 times a week after initial doses of 3mg and 10mg (FDA approved dose for Chronic Lymphocytic Leukemia)

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

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

Adverse effects: Nausea, vomiting, fever, chills, rigors, hypotension, rash, headaches, abdominal pain, myalgias, arthralgia, diarrhea, dyspnea, bronchospasm, angioedema, tumor lysis syndrome, infections, transient neutropenia and thrombocytopenia, pancytopenia, impaired sperm motility.

11.2 Daclizumab (Zenapax®)

General: Zenapax® (Daclizumab) is an immunosuppressive, humanized immunoglobulin-G1 monoclonal antibody. It binds to the CD-25 or Tac subunit of human IL-2 receptors on T-lymphocytes. The volume of distribution is approximately 6 liters and the systemic clearance is about 15 mL/hour. The elimination half-life is approximately 480 hours (20 days).

Administration: Daclizumab should be mixed with 50 mL of sterile 0.9% sodium chloride solution and administered via a peripheral or central vein over a 15 minute period.

Adverse Drug Reactions: Generally, daclizumab is a well tolerated agent. In clinical trials the most frequently reported adverse events were gastrointestinal disorders. Also reported are hypertension, hypotension, chest pain, tachycardia, edema, dyspnea, pulmonary edema, coughing, thrombosis, bleeding, and renal tubular necrosis.

Dose: In the treatment of GvHD: 1mg/kg IV on Days 1, 4, 8, 15, and 22.

11.3 Human Recombinant Granulocyte Colony Stimulating Factor

Generic: G-CSF or Filgrastim (Amgen; Neupogen®)

Classification: glucoprotein

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

Metabolism: Absorption and clearance of G-CSF follow first order kinetics. A positive linear correlation occurs between the parenteral dose and both the serum concentration and area under the concentration-time curves. The elimination $T_{1/2}$ is 3.5 hours.

Administration: G-CSF may be given, as a single daily dose, SC or IV over 30 min.

Adverse effects: Medullary bone pain, myalgia, headache, insomnia, chills, low grade fever, rash, injection site irritation, exacerbation of preexisting inflammatory conditions, splenomegaly, mild to moderate decreases in platelet number, and reversible elevations in uric acid, lactate dehydrogenase, and leukocyte alkaline phosphatase.

G-CSF is a protein that is produced by normal human bone marrow cells. G-CSF used in this study is produced by *Escherichia coli* with recombinant DNA technology. The only differences between this product and natural G-CSF are an additional N-terminal methionine necessary for expression in *E. coli* and the fact that this product is unglycosylated. G-CSF is approved by the US Food and Drug Administration for use in preventing chemotherapy-induced leukopenia. This drug has been used in cancer patients for prolonged periods of time without major toxicities. The extensive use of this drug in many patients has also proven its safety at a dose of 10 mg/kg/day, an effective dose for up to 80% of subjects. The most frequent adverse effect is bone pain originating from the stimulated proliferation of cell mass within the bone marrow. The pain is usually mild, can be alleviated most often by acetaminophen or non-steroidal analgesics, and will cease altogether once G-CSF is discontinued. G-CSF administration is associated with increased inflammation in patients with autoimmune diseases or subjects with infections, but such patients are excluded from this study. Leukocytosis is expected after

administration of G-CSF. Other adverse reactions associated with the use of G-CSF as cited in the literature include: fatigue (50%), headache (30%), muscle aches (25%), insomnia (rare), fever (rare), transient but reversible increases of alkaline phosphatase, lactate dehydrogenase, and uric acid levels, exacerbation of preexisting skin rashes, and thrombocytopenia (mild, reversible).

