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CLINICAL RESEARCH PROTOCOL

Project: **#99-H-0050**

Date: February 22, 2022

Title: Non-Myeloablative Allogeneic Peripheral Blood Mobilized Hematopoietic Precursor Cell Transplantation For Hematologic Malignancies In High Risk Patients and In Patients With Debilitating Hematologic Diseases

Other Underlying Words: Peripheral blood stem cells, non-myeloablative bone marrow transplantation, engraftment, graft-versus-host disease, graft-versus-leukemia, graft-versus-tumor, cyclophosphamide, fludarabine, donor apheresis

Principal Investigator:

Richard Childs, M.D., CMTB, NHLBI (E)	594-8008	Bldg 10, CRC 3-5332

Medically Responsible Investigator:

Richard Childs, M.D., CMTB, NHLBI (E)	594-8008	Bldg 10, CRC 3-5332

Subjects of Study	Group	Number	Sex	Age range
Recipients	A	45	either	$\geq$ 10 and $\leq$ 75 $\geq$ 8 and $\leq$ 65(multiple myeloma)
Recipients	B	62	either	$\geq$ 8 and $\leq$ 80
Total Recipients	Both A and B	107	either	
Donors	-	107	either	$\geq$ 2 and $\leq$ 80

Project Involves Ionizing Radiation?	Yes (only if medically indicated)
Off Site Project?	No
Multi-Institutional Project?	No
DSMB Involvement?	Yes

PRECIS

Patients with malignant and non-malignant hematologic diseases including severe aplastic anemia (SAA), paroxysmal nocturnal hemoglobinuria (PNH), myelodysplastic syndrome (MDS), acute and chronic leukemias, Hodgkin's and non-Hodgkin's lymphoma and multiple myeloma (MM) can now be cured by allogeneic bone marrow transplantation (BMT). This curative effect has been ascribed to the use of high dose chemo-radiotherapy and the anti-tumor or anti-bone marrow effect of the allograft. Dose intensification of conditioning regimens in attempts to reduce disease recurrence has been largely unsuccessful because of increased toxicity and mortality. Indeed, most evidence now points to donor- derived T-cells as being the principal modality leading to the complete eradication of both malignant and non-malignant host hematopoietic cells.

The assumption that successful allogeneic BMT relies on the myeloablative effect of intensive but hazardous chemo-radiotherapy has largely restricted this therapeutic modality to patients with malignant or life-threatening hematologic disorders under the age of 55 years. Treatment-related mortality increases substantially with age, prior intensive treatment with chemo-radiotherapy, worsening performance status, and co-morbid medical conditions. An unacceptable risk of death from conventional BMT renders many patients ineligible for what may otherwise be curative therapy.

Several in vitro studies have demonstrated the existence of donor-derived CD4 and CD8 positive lymphocytes with specific reactivity for the patient's leukemia. These cells provide a potent graft-versus- leukemia (GVL) effect. This GVL effect is best seen in patients with CML relapsing after BMT, where a single infusion of donor lymphocytes has been shown to induce complete remission. In addition to the potent anti-leukemia effect of these cells, there is now strong evidence that donor T-cells are capable of completely eradicating residual host hematopoietic cells in a non-myeloablative transplant setting (graft-versus-marrow) leading to successful and complete donor hematopoietic engraftment.

Non-myeloablative allogeneic peripheral blood stem cell transplants are currently being investigated in phase I/II trials assessing engraftment efficacy and toxicity at a number of transplant centers. Preliminary data, including our own experience with >150 patients undergoing this type of procedure, have shown a high rate of complete donor engraftment with a low toxicity profile. Two recent studies investigating non-myeloablative allo-transplantation in standard risk patients revealed an extremely low rate of transplant- related complications and mortality.

The decreased risk of transplant-related complications associated with non-myeloablative transplants expands the eligibility of transplant candidates as well as opens the possibility to evaluate non-myeloablative regimens in patients at high risk for complications with standard transplantation. Besides hematologic malignancies, allogeneic BMT has been shown to be curative in a number of debilitating hematologic diseases which may behave in a relatively indolent fashion, such as paroxysmal nocturnal hemoglobinuria (PNH) and refractory anemia (RA) or refractory anemia with ringed sideroblasts (RARS). However, the 30% risk of treatment-related mortality (TRM)with standard myeloablative allotransplantation usually precludes these patients from potentially curative therapy, because of concerns about shortening life in patients with these disorders. In this protocol we investigate non-myeloablative allogeneic peripheral blood stem cell (PBSC) transplantation in two groups of subjects where standard allogeneic transplantation is considered to have unacceptable toxicity

Group A: Subjects with hematologic malignancies with factors putting them at high risk for transplant related complications and mortality, including prior intensive chemo-radiotherapy and co-morbid diseases.

Group B: Subjects with hematologic diseases (both clonal and non-clonal) associated with reasonable longevity not currently considered for allogeneic BMT because of prohibitive procedural mortality with conventional

BMT (enrollment closed October 2010).

In this protocol, eligible subjects are treated with an allogeneic PBSC transplant from an HLA identical or single HLA antigen-mismatched family donor, using an intensive immunosuppressive regimen without myeloablation in an attempt to decrease the transplant related toxicities while preserving the anti-malignancy and/or anti-host marrow effect of the graft. The low intensity non-myeloablative conditioning regimen should provide adequate immunosuppression to allow stem cell and lymphocyte engraftment. T -cell replete, donor-derived, granulocyte colony stimulating factor (G-CSF)-mobilized PBSCs will be used to establish hematopoietic and lymphoid reconstitution. We will add back lymphocytes in recipients with <100% donor T-cell chimerism in an attempt to prevent graft rejection and enhance a graft-versus-malignancy effect.

The primary endpoint of this study is transplant related mortality (200 day survival). Other end points include engraftment, degree of donor-host chimerism, incidence of acute and chronic graft versus host disease (GVHD), transplant related morbidity, as well as disease-free and overall survival.

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SEE ALSO

Blood & Marrow Transplant Consortium Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant Recipients
Available online at:
http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml

1.0 OBJECTIVES

1.1. To evaluate the safety and toxicity of a low intensity non-myeloablative preparative regimen followed by a granulocyte colony stimulating factor (G-CSF) mobilized peripheral blood stem cell (PBSC) transplant in a population of subjects with malignant hematologic disorders at increased risk for complications with standard myeloablative allo-transplantation, and in subjects with hematologic diseases not usually considered for allogeneic bone marrow transplantation (BMT) because of prohibitive procedural mortality with conventional BMT .

1.2. To evaluate engraftment by bone marrow chimerism analysis.

1.3. To determine the incidence and severity of acute and chronic graft-versus-host-disease (GVHD) following the transplant.

1.4. To examine the possibility of controlling hematologic malignancies by induction of a graft-versus-malignancy effect (GVM).