Recombinant G-CSF is available from the pharmacy at the National Heart, Lung and Blood Institute in colorless glass, single-use vials containing either 300mg in 1.0 mL vials or 480mg in 1.6 mL vials (300 mg/mL). It is formulated as a sterile, clear, colorless liquid in a 10 mM sodium acetate buffer at pH 4.0. The quantitative composition (per mL) is:

Recombinant G-CSF	300 mg
Acetate	0.59 mg
Mannitol	50 mg
Tween 80™	0.004%
Sodium	0.035 mg
Water for injection (qs ad) to	1.0 mL

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

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

11.4 Infliximab (Remicade®)

General: Remicade® (Infliximab) is a chimeric monoclonal antibody directed against tumor necrosis factor-alpha (TNF-α). TNF-α, a pro-inflammatory protein produced by immune cells, is believed to play a role in the pathogenesis of GvHD. Infliximab is a human/murine chimeric monoclonal antibody that binds specifically to both membrane-bound and soluble TNF-α.

Administration: Infliximab should be mixed with 250 mL of sterile 0.9% sodium chloride solution and administered via a peripheral or central vein over a period of not less than 2 hours. An in-line, sterile, non-pyrogenic, low-protein-binding filter with a pore size of 1.2um or less should be used for administration.

Adverse Drug Reactions: Infliximab is generally well tolerated. The most common adverse effect has been nausea. Infusion reactions that have been reported include chest pain, nausea, fever, facial flushing, headache, urticaria, dyspnea, and hypotension. Patients treated with infliximab are at an increased risk of developing respiratory infections. Other serious infections have also been reported including sepsis, reactivation of Hepatitis B, and disseminated tuberculosis. A tuberculin skin test is recommended prior to initiating therapy. Patients are at an increased risk of lymphoma (non-Hodgkin's lymphoma and Hodgkin's disease). Severe hepatic reactions, including acute liver failure, jaundice, hepatitis, and cholestasis have been reported rarely in post-marketing data.

Dose: In the treatment of GvHD: 10 mg/kg IV every week for a maximum of 4 doses.

11.5 Sirolimus (Rapamune®)

General: Sirolimus is an immunosuppressant that is FDA approved for use in combination with cyclosporine and corticosteroids for prophylaxis of organ rejection after kidney transplantation.

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

Adverse Drug Reactions: Common adverse effects attributed primarily to sirolimus include anemia, thrombocytopenia, hyperlipidemia, and hypertension. When used in combination with cyclosporine, careful monitoring of renal function is required because this combination has been associated with increases in serum creatinine. Co-administration of sirolimus with strong inhibitors or inducers of CYP3A4 and/or P-gp is not recommended.

Dose: Variable dosing. FDA approved adult dose for renal transplant rejection: loading dose of 6 mg followed by a maintenance dose of 2 mg once daily. For patients 13 years or older who weigh less than 40 kg, the 1-time loading dose is 3 mg/m^2 followed by a maintenance dose of 1 mg/m^2/day; the dose should be decreased by one-third for patients with hepatic impairment.

Metabolism: The drug is metabolized in the liver by cytochrome P450 3A enzymes and has an elimination half-life of about 60 hours.

Vaccination: Use of live vaccines during treatment with sirolimus should be avoided as vaccination may be less effective.

11.6 Busulfan

Source: ESP Pharma

Generic: busulfan

Other: Busulfex® (IV formulation), Myleran® (PO formulation)

Classification: Alkylating agent that prevents cell division by altering DNA

Metabolism: Via the liver; sulfoxane, 3-hydroxysulfoxane, and other metabolites are formed and excreted in the urine.

Formulation and Preparation

Tetramethylene di(methanesulphonate); Butane-1,4-diol di(methanesulphonate).; supplied as an ampoule containing 60 mg/10 mL injection.

Stability and Solubility

Busulfan for injection should be refrigerated at 2 to 8°C.

Incompatibilities and Drug Interactions

Itraconazole decreases busulfan clearance. Phenytoin increases busulfan clearance by 15%.

Administration Procedure

IV busulfan must be diluted prior to use with either 0.9% Sodium Chloride Injection, USP (normal saline) or 5% Dextrose Injection, USP (D5W). The diluent quantity should be 10 times the volume of busulfan injection, so that the final concentration is approximately 0.5 mg/mL.

Busulfan will be administered intravenously via a central venous catheter as a 2-hour infusion on 2 consecutive days for a total dose of 10 mg/kg. Antiemetics of the 5-HT3 class should be administered prior to the first dose of busulfan and continued on a fixed schedule through busulfan administration.