1.5. To determine the disease-free survival, relapse, transplant related mortality (TRM) and death from all causes.

2.0 BACKGROUND

2.1 Introduction

It is now well established that chronic myelogenous leukemia (CML), myelodysplastic syndromes (MDS), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), Hodgkin's lymphoma/disease (HD) and non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM) can be cured by allogeneic BMT[(1)(2)(23)]. This curative effect has been ascribed to the use of myeloablative chemo-radiotherapy and the antileukemic effect of the transplant (the graft-versus-leukemia [GVL] effect) [(3)]. 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 [(4)(5)(6)(7)]. This observation has important implications. First, it may be possible to cure some hematological 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 hematological diseases as well as patients with co-morbid diseases and older patients. Several groups, including our own, have begun to investigate this approach to improve the applicability and outcome following allogeneic BMT [(10)(17)(20)]. Our experience at the NIH has included >150 subjects and has also been quite favorable, with a lower incidence of treatment-related complications than is typically observed with a standard "myeloablative" transplant approach.

We now plan to extend the evaluation of low-intensity marrow stem cell transplants to include all subjects at high risk for treatment-related complications with standard myeloablative allotransplantation, as well as to subjects with a number of debilitating hematologic diseases where allotransplantation has been shown to be curative but is usually not performed, due to unacceptable toxicity with standard transplantation [(18)(19)]. The design of the study incorporates the following features:

1) This is a phase II trial to determine the therapeutic potential of a new transplant approach (disease-free survival, GVM effect) and to evaluate its toxicity profile (immediate toxicity, graft-vs.-host disease, graft rejection, mortality).

2) Two subject cohorts will be studied:

Group A: Heavily treated and/or debilitated subjects:

Several groups of patients with hematologic malignancies have been found to be particularly poor risks for standard allogeneic transplantation due to extremely high TRM. These include patients with a history of previous treatment with dose-intensive chemotherapy (including previous allo/auto-transplant) and/or prior radiotherapy, debilitated patients with co-morbid diseases, and patients with MM. The decreased procedure-related toxicity and mortality associated with the use of a non-myeloablative preparative regimen should be most evident in this high risk group where treatment related mortality is >40% in most studies. Because the transplant does not incorporate intensive chemotherapy and relies instead on a graft-versus-malignancy (GVM) and graft-versus-marrow effect, which may take months to be effective, only subjects in remission or with a stable or slowly proliferating disease will be selected for transplant. These diseases include hematologic malignancies such as all acute leukemias in complete remission, CML in chronic phase, MDS, CLL, MM in remission or in stable phase following chemotherapy, and relapsed HD and NHL. These disorders are more common in older adults and at present there are no other potentially curative options for such patients.

Group B:Debilitating hematologic disease (closed to enrollment in Oct. 2010):

Some hematological disorders (both clonal and nonclonal) behave in a relatively indolent fashion but nonetheless may be associated with life-threatening neutropenia and/or significant debilitation resulting in poor quality of life. Patients with severe aplastic anemia (SAA), acquired or congenital pure red cell aplasia (PRCA), refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) MDS and PNH often have recurrent needs for platelet and/or red blood cell transfusions, and may have associated iron overload resulting in end organ damage. In addition, PNH patients are at increased risk for thrombotic events, some of which may be life threatening. Allogeneic marrow cell transplantation has been shown to be curative for these patients but is not attempted in every patient who has a suitable donor due to concerns of unacceptable mortality associated with standard myeloablative procedures [18][19]. Median survival in these groups without transplantation is variable and ranges from 10 years in PNH patients, 7 -8 years in RA and RARS MDS patients, to 12-14 months in SAA patients failing immunosuppression. The low incidence of treatment related complications and mortality associated with non-myeloablative PBSC transplantation now makes non-myeloablative transplantation a potentially curative therapeutic option with an acceptable risk profile. These disease categories include hematopoietic diseases such as PNH, SAA in patients who have failed immunosuppression, and RA and RARS MDS.

3) Immunosuppression without myeloablation: Subjects will receive chemotherapy sufficient to allow donor lympho-hematopoietic engraftment without complete marrow ablation. If the graft is rejected, the subject will reconstitute autologous marrow function to pre- transplantation levels. We will use a combination of two or three agents with known immunosuppressive activity in marrow transplantation: cyclophosphamide, which produces transient pancytopenia; fludarabine, which causes less cytopenia but equivalent immunosuppression; and in some subjects at increased risk for graft rejection antithymocyte globulin (ATG) which is potently immunosuppressive.

4) Peripheral blood hematopoietic progenitor cell (PBPC) transplant: An unmanipulated PBSC collection

from a G-CSF-stimulated HLA-matched donor should improve the chance of engraftment because of the high stem cell dose (≥5 x 10^6/kg CD34+ cells) and the presence of donor lymphocytes. Low-intensity preparative regimens and PBPC transplants have a low incidence of severe GVHD [20]. To further reduce the risk of GVHD, subjects will receive cyclosporine (CSA) and methotrexate (MTX) after the transplant. Subjects with mixed T-cell chimerism at day 30 will be tapered off CSA over 2 weeks and subjects with 100% donor T-cell chimerism will begin a CSA taper at day 60 (through day 100) if no GVHD is present.

5) Donor lymphocyte infusion (DLI): Subjects who show progressive disease in the absence of GVHD or who are at risk for graft failure due to incomplete donor T-cell engraftment will receive DLI (section 7.11).

3.0 SCIENTIFIC AND CLINICAL JUSTIFICATION

There is sufficient experimental evidence and clinical data to indicate that the low dose preparative regimen is capable of allowing lympho-hematopoietic engraftment with minimal toxicity. Cyclophosphamide alone permits engraftment in patients with SAA [9], and fludarabine has been used either alone or in conjunction with ATG and other agents to achieve engraftment [10].

Early data indicate that non-myeloablative stem cell transplants are associated with an excellent transplant outcome in younger patients. The presence of high stem cell doses and donor lymphocytes is known to facilitate engraftment [11][12][13]. Preliminary studies, including our own experience with PBPC transplants and low intensity preparative regimens, indicate that the risk of transplant related mortality appears to be lower than historical controls receiving a myeloablative transplant (10).

4.0 STUDY DESIGN

T-cell replete PBPC allograft: Subjects will receive a non-myeloablative preparative regimen of cyclophosphamide 60mg/kg/d x 2 days, and fludarabine 25mg/m^2 intravenously (IV) over 30 minutes daily x 5 days followed by a PBPC graft targeted to deliver >5x10^6 CD34+ cells/kg. CSA and MTX for GVHD prophylaxis will be used initially with target CSA levels in the lower therapeutic range. The HLA matched donor will receive granulocyte colony-stimulating factor (G-CSF) with apheresis collections of PBPC on day 5 and day 6 if required.

CSA beginning day -4 and IV MTX on days +1, +3, and +6 will be given. Subjects with mixed T-cell chimerism on day 30 will begin a CSA taper. Subjects with 100% donor T-cell chimerism by day 30 will be tapered off CSA from days 60 through 100 (25% reduction in dose every 10 days-off by day 100). CSA will not be tapered in any subjects with grade ≥ II acute GVHD regardless of chimerism results. In addition, subjects with evidence of disease progression without grade ≥ II GVHD will have CSA discontinued regardless of chimerism results.

On day +30, +60 and +100 disease status will be assessed and subjects with disease progression or relapse will be treated on protocol as indicated (Section 7.11).

In addition, on days +30, +60 and +100 the chimeric status of subjects will be assessed by microsatellite analysis of the peripheral blood and sometimes bone marrow. Subjects demonstrating less than 100% donor T-cell chimerism on day 30 will be tapered off CSA over a 12 day period and will be re-evaluated.

Should T-cell chimerism remain less than 100% donor, then infusions of previously collected and cryopreserved donor lymphocytes will be given to subjects (in the absence of grade ≥ II GVHD) in an attempt to prevent graft rejection.

5.0 ELIGIBILITY ASSESSMENT

5.1 Inclusion Criteria-Recipients

5.1.1 Group A: Subjects at high risk for transplant related complications and mortality as defined below:

Ages 10 to 75 (both inclusive) with a history of one of the following:

1) Treatment with dose intensive chemotherapy and/or radiotherapy
2) Previous history of allo/auto transplant
3) History of multiple myeloma or extramedullary plasmacytoma
4) Chronic disease or co-morbid medical condition including subjects with symptoms or signs of significant pulmonary disease, hepatic disease, kidney disease, cardiac disease or disease of other organ systems which would result in increased risk of morbidity or death from a standard myeloablative transplant.

Diseases to be included:

1) CML, chronic phase
2) Acute lymphoblastic leukemia (ALL), all subjects in complete or partial remission
3) AML: AML in first complete or partial remission. Exceptions: AML with good risk karyotypes: AML M3 t(15:17), AML M4Eo (inv. 16), AML t(8;21). All AML in second or subsequent complete remission
4) MDS: refractory anemia with excess blasts (RAEB), or chronic myelomonocyte leukemia (CMML)
5) Myeloproliferative diseases associated with either cytopenia or uncontrolled proliferation
6) CLL or small lymphocytic lymphoma (SLL) with bulky or progressive disease despite prior treatment with chemotherapy which includes purine analogs
7) NHL
   A) Intermediate or high grade relapsed or progressive despite treatment with standard therapy ineligible for autologous PBSC transplant
   B) NHL intermediate or high grade relapsing despite prior autologous transplant
   C) Low grade follicular or small lymphocytic lymphoma: (1) high risk patients who have relapsed following conventional chemotherapy, (2) relapsed following autologous marrow or PBSC transplant, or (3) chemo-resistant disease
   D) Mantle cell lymphoma

E) NHL intermediate or high grade with concurrent BCL2 and MYC translocations who are at high risk for relapse and who have low survival with conventional chemotherapy [25-29]8) HD, relapsed after prior autologous transplant or after 2 or more combination chemotherapy regimens and ineligible for autologous PBSC transplant

9) EBV driven lymphoproliferative disorders progressing despite standard therapies
10) MM: MM subjects must be between the ages of 8 and 65 (both inclusive)
11) Mycosis fungoides, which has been shown to be amenable to allogeneic stem cell transplants.

5.1.2 Group B (closed to enrollment Oct. 2010): Subjects with hematologic diseases associated with reasonable longevity, shown to be curable by allogeneic BMT but where concern for a high procedural mortality with conventional BMT may delay or prevent such treatment.

Ages 8 to 80 (both inclusive) with a history of one of the following

1) PNH associated with either life-threatening thrombosis, cytopenia, transfusion dependence or recurrent and debilitating hemolytic crisis
2) Aplastic anemia or PRCA (acquired or congenital) in subjects associated with transfusion dependence and/or neutropenia who are not candidates for or who have failed immunosuppressive therapy
3) RA or RARS MDS subjects who have associated transfusion dependence and/or neutropenia.

5.1.3 Ability to comprehend the investigational nature of the study and provide informed consent. The procedure will be explained to subjects age 8 -17 years with formal consent being obtained from parents or legal guardian.

5.1.4 Availability of HLA identical or single HLA locus mismatched family donor.

5.2 Inclusion Criteria -Donor

5.2.1 HLA identical or single HLA mismatched family donor.
5.2.2 Age $\geq$ 2 up to 80 years old.
5.2.3 Weight $\geq$ 18 kg
5.2.4 Ability of donor or guardian of donor to comprehend the investigational nature of the study and provide informed consent.

5.3 Exclusion Criteria-Recipient: any of the following:

5.3.1 Pregnant or lactating
5.3.2 Group A: age < 10 or >75 (multiple myeloma age <8 or > 65)
Group B: Age < 8 or >80 years
5.3.3 ECOG performance status of 3 or more (See NIH Bone and Marrow Consortium Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant Recipients - http://intranet.cc.nih.gov/bmt/_pdf/ECOG_Karnofsky_Lansky_Scales.pdf)
5.3.4 Psychiatric disorder or mental deficiency severe as to make compliance with the BMT treatment unlikely and making informed consent impossible
5.3.5 Major anticipated illness or organ failure incompatible with survival from PBSC transplant
5.3.6 Diffusion capacity of carbon monoxide (DLCO) < 40% predicted
5.3.7 Left ventricular ejection fraction: < 30%
5.3.8 Serum creatinine > 2.5mg/dl or creatinine clearance < 50 cc/min by 24 hr urine collection
5.3.9 Serum bilirubin > 4 mg/dl, transaminases > 5x upper limit of normal
5.3.10 Other malignant diseases liable to relapse or progress within 5 years, with the exception of a separate hematologic malignancy where allogeneic stem cell transplant is shown to be potentially curative.

5.4 Exclusion Criteria-Donor: any of the following

5.4.1 Pregnant or lactating
5.4.2 Donor unfit to receive G-CSF and undergo apheresis (uncontrolled hypertension, history of congestive heart failure or unstable angina, thrombocytopenia)
5.4.3 HIV positive donor. Donors who are positive for hepatitis B (HBV), hepatitis C (HCV) or human T-cell lymphotropic virus (HTLV-I/II) will be used at the discretion of the investigator following counseling and approval from the recipient.

6.0 CLINICAL EVALUATION OF THE RECIPIENT

-Human biologic materials are collected as follows for the clinical evaluation and management of the subject. Bone marrow aspirates will be read by a pathologist. Samples will be ordered and tracked through the CRIS screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

6.1 Pre-Study Evaluation

6.1.1 High resolution molecular HLA- A, B, DR typing
6.1.2 Bone marrow aspiration and biopsy with chromosome analysis, PCR, and flow cytometry as appropriate to stage and classify underlying disorder. Myeloma subjects will additionally have an SPEP, serum immunofixation, urine protein electrophoresis, urine immunofixation, quantitative immunoglobulins and 24 hour urine for creatinine clearance and protein evaluation. PCR to detect BCR.ABL when appropriate
6.1.3 Antibody screen for HBV, HCV, HIV, HTLV-VII, CMV, EBV, toxoplasma, syphilis and VZV serology. Consider PPD test for subjects from areas where tuberculosis is prevalent
6.1.4 Coagulation screen, CBC with differential
6.1.5 Acute care panel, hepatic panel and mineral panel
6.1.6 24 hour creatinine clearance
6.1.7 - Pulmonary function testing: vital capacity FEV-1, DLCO
6.1.8 Sinus CT scan. Lymphoma subjects will additionally have baseline CT scans of the chest, abdomen, pelvis and any other areas of evaluable disease within 30 days of the transplant.MM subjects will, in addition, undergo a baseline skeletal series.
6.1.9 Cardiac function: EKG, - and baseline echocardiogram for all subjects
6.1.10 H&P and ECOG status - All subjects age 50 or greater, or age 40 or over, with one of the following risk factors: a history of high blood pressure or increased cholesterol, family history of coronary disease, smoking or diabetes, will have a baseline cardiac workup, which will include:
    1. Stress nuclear perfusion imagery
    2. Cardiac consultation in subjects age 50 and over
6.1.11 Nutritional assessment
6.1.12 Dental review
6.1.13 Social worker interview and complete durable power of attorney form
6.1.14 Ophthalmology consultation
6.1.15 Interview with members of primary care team and visit to unit
6.1.16 Consent form signed
-
6.1.18 Pregnancy test for women of child bearing potential

6.2 In-Patient Monitoring

Once daily: CBC with differential, acute care, hepatic, and mineral panels, direct bilirubin, temperature, pulse, blood pressure, respiratory rate, weight, caloric intake, abdominal girth.