Clonazepam IV will be given daily for the 2 days of the infusion as anti-epileptic prophylaxis.

Adverse effects: please see Section 9.8.5 for discussion on this.

12.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control.

All infectious specimens will be sent using the ISS-1 SAF-T-PAK mandated by the International Air Transport Association Dangerous Goods Regulations-Packing Instruction 602.
Please refer to individual carrier guidelines, e.g., Fed Ex, Airborne, for specific instructions.

13.0 DATA COLLECTION, MANAGEMENT AND STORAGE

13.1 Data and Safety Monitoring Plan

Study data will be collected and maintained using the Crimson computerized system . The Principal Investigator is responsible for assuring that the data collected is complete, accurate, and recorded in a timely manner. Source documentation (the point of initial recording of a piece of data) should support the data collected on the case report form, and be signed and dated by the person recording and/or reviewing the data. Source documents must include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Source documents include, but are not limited to, the patient medical records, laboratory reports, ECG tracings, x-rays, radiologist reports, patient diaries, biopsy reports, ultrasound photographs, patient progress notes, pharmacy records, and any other similar reports or records of procedures performed during the subject's participation in the protocol. Data for Crimson will be collected during patient visits, phone calls with subjects and health care providers, patient diaries, and information abstracted from the patient's medical record. It is not acceptable for Crimson to be the only record of the patient's participation in the study. This is to ensure that anyone who would access the patient medical record has adequate knowledge that the patient is participating in a clinical trial.

13.2 Study Monitoring

The trial will be conducted in compliance with this protocol, International Conference on Harmonization (ICH) Guideline for Good Clinical Practices (GCP), and any applicable regulatory requirement(s). Monitors under contract to the NIAID will visit the clinical research site to monitor all aspects of the study in accordance with the appropriate regulations. The objectives of a monitoring visit will be:

1) To verify the prompt reporting of all data points, including reporting SAEs, checking availability of signed informed consent

2) To compare individual subject records, data table pulls and the source documents (supporting data, laboratory specimen records and medical records to include physician progress notes, nurses' notes, subjects' hospital charts)
3) To ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records.

The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (OHRP) and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms, data table pulls) and pertinent hospital or clinical records readily available for inspection by the local IRB, , the site monitors, and the NIAID staff for confirmation of the study data.

The investigator is responsible for retaining all essential documents listed in the ICH Good Clinical Practice Guideline. The essential documents should be maintained until at least 2 years after the last approval of a marketing application, or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational agent, or at least 3 years per NIH FWA, whichever is longest. Storage of all trial-related documents will be such that confidentiality will be strictly maintained.

14.0 PLAN FOR USE AND STORAGE OF BIOLOGICAL SAMPLES

Patients will be asked to provide 10 mL of blood so that genomic DNA can be isolated and stored for future genetic studies. These studies will concern genes that are involved with regulation of inflammation, and innate or adaptive immune responses to infections. The tissue specimen blocks requested for this study, both paraffin-embedded and fresh-frozen, will also be stored. These specimens will be coded separately with a unique number and not by patient name. A separate file will be maintained that will include the key for coupling the code number to a specific patient. Both the specimens and the file will be stored in a locked room, in locked storage units for the purpose of maintaining confidentiality. Access to research samples will be limited using either a locked room or a locked freezer. Data will be kept in password-protected computers. Only investigators or their designee(s) will have access to the samples and data. We anticipate the storage of these samples for 10 years after the completion of the protocol. These specimens may be used in collaborative research, but the specimens will not be able to be linked with specific patients. The research use of these stored specimens will be conducted in accordance with policy set by the NIH Office of Human Subjects Research and only with appropriate IRB approval. Informed consent will be obtained for these purposes (see Consent Form). The Prinicipal investigator will notify the NIAID IRB under any of the following circumstances:

a. The loss of key for coupling the code number to a specific patient
b. An unauthorized entry into the locked room where samples are stored.
c. An unauthorized entry into computerized data

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16.0

APPENDIX A: GvHD PROPHYLAXIS: SIROLIMUS (RAPAMUNE®)

Dose

Sirolimus Day -1 to Day 100 in all patients. Subjects will be advised not to take medication with grapefruit juice and not to take St. John's wort while on medication. Subjects must also be advised to limit exposure to sunlight and UV light due to an increased risk of skin cancer. Women of child-bearing potential will be informed of the potential risks during pregnancy and that they should use effective contraception prior to initiation of drug.