Twice weekly: reticulocytes, pre-albumin, coagulation screen.
Weekly: CMV surveillance, C-reactive protein
Stool for C *difficile* when appropriate
Drug levels when appropriate (e.g., gentamicin, vancomycin, CSA).

6.3 Follow Up to Day 100: Out-patient

At least weekly: CBC, acute care panel, hepatic and mineral screen, temperature, pulse, blood pressure, respiratory rate, weight; C-reactive protein: CMV surveillance. A complete physical exam will be repeated weekly.

Peripheral blood will be drawn and in some recipients a bone marrow aspirate will be taken on days +15, +30, +45, +60 and + 100 to assess for donor-host chimerism in the lymphoid and myeloid cell lines. A sample of peripheral blood will also be obtained for chimerism analysis at the time of neutrophil recovery (ANC > 500).

Day 30, 60, and 100: Recipients with hematological malignancies or where clinical conditions warrant, will have bone marrow aspirate to quantitate engraftment. All recipients will receive a repeat PFT on day 30.

MM recipients will have staging labs repeated and appropriate radiographic studies.

Lymphoma recipients (HD, NHL, CLL with adenopathy, and SLL recipients) will have baseline CT scans of the chest, abdomen and pelvis performed pre-transplant and on days 30, 60, 100.

6.4 Beyond Day 100

At 6, 12, 18, 24, 36, 48 and 60 months (+/- 1 month): CBC, acute care panel, hepatic and mineral screen, ferritin, iron, TSH, T4, LH, quantitative immunoglobulins, CD4+ count. Estradiol, testosterone, FSH, chest radiograph, bone marrow aspirate or blood sample for morphology, chimerism studies, and karyotype, pulmonary function, and an ophthalmology assessment.

Repeat staging labs in myeloma recipients.

Lymphoma recipients (HD, NHL, CLL with adenopathy, and SLL recipients) will have CT scans every 3 months for the first 2 years (after day 100), then at 6 to 12 month intervals until 5 years post transplant.

After 5 years follow up visits are not mandatory, but yearly communication with the recipient and the referring physician is continued.

7.0 TREATMENT PLAN

7.1 Pre-transplant Treatment of MDS, Leukemia, MM, and Lymphoma

Recipients with active disease may receive appropriate chemotherapy according to standard indications to control disease prior to the preparative regimen.

7.2 Apheresis of Recipient

One collection of 10^{10} leukocytes by a 10-liter leukapheresis before transplant preparative regimen begins and at the time of disease response or acute graft-vs.-host disease for cryopreservation of leukemia cells and/or lymphocytes to be used for laboratory investigation of graft-versus-malignancy, graft-versus-marrow, or GVHD effectors.

7.3 Central Venous Line Placement

A triple lumen Hickman catheter will be placed by an interventional radiologist or a surgeon.

7.4 Infection Prophylaxis (See Supportive Care Guidelines)

7.4.1 **Strongyloides prophylaxis:** Ivermectin, will be given per NIH Bone and Marrow Consortium Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant Recipients prior to transplant as prophylaxis against Strongyloides stercoralis (threadworm), a known human parasite (recipients at risk).

7.5 Preparative Regimen (see Appendix A)

7.5.1 Antithymocyte Globulin (ATG or Atgam) (See also NIH Bone and Marrow Consortium Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant Recipients)

Subjects will receive one of two preparative regimens as described below. Recipients at higher risk for graft rejection with this non-myeloablative approach (for example, subjects with aplastic anemia, heavily transfused subjects and single HLA locus mismatched subjects) will have ATG added to the preparative regimen unless a contraindication exists (i.e. prior life threatening toxicity associated with ATG treatment).

7.5.2 Dose Levels

1) Cyclophosphamide 60 mg/kg/d IV day -7, -6
Fludarabine 25 mg/m^2/d IV day -5, -4, -3, -2, -1

2) Aplastic anemia or recipients at increased risk for graft rejection as defined above, 7.5.1
Cyclophosphamide 60 mg/kg/d IV day -7, -6
Fludarabine 25 mg/m^2/d IV day -5, -4, -3, -2, -1
Antithymocyte globulin 40mg/kg IV days -5,-4,-3,-2

7.6 Peripheral Blood Progenitor Cell Transplant (See also Appendix B)

The target for progenitor cells is $\geq 5 \times 10^6$ CD 34+ cells/kg.
Minimum dose for transplant is 2×10^6 cells/kg.

7.7 GVHD Prophylaxis

(For grading - see NIH BMT Consortium Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant)

All recipients will receive CSA and MTX as GVHD prophylaxis. CSA (1.5 mg/kg/dose IV BID) will begin on day -4. Dosing will be based on actual body weight unless the recipient is "obese" (BMI > 35) when practical weight will be used. Switch to the equivalent oral CSA dose divided bid on day 14 or when able to tolerate PO. CSA doses will be adjusted to achieve serum trough levels of 200-400 mcg/mL. MTX (5 mg/m^2/dose) will be given IV on days +1, +3 and +6. Refer to NIH Bone and Marrow Consortium Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant Recipients for MTX dose adjustments based on serum creatinine and total bilirubin values on the day of administration.

Recipients with <100% donor T-cell chimerism on day 30 will begin a 12 day CSA taper (i.e. 25% reduction in

dose every 3 days). Recipients with 100% donor T-cell chimerism by day 30 will be tapered off CSA from days 60 through 100 (25% reduction in dose every 10 days-off by day 100). CSA will not be tapered in any recipients with grade ≥ II AGVHD regardless of chimerism results. In addition, recipients with evidence of disease progression without grade ≥ II acute GVHD will have CSA tapered regardless of chimerism results.

7.8 Transfusion Support

(See - NIH BMT Consortium Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant): Filtered and irradiated blood products. CMV negative recipients of CMV negative or positive marrow will receive CMV negative blood products.

7.9 Nutrition:

Parenteral nutrition will be instituted if daily caloric intake <1000KCal/day in recipients > 18 years. Recipients 8 to 18 years will start parenteral nutrition if they are unable to meet at least half their daily caloric needs by mouth (i.e. 50 KCal/kg/day).