Levels

Serum levels will be monitored as appropriate to maintain trough levels between 10-20 ng/mL.

Mechanism of Action

Sirolimus blocks T cell activation by inhibiting transport of cytoplasmic nuclear factor of activated T-cells (NFAT) into the cell nucleus, thereby preventing IL-2 transcription.

Adverse Drug Reactions: (See Section 11.5)

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

Major ABO incompatible recipient - donor

Patient	Donor	Transfused RBC = patients
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

APPENDIX C: IMMUNODEFICIENCY AlloBMT

DONOR

16.1.1 Schedule of Events

Patient's Name: _____ MRN:_____

Protocol Requirement	Visit 1 (Screen)	Visit 2 (Day 1)	Visit 3 (Day 2)	Visit 4 (Day 3)	Visit 5 (Day 4)	Visit 6 (Day 5)	Visit 7 (Day 6)
DATE:							
CONSENT	X						
H &P (C=complete; D=directed)	C						
CBC with diff	X						
Chem 20	X						
COAG Panel (PTT/PT/FIB/TT)	X						
High resolution HLA typing	X						
HLA Ab screening	X						
HIV, Hep B, C antibody, HTLV I/II	X						
EBV PCR	X						
CMV antibodies	X						
Serum Pregnancy test	X						
RPR	X						
Inspection of vessels by DTM to determine the need for CVC catheter for apheresis	X						
LYMPH PHENO BMT	X						
G-CSF injections		X	X	X	X	X	X*
Apheresis for PBPC collection						X	X*
OTHERS:							

X*- Day 6 of G-CSF injections followed by a second apheresis will only occur for patients whose product contains less than the minimum dose of CD34+ cells/kg after the first apheresis collection.

Note:

1. **All pediatric patients and adult patients requiring a CVC catheter for collection will be admitted as inpatients.**
2. The amount of blood that will be drawn from adult donors will not exceed 450 ml over any six- week period. For pediatric patients, we will not draw more than 3 ml/kg in a single blood withdrawal, and no more than 7 ml/kg will be drawn over any six-week period.

Please see the excel spread sheet for the following appendices:

Appendix D: Patient/Recipient AlloBMT INITIAL SCREENING

Appendix E: Group 1: AlloBMT Recipient INPATIENT schedule of Event

Appendix F: Group 2: MUD Recipient INPATIENT schedule of Events

Appendix G: AlloBMT and MUD Recipient DISCHARGE TO DAY +100

Appendix H: AlloBMT and MUD Recipient BEYOND DAY 100

Commented [c20]: Note that these were not provided for review.

Commented [JPK21]: Please convert each of these to MSWord documents and incorporate into protocol document.