7.10 Hospital Discharge:

Recipient will be in the hospital for about 4 weeks and will be discharged when the following criteria are fulfilled:

- Recipient afebrile, positive weight balance without requirement for parenteral nutrition
- Platelet transfusion requirement absent or less than 2 x weekly
- Recipient or family able to care for Hickman line

7.11 Donor Lymphocyte Transfusions:

Recipients with disease progression or donor T-cell chimerism of <100% following CSA tapering without grade ≥ II GVHD will receive donor lymphocyte infusions (previously collected and cryopreserved) based on the following algorithm:

7.11.1 Mixed chimerism without disease progression
Monthly increments of DLI 5 x 10^6, 1 x 10^7, 5 x 10^7 CD3+ cells/kg.

7.11.2 Disease progression (independent of chimerism status)
DLI 1 x 10^7 CD3+ cells/kg and repeat doses depending on response and acute GVHD.

8.0 DONOR EVALUATION AND PLAN

8. 1 Pre-study Consult and Evaluation

8.1.1 Pregnancy test for women of child bearing potential
8.1.2 HLA-A,-B,-DR typing
8.1.3 Confirm HLA identity of donor with recipient
8.1.4 History and physical examination
8.1.5 Chest X-ray in donors with underlying pulmonary disease or history of smoking
Hepatitis B, C, HIV, HTLV I/II, CMV antibodies, RPR, VZV serology, anti- T. cruzi, West Nile virus NAT (nucleic acid test) and human transmissible bovine spongiform encephalopathy (BSE is done by questionnaire of associated risk factors)

8.1.6 Red blood cell ABO group, Rh type, antibody screen
8.17. CBC, coagulation screen, acute care, hepatic, mineral screen
8.1.8 If eligible: Orientation - visit to Department of Transfusion Medicine inspection of veins to determine the need for a central line for apheresis.

8.2 Pre-consent Evaluation and Concurrent Care of Minor Donors (donors less than age 18 only)

For donors less than age 18, a social worker and mental health specialist (psychologist or psychiatrist) will meet with the minor prior to the assent process to determine willingness to participate.

Donors who are less than 18 years of age will see a pediatric provider (pediatrician, pediatric nurse practitioner or pediatric physician's assistant) who is separate from the transplant team. This practitioner will serve as the donor's health care provider and advocate during the minor's participation on the clinical trial.

8.3 Consent to Undergo G-CSF Mobilization and Donate Leukocytes

8.4 PBPC Collections (See Appendix B) Algorithm for mobilization, collection, and processing:

8.4.1 Minor donor mobilization with G-CSF (≤ 18 years old)

After medical evaluation and clearance for suitability as an allogeneic donor by the BMT service in consultation with DTM, the donor will undergo mobilization with filgrastim (G-CSF) as an outpatient. G-CSF will be administered based on body weight (see below) for at least 5, and up to 7 days, subcutaneously.

Dosing will be based on actual body weight unless the donor is "obese" (BMI > 35) when practical weight will be used. G-CSF will be administered according to a vial based algorithm to reduce wastage, improve donor compliance, and optimize CD34+ yields. The doses for days 1-4 may be given at any time of day, but the doses for days 5 (and if necessary, days 6 and 7) must be given very early in the morning, prior to apheresis. Predictable side effects of G-CSF including headache, bone pain, and myalgia, will be treated with acetaminophen or ibuprofen. Prophylactic treatment of these side effects with the same medications may be elected. Other side effects will be evaluated and treated accordingly.

Donor Wt	Total G-CSF	Dose (range)
19-30.9 kg	300 mcg	(10.0 to 15.8 mcg/kg)
31-37.9 kg	480 mcg	(10.0 to 13.0 mcg/kg)
38 – 48.9 kg	600 mcg	(12.5 to 15.8 mcg/kg)
49 – 56.9 kg	780 mcg	(13.9 to 15.9 mcg/kg)
57 – 60.9 kg	900 mcg	(15.0 to 15.8 mcg/kg)
61 – 67.9 kg	960 mcg	(14.3 to 15.7 mcg/kg)
68 – 108.9 kg	1080 mcg	(10.0 to 15.9 mcg/kg)
> 109 kg	1200 mcg	(11.0 or less)

8.4.2 Adult donor mobilization with G-CSF (> 18 years old) – per NIH BMT Consortium Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant **Dose algorithms:** Two dosing algorithms are recommended for use in the Clinical Center. The **standard dose algorithm** uses doses in the range of 10 to 12 mcg/kg /day, with a higher dose given to lighter weight adult donors to improve CD34+ yields. The **higher dose algorithm** is intended for use in (1) adult autologous donors who have received prior myelotoxic agents; (2) adult donors whose components will undergo further processing; (3) protocols in which a high transplant cell dose is required (CD34+ $\geq 8 \times 10^6$); and (4) situations with large weight discrepancy between the adult

donor and recipient (recipient > donor) (as assessed by the Principal Investigator). The total dose in both regimens is capped at 1200 mcg/day. The Principal Investigator (PI) or his designee will determine the dosing algorithm to be utilized.

I. Standard-Dose Filgrastim Algorithm

Donor Weight	Total Daily Filgrastim	Dose (range)
38 – 60.9 kg	600 mcg	(10.0 to 15.8 mcg/kg)
61 – 78.9 kg	780 mcg	(10.0 to 12.8 mcg/kg)
79 – 90.9 kg	900 mcg	(10.0 to 11.4 mcg/kg)
91 – 96.9 kg	960 mcg	(10.0 to 10.5 mcg/kg)
97 – 108.9 kg	1080 mcg	(10.0 to 11.0 mcg/kg)
≥ 109 kg	1200 mcg	(11.0 or less)

II. Higher-Dose Filgrastim Algorithm

Donor Weight	Total Daily Filgrastim	Dose (range)
38 – 48.9 kg	600 mcg	(12.5 to 15.8 mcg/kg)
49 – 56.9 kg	780 mcg	(13.9 to 15.9 mcg/kg)
57 – 60.9 kg	900 mcg	(15.0 to 15.8 mcg/kg)
61 – 67.9 kg	960 mcg	(14.3 to 15.7 mcg/kg)
68 - 108 kg	1080 mcg	(10.0 to 15.9 mcg/kg)
≥109 kg	1200 mcg	(11.0 or less)

Collection and processing of donor cells

Donors will receive divalent cation prophylaxis to prevent citrate toxicity during apheresis, in accordance with standard DTM policies. The volume processed per apheresis procedure will be determined by DTM medical staff on the day of apheresis, based on peak CD34+ cell mobilization response to GCSF and the CD34+ cell dose needed, based on kilogram weight of recipient. This will range from 15 to 35 liters processed per day for 1 to 3 days, not to exceed a total of 75 liters over 3 days. In pediatric subjects, defined as less than 40 kg, a maximum of 8 blood volumes will be processed per day, for up to 1-3 days.

Obtaining a dose of > $3x10^6$ CD34+ cells/kg will be considered adequate, and no further apheresis will be performed. If the target CD34+ cell dose of 3 x 10^6/kg is not reached, then a 6^{th} (and possibly 7th) dose of G-CSF will be given; and an apheresis procedure will be performed on day 6 (and possibly day 7).

If the minimum CD34+ cell dose is not achieved with one mobilization and 3 apheresis collections (i.e.2 x 10^6 CD 34+ cells/kg), then the PI will consider options of a bone marrow collection, a second course of mobilization and apheresis, or no further donor collections and taking the subject off protocol.