APPENDIX I: Study Drug Information Sheets

Help | Main | New Search | Search Results

DrugPoints® System

BUSULFAN

- **Common Tradenames** *(See Complete Tradename Listing)*
  - Busulfex
  - Myleran
- **Class**
  - Alkylating Agent
  - Antineoplastic Agent
- **Dosage, Adult (usual)**
  - Chronic myeloid leukemia, Palliative: induction, 60 mcg/kg or 1.8 mg/m(2) ORALLY every day; usual dose is 4-8 mg
  - Chronic myeloid leukemia, Palliative: maintenance, 1-3 mg ORALLY every day
  - Chronic myeloid leukemia - Hemopoietic stem cell transplant: conditioning agent, 0.8 mg/kg IV every 6 hours for 4 days (16 doses) in combination with cyclophosphamide 60 mg/kg as a 1-hour infusion on bone marrow transplant day minus 3, six hours after the final dose of busulfan, and again on bone marrow/progenitor cell transplant day minus 2
  - Chronic myeloid leukemia - Hemopoietic stem cell transplant: (oral dosage not FDA approved) conditioning agent, 1 mg/kg ORALLY every 6 hours for 4 days (16 doses) in combination with cyclophosphamide 60 mg/kg as a 1-hour infusion on bone marrow transplant day minus 3, six hours after the final dose of busulfan, and again on bone marrow/progenitor cell transplant day minus 2
- **Dosage, Pediatric, (usual)**
  - Although ORAL busulfan is indicated for the treatment of chronic myelogenous leukemia in children, the safety and efficacy of INTRAVENOUS busulfan in pediatric patients have not been determined
  - Bone marrow transplant - Hemopoietic stem cell transplant: conditioning agent, 1.1 mg/kg INTRAVENOUS busulfan if pt weighs less than or equal to 12 kg, or 0.8 mg/kg if more than 12 kg, infused over 2 hours every 6 hours for 4 days (16 total doses) followed by 4 once daily infusions of cyclophosphamide 50 mg/kg; hematopoietic progenitor cells are infused after 1 day of rest
  - Chronic myeloid leukemia, Palliative: induction, 60 mcg/kg or 1.8 mg/m(2) ORALLY every day; usual dose is 4-8 mg
  - Chronic myeloid leukemia, Palliative: maintenance, 1-3 mg ORALLY every day
  - Chronic myeloid leukemia - Hemopoietic stem cell transplant: (oral dosage not FDA approved) conditioning agent, 1 mg/kg ORALLY every 6 hours for 4 days (16 doses) in combination with cyclophosphamide 60 mg/kg as a 1-hour infusion on bone marrow transplant day minus 3, six hours after the final dose of busulfan, and again on bone marrow/progenitor cell transplant day minus 2
- **Dose Adjustments:**
  - Therapeutic drug monitoring: The following formula can be used to adjust subsequent doses of IV busulfan to achieve the desired target (1125 micromolar X minute (mM X min))area under the curve (AUC): adjusted dose (mg) = actual dose (mg) X target AUC (mM X min)/actual AUC (mM X min)
  - Geriatrics: dose selection in the elderly should be cautious, usually starting at the low end of the

Help | Main | New Search | Search Results

DrugPoints® System

ALEMTUZUMAB

- **Common Tradenames** *(See Complete Tradename Listing)*
  - Campath
- **Class**
  - Immunological Agent
  - Monoclonal Antibody
- **Dosage, Adult (usual)**
  - B-cell chronic lymphocytic leukemia, In patients who have been treated with alkylating agents and who have failed fludarabine therapy: initiate therapy at 3 mg as a 2-hour IV infusion daily. When the 3-mg daily dose is tolerated (e.g., infusion-related toxicities are Grade 2), escalate to 10 mg and continue until tolerated. When the 10-mg dose is tolerated, the maintenance dose (30 mg) may be initiated.
  - B-cell chronic lymphocytic leukemia, In patients who have been treated with alkylating agents and who have failed fludarabine therapy: maintenance dose is 30 mg/day IV administered three times per week on alternate days for up to 12 wk
- **Dosage, Pediatric, (usual)**
  - safety and effectiveness have not been established in children
- **Dose Adjustments:**
  - hematologic: FIRST OCCURRENCE OF ANC less than250/microliter and/or platelet count 25,000/microliter- withhold therapy. When ANC 500/microliter and platelet count 50,000/microliter, resume at same dose. If delay between dosing is 7 days, initiate therapy at 3 mg and escalate to 10 mg and then to 30 mg as tolerated. SECOND OCCURRENCE OF ANC less than250/microliter and/or platelet count 25,000/microliter- withhold therapy. When ANC 500/microliter and platelet count 50,000/microliter, resume 10 mg. If delay between dosing is 7 days, initiate therapy at 3 mg and escalate to 10 mg only. THIRD OCCURRENCE OF ANC less than250/microliter and/or platelet count 25,000/microliter- Discontinue therapy permanently. For a decrease of ANC and/or platelet count to 50% of the baseline value in patients initiating therapy with a baseline ANC 500/microliter and/or a baseline platelet count 25,000/microliter- withhold therapy. When ANC and/or platelet count return to baseline value(s), resume therapy. If the delay between dosing is 7 days, initiate therapy at 3 mg and escalate to 10 mg and then to 30 mg as tolerated.
- **Administration**
  - do not administer as IV bolus or push
  - single-use vial concentration 30 mg/mL; single-use ampoule concentration 10 mg/mL
  - do not shake vial; use 5-micron filter/dilute in 100 mL NS/D5W; protect from light; use within 8 hr
  - escalation to 30 mg can usually be accomplished in 3-7 days
  - premedication (diphenhydramine 50 mg, acetaminophen 650 mg) should be given prior to the first dose, at dose escalations, and as clinically indicated
  - Pneumocystis carinii and herpes virus infection prophylaxis is recommended upon initiation of alemtuzumab therapy and should be continued for a minimum of 2 months following the last dose or until CD4 counts are 200 cells/liter, whichever is later
- **Monitoring**

Help | Main | New Search | Search Results

DrugPoints® System

SIROLIMUS

- Common Tradenames *(See Complete Tradename Listing)*
  - Rapamune
- **Class**
  - Immune Suppressant
- Dosage, Adult (usual)
  - Renal transplant rejection, As part of a regimen that includes cyclosporine and corticosteroids; Prophylaxis: loading, 6 mg ORALLY
  - Renal transplant rejection, As part of a regimen that includes cyclosporine and corticosteroids; Prophylaxis: maintenance, 2 mg ORALLY once daily
  - Renal transplant rejection, Cyclosporine-sparing regimen, for patients with low to moderate immunologic risk of organ rejection; Prophylaxis: cyclosporine should be withdrawn 2-4 months posttransplant over a 4-8 week period; adjust sirolimus dose for whole blood trough concentration 12-24 ng/mL (chromatographic method); sirolimus dose will need to be approximately 4 fold higher in absence of cyclosporine
- Dosage, Pediatric, (usual)
  - safety and efficacy in pediatric patients below the age of 13 years has not been established
  - Renal transplant rejection, As part of a regimen that includes cyclosporine and corticosteroids; Prophylaxis: (age 13 yr and older, weight 40 kg or greater) loading, 6 mg ORALLY
  - Renal transplant rejection, As part of a regimen that includes cyclosporine and corticosteroids; Prophylaxis: (age 13 yr and older, weight less than 40 kg) loading, 3 mg/m(2) ORALLY
  - Renal transplant rejection, As part of a regimen that includes cyclosporine and corticosteroids; Prophylaxis: (age 13 yr and older, weight 40 kg or greater) maintenance, 2 mg ORALLY once daily
  - Renal transplant rejection, As part of a regimen that includes cyclosporine and corticosteroids; Prophylaxis: (age 13 yr and older, weight less than 40 kg) maintenance, 1 mg/m(2)/day ORALLY once daily
- Dose Adjustments:
  - hepatic impairment: reduce maintenance dose by one-third; not necessary to reduce loading dose
- **Administration**
  - oral solution: administer in glass or plastic with 2 oz water or orange juice ONLY, mix and take, after taking add 4 oz orange juice or water to container, mix vigorously and take; administer tablets and solution consistently with or without food
  - take sirolimus at least 4 hours after cyclosporine dose
  - do NOT administer with grapefruit juice; grapefruit juice reduces CYP3A4-mediated drug metabolism and may enhance P-glycoprotein (P-gp) mediated drug counter-transport from enterocytes of small intestine
- **Monitoring**
  - development of hyperlipidemia
  - development of rhabdomyolysis (if receiving concomitant HMG-CoA reductase inhibitor)
  - blood sirolimus levels in patients likely to have altered drug metabolism (pediatric patients, hepatic impairment, CYP3A4 inducers and inhibitors, changes in cyclosporine dosing