The PBSC collections will not be manipulated except for plasma removal or red blood cell depletion as needed for ABO or other red blood cell antigen incompatibility. PBSC will be either cryopreserved for later thawing and infusion, or infused as fresh products within 48 hours of collection.

On rare occasions, recipients may develop marrow failure as a consequence of graft-vs.-host hematopoiesis or from post transplant drug treatment (i.e. ganciclovir). When such situations arise, recipients may require a stem cell "boost". Donors will undergo a repeat mobilization with G-SCF (as outlined above) followed by CD34+ selection of hematopoietic progenator cells prior to being infused.

8.5 Donor Lymphocyte Collection

Prior to undergoing G-CSF mobilization, the donor will undergo a ten liter apheresis for cryopreservation of donor lymphocytes to be infused as necessary after transplantation (section 7.11). Aliquots of 5 x 10^6 CD3+ cells/kg, 1 x 10^7 CD3+cells/kg and 5 x 10^7 CD3+cells/kg will be cryopreserved.

9.0 MANAGEMENT OF RECIPIENT COMPLICATIONS

The major complications are cytomegalovirus reactivation, acute GVHD, chronic GVHD, relapse of the original disease, donor-recipient ABO incompatibility and pulmonary engraftment syndrome. Recipients with these complications will be treated along the following lines:

9.1 CMV Reactivation

(See NIH BMT Consortium Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant)

9.2 Acute GVHD

(See NIH BMT Consortium Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant)

9.3 Chronic GVHD

(See NIH BMT Consortium Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant)

Cyclosporine at standard dose (see Pharmaceuticals, section 15.3).
Prednisone 20-60 mg daily according to severity.
Change to alternate day steroid and cyclosporine therapy when response is established.
Non-responding recipients may be treated with other standard of care therapies such as azathioprine, sirolimus, tacrolimus, rituximab, mycophenolate, daclizumab or infliximab, PUVA, photopheresis, and/or thalidomide at the discretion of the attending physician.

9 .4 Graft Rejection

This transplant protocol uses a non-myeloablative preparative regimen. Therefore, auto-recovery is anticipated in recipients who fail to engraft. Recipients who fail to demonstrate donor T-cell engraftment will be taken off study and referred back to their primary physician for further therapy.

9.5 Relapse of Hematologic Malignancy:

Recipients may be treated at the discretion of the PI with donor lymphocyte transfusions, standard of care treatment approaches (i.e. interferon-alpha, GM-CSF, low dose IL-2 by subcutaneous injection, Rituximab, chemotherapy, Gleevec) with or without stem cell rescue.

9.6 Donor-recipient ABO Incompatibility

(See NIH BMT Consortium Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant)

9.7 Pulmonary Engraftment Syndrome:

Recipients who develop pulmonary engraftment syndrome (around 10-14 days post-transplant) will be treated with steroids.

10.0 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 7.5.

11.0 ANCILLARY LABORATORY RESEARCH STUDIES

11.1 Sample Collection

During the course of participating on this study, blood and tissue as detailed below will be collected for correlative laboratory research studies:

11.1.1 Recipient before transplant
One apheresis collection of approximately 10^{10} leukocytes in a volume of 200 ml.

11.1.2 *Recipient in hospital:* Weekly lymphocyte subsets

11.1.3 Planned **outpatient** visits: Cytotoxic T-cell precursor frequency (CTLPf) assays and lymphocyte subsets on day 60 and 100 then at each scheduled follow-up.

11.1.4 Donor at time of leukapheresis
Donor leukocytes obtained at leukapheresis will be used for research studies investigating graft-versus-leukemia effects. The recipient's leukemia cells and normal lymphocytes will serve as targets for all subsequent testing of donor-anti host responses.

11.1.5 Recipient bone marrow samples
An extra volume (up to 25 ml) of bone marrow aspirate will be collected for research studies at the pre-transplant evaluation and for monitoring minimal residual disease. The cells will be used to investigate lymphocyte interactions with bone marrow progenitor cell proliferation. No marrow aspirates solely for research purposes are planned.

11.1.6 Pulmonary function tests
Pulmonary status at baseline, day 30 and after day 100 will be analyzed to see if reduced intensity conditioning (RIC) effects lung function before day 100 or if the decline in lung function is only seen later consistent with bronchiolitis obliterans syndrome (BOS).

11.2 Intended Use

These specimens will not be read by a pathologist or used for diagnostic purposes. Studies will not be used in assessing the primary endpoint, but are undertaken for descriptive or exploratory ancillary research as detailed below:

11.2.1 GVL and GVHD studies: Blood and serum from recipients and donors will be obtained by leukapheresis to study genomic and proteomic pathways involved in mediating GVHD and in vitro donor T-cell responses to recipients leukemia. The cytotoxic T-cell precursor frequency (CTLPf) assay will be used to evaluate the donor immune function at various periods after BMT.

11.2.2 Chimerism studies: PCR of microsatellites of post-transplant peripheral blood T-lymphocytes, B-cells, NK cells and blood myeloid cells will be assessed in serial fashion to determine the chimeric status of the recipient as a function of time. These studies will be repeated after lymphocyte infusions to assess their effect on host chimeric status as well. The relationship between degrees of donor host chimerism, GVHD and tumor response will be analyzed.

11.2.3 Nephrotic syndrome studies: We have reported an unusually high incidence of nephrotic syndrome (6.1% with a median follow up of 2.8 years) in recipients following fludarabine/cyclophosphamide conditioning prior to undergoing non-myeloablative stem transplantation. In order to understand the pathogenesis of this condition in this recipient population, samples from recipients and when available their associated donors may be sent to Pierre Ronco, M.D., Ph.D., Head, Nephrology Department, Unit 702 At Tenon Hospital, Paris, France tel : + 33 1 56 01 66 39/38 pierre.ronco@tnn.aphp.fr for further evaluation. We will use a numerical code to indicate if the samples were drawn before the onset of or during the nephrotic syndrome. Samples will be free of any identifying information in order to protect the identity of the subjects providing samples.

Transplant recipients and their associated donor will be asked to sign the revised consent documenting their approval to have an anonymized sample of their blood sent to an investigator outside the NIH. See section 14.5 for telephone consenting procedure to be utilized if recipient is unavailable for an NIH visit. A material transfer agreement (MTA) will be in place prior to transfer of any anonymized samples to this external lab.

11.2.4 Ex vivo expanded autologous double negative (DN) T cells: In order to determine the feasibility and safety of using ex vivo expanded autologous double negative (DN) T cells as a novel cell therapy for treating leukemia, it is necessary to determine whether DN T cells can be expanded from apheresis/peripheral blood mononuclear cells (PBMCs). It is critical to understand if these ex vivo expanded DN T cells kill autologous tumor cells but not normal bone marrow cells. To address these questions, Dr. Zhang at University of Toronto will utilize apheresis/PBMC samples from NIH healthy donors to isolate and expand their DN T cells ex vivo. The expanded DN T cells will then be utilized in an assay to determine their ability to kill enriched autologous AC133+ cells (provided by NIH) as well as autologous EBV-transformed B cells (provided by NIH). This will be evaluated both in vitro and in NOD-SCID mice. The goals are twofold:

1. To determine if DN Tcell expansion is feasible from healthy donor apheresis/PBMC.
2. Determine DN T cell toxicity to autologous Epstein Barr Virus transformed B cells and normal autologous AC133+ cells both in vitro and in a NOD-SCID mouse model.

Other research labs as described on the NHLBI Laboratory Research Studies submitted to and reviewed by the IRB (Appendix C).

Etiology of post-transplant syndromes: We will share de-identified blood plasma and/or serum with Dr.

Waldman at NIDDK. Dr. Waldman will use blood plasma and or serum to conduct studies on the etiology of post-transplant syndromes that cause renal failure including nephrotic syndrome, TMA, and glomerulonephritis.

We will share the following de-identified samples to Dr. Li Zhang, Senior Scientist, Toronto General Research Institute (TGRI) at University Health Network: peripheral blood stem cells (PBSC), peripheral blood mononuclear cells (PBC,) coded as TRA-PBMC, GRE-PBMC and WYN-PBMC, and coded Epstein-Bar-Virus (EBV) transformed lymphoblastoid cell line (LCL), coded as TRA-EBV-LCL, GRE-EBV-LCL and WYN-EBV-LCL, derived from healthy donors enrolled in protocols 97-H-0196 and 99-H-0064 and 99-H-0050 .

We may share the following coded samples with Dr. Michael Nishimura at Loyola University Chicago, Chicago Illinois: frozen PBMC from healthy donors from this protocol to sequence the T cell receptor (TCR) genes and clone them into retroviral vectors for the transduction of T cells.

11.3 Tracking, Storage and Disposition of Samples

Storage: Research samples will be stored coded in the secure laboratory of the PI.

Tracking: Samples will be ordered and tracked through the CRIS research screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Specimens will be entered in the NHLBI Biospecimen Inventory System (BSI). Samples will not be sent outside NIH without IRB notification and an executed MTA.

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

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

11.4 Technology Transfer:

This protocol has the following MTAs

Between NHLBI and Pierre Ronco for the transfer of samples to Pierre Ronco, MD, PhD, Head, Nephrology Department, Unit 702 At Tenon Hospital, Paris, France tel : + 33 1 56 01 66 39/38 pierre.ronco@tnn.aphp.fr

Between NHLBI and Li Zhang, M.D., Ph.D. Professor Departments of Lab Medicine& Pathobiology, Immunology University of Toronto , Toronto General Hospital, Norman Urquhart , 1st Floor Rm 1NU119, 200 Elizabeth St., Toronto, Ontario, Canada M5G 2C4.

Between NHLBI and Dr. Michael Nishimura, Loyola University Chicago, Chicago, Illinois.

12.0 BIOSTATISTIC CONSIDERATIONS

12.1 Design and Sample Size

This trial is designed to estimate the 200 day survival proportion in each of Groups A and B. The expected 200 day survival for Group A (recipients between ages 10 and 75 (MM recipients between 8 and 65) inclusive who are at high risk for transplant-related complications and mortality) is 60% and for Group B (debilitating non-malignant hematological diseases) is 70%. Up to forty-five recipients will be enrolled in group A. Up to 62 subjects could be enrolled in group B (enrollment closed Oct. 2010 with 57 subjects). After all recipients have been followed for 200 days, a 95% confidence interval for the 200 day survival of each subgroup of will be given based on the Kaplan-Meier estimate and Greenwood's formula for the variance [21]. This sample size will assure that the true 200 day survival proportion will be estimated within approximately $\pm$ 14% with 95% confidence if the Kaplan-Meier estimate of 200 day survival is in the range of 60% - 70% [21,22]. If 90% of the patients survive beyond 200 days, the 95% confidence interval for survival will be estimated to within approximately +/-9%.

In February 2001, the protocol was amended to add an additional immunosuppressive agent, Mycophenolate mofetil (MMF), to the transplant regimen to decrease the incidence of life-threatening GVHD. This drug has been used in combination with CSA by a number of other transplant centers using a similar treatment algorithm, and has resulted in a significant reduction in the incidence of life-threatening GVHD (unpublished personal communication, Dr. Champlin, M.D. Anderson and Dr. Sandameir, Fred Hutchinson Cancer Center). Acute GVHD has been the major toxicity associated with the procedure and has been fatal in two cases. The majority of recipients suffering severe GVHD has done so within the first 60 days following transplantation and has had 100% donor T-cell engraftment by chimerism analysis. Therefore, we wish to cover all recipients with an additional immunosuppressive agent from days 0 to at least 30 days post-transplant. Recipients with mixed T-cell chimerism on day 30 will be tapered off MMF over the next 2 weeks concomitantly with their CSA taper (the group we have previously defined to be at low risk for GVHD). Recipients with 100% donor T-cell chimerism on day 30 (high-risk GVHD group) will remain on MMF until day 60 at which point, they will begin a taper over the next 40 days concomitantly with their CSA taper. Recipients with active GVHD at a time point which precedes their planned MMF taper, will remain on this agent, having their MMF taper delayed until symptoms of GVHD have resolved. The addition of MMF to CSA prophylaxis will be incorporated into all the non-myeloablative protocols. In November 2002, a retrospective analysis for all recipients on HB protocols undergoing non-myeloablative allogeneic stem cell transplantation following cyclophosphamide/fludarabine based conditioning was conducted to determine the impact of adding MMF to CSA as GVHD prophylaxis. In this analysis we evaluated the incidence and severity of acute GVHD in our first 66 consecutive recipients who received CSA alone (Group I) versus the next 78 consecutive recipients who received CSA + MMF (Group II). We saw no beneficial impact of adding MMF to CSA on the incidence of acute grade II-IV, acute grade III-IV GVHD or chronic GVHD. As a consequence, on 2/7/2003 we amended the protocol to replace MMF with the more conventional GVHD prophylactic agent MTX as follows:

1) Discontinued the use of MMF as it has clearly failed to reduce the incidence of acute or chronic GVHD.
2) Continued CSA as GVHD prophylaxis using the same dose and scheduling regimen that was used for recipients in Group I and Group II.
3) Added MTX to CSA as GVHD prophylaxis in the same fashion and doses as are currently given on protocol 97-H-0196. CSA and MTX have been used together for over a decade and are considered "standard agents" for GVHD prophylaxis.

1/30/2007: As a consequence of interim analysis of the first 43 recipients accrued to the Group B arm, an amendment was requested to increase accrual to the Group B arm with the following justification: 31 subjects had received the CSA + MTX as conditioning regimen and 200 day survival had been approximately 90%. Because results with CSA + MTX also appear to decrease GVHD, we would like to extend accrual to 17

additional recipients, making a total of 62 subjects in the Group B arm (50 of whom who received CSA + MTX), in order to be able to estimate the 200 day survival for recipients who received CSA + MTX to within approximately +/- 8% with 95% confidence. This will also provide a stronger basis for using this regimen as a control group for a future trial which will add an additional agent to the conditioning regimen.

12.2 Endpoints

The primary endpoint of this study is transplant related mortality (200 day survival).

Secondary end points include engraftment, degree of donor-host chimerism, incidence of acute and chronic graft versus host disease (GVHD), transplant related morbidity, death from all causes as well as disease-free and overall survival.

The parameters to be monitored include:

1) CD34+ cell dose, CD3+ cell dose
2) Degrees of donor-recipient lymphoid and myeloid chimerism by microsatellite probe analysis of peripheral blood and marrow cells (marrow will be collected on days +30, +60, and +100)
3) Neutrophil recovery (days to neutrophil count of 0.5 x 10^9/L and 1.0 x 10^9/L)
4) Platelet recovery (days to platelet count of 50 x 10^9, days to transfusion independence)
5) Red cell recovery (days to transfusion independence)
6) Effect of DLI on donor-host lymphoid and myeloid chimerism measured by microsatellite DNA analysis of lymphocytes and myeloid cells
7) Incidence and severity of acute GVHD
8) Incidence and severity of chronic GVHD
9) Non-hematologic effects attributable to the preparative regimen
10) Relapse of malignancy or disease control
11) TRM by day 200
12) Disease-free survival and overall survival

12.3 Other Analyses

12.3.1. The proportion of graft failures will continue to be monitored. If there are 6 among the first 42 recipients, we will reassess our preparative regimen because such results are inconsistent with prior beliefs that the graft failure rate is most likely to be 5% and unlikely to be greater than 15%. A beta distribution with parameters 2 and 38 was used as the prior distribution for this calculation.

12.3.2. The proportion of recipients with Grade 3 or higher acute (day 100 or less) and chronic graft versus host disease will also be estimated with 95% confidence intervals.

12.3.3 Although the primary endpoint for this protocol occurs at 200 days, recipients will be followed up for 60 months so that long-term survival can be estimated.

12.4 Stopping Rules for Safety

12.4.1 Stopping rule for primary endpoint

We wish to stop this trial if the 200 day survival proportion for either Group A or B is lower than the expected 60 and70% respectively with conventional transplantation. Stopping rules will be applied to Group A and Group B separately. For each, an interim analysis is planned at 200 days after the 15th and

30th recipients in each group receive their transplant. The final analysis will be undertaken 200 days after all recipients in each group receive their transplant. At each interim analysis, the proportion of 200 day survivors will be estimated and a 2-sided 99.9% confidence interval given. If the upper bound of the confidence interval lies below 60% this suggests that that part of the trial should stop for lack of efficacy. This procedure assures that the 95% confidence interval specified in section 12.1. for the final analysis in each group can be used with approximately 95% confidence. Operationally, the Z-value 3.29 is used to compute the confidence interval at the two interim looks and the Z-value 1.96 is used for the final confidence interval.

This results in the following stopping rule:
1st interim analysis ($n_1 = 15$): Stop for failure if 0, 1 or 2 are alive at day 200.

2nd interim analysis ($n_2 = 30$): Stop for failure if 9 or fewer are alive at day 200.

It should be noted that the boundaries may be crossed even if all recipients are not 200 days post-transplant.

12.5 Off Study Criteria

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

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

12.5.2 Withdrawal *by the physician* from experimental protocol
Recipients with disease relapse will be taken off protocol and the attending physician may carry out other treatments than described in this protocol if they are considered to be in the best interests of the recipient.

Recipients who fail to achieve allogeneic marrow engraftment (donor chimerism or mixed chimerism) will be removed from study after full autologous marrow recovery is achieved.

Recipients with disease progression after 6 month restaging who have active grade ≥II GVHD or who have failed to respond to a previous lymphocyte transfusion and/or to interferon-alpha or IL-2 may be removed from study.

Recipients with disease progression associated with a significant decline in performance status which negates further treatment on protocol (i.e. DLI) will be removed from study.

12.6 Data Management

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The PI, associate investigators (AI) /research nurses and/or a contracted data manager will assist with the data management efforts. All human subjects personally identifiable information (PII) as defined in accordance to the Health Insurance Portability and Accountability Act, eligibility and consent verification will be recorded. Primary data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant, e.g., study-specific identifying

number (SSPIN) or other unique code or minimum PII required for subject identification.

Laboratory values from referring home physicians may also be entered into the system.

CIBMTR: For the purposes of quality assurance (i.e. accreditation of the Transplant program), anonymized data will be released to the Center for International Blood and Marrow Transplant Research (CIBMTR) according to federally mandated policies and procedures.

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

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

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

13.0 DATA SAFETY AND MONITORING PLAN

13.1 Safety Monitoring

Principal Investigator: Accrual, efficacy and safety data will be monitored by the PI. The study will be continuously evaluated for any unusual or unpredicted complications with the aim of detecting and preventing unacceptable increase in morbidity and mortality over that anticipated from unmanipulated bone marrow transplantation.

***IRB*:** Accrual and safety data will be monitored reviewed annually by the Institutional Review Board (IRB). Prior to implementation of this study, the protocol and the proposed subject consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to the 45 CFR 46 Code of Federal Regulations. This committee will also approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

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

13.2 Definitions

Adverse Event (AE): Any untoward medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of

the following:

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

Serious Adverse Event (SAE): A serious adverse event that:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in in-patient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant incapacity;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

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

Serious event: An event is serious if it meets the definition of a serious adverse event (above) or if it requires immediate corrective action by a PI and/or IRB to protect the safety, welfare or rights of subjects.

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

Unanticipated Problem (UP): Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

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

Unanticipated Problem that is not an Adverse Event: An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of

breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Protocol Deviation (PD): Any change, divergence, or departure from the IRB approved research protocol.

Non-compliance: The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:

1. **Serious non-compliance:** Non-compliance that:
   a. Increases risks, or causes harm, to participants.
   b. Decreases potential benefits to participants.
   c. Compromises the integrity of the NIH HRPP.
   d. Invalidates the study data.

2. **Continuing non-compliance:** Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.

3. **Minor (non-serious) non-compliance:** Non-compliance that, is neither serious nor continuing.

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

All reportable events will be reported to the NIH intramural IRB in accordance with the Policy 801.
The PI or designee will refer to NHLBI DIR Policy to determine Clinical Director reporting requirements and timelines.

13.3 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 (i.e. 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 (see section 12.2 parameters to be monitored).

The following are expected outcomes and will not be reported to the IRB (per review and approval by the IRB) unless they meet the criteria for an SAE (life threatening):

- Renal insufficiency
- Hepatic insufficiency
- Transient cardiac arrhythmias
- Transient cardiac insufficiency
- Pulmonary insufficiency
- Neutropenia and its complications
- Thrombocytopenia 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 manifestations, "red man" syndrome
- Well-characterized drug side effects from drugs used routinely in transplant recipients (e.g.; preparative regimen chemotherapy, immunosuppressive drugs, antimicrobials)
- Common side 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 are expected outcomes and will not be reported to the IRB at each occurrence unless they meet the criteria of an SAE. They will be reported in summary form at the time of continuing review and at termination of the protocol. The PI will incorporate these events into the protocol and consent as appropriate:

- Acute graft-versus-host disease
- Chronic graft-versus-host disease
- Graft failure / graft rejection
- Veno-occlusive disease
- Hemorrhagic cystitis
- Nephrotic syndrome
- Regimen-related toxicity
- Cytomegalovirus reactivation and disease
- Disease relapse or progression

Adverse events that are deemed possibly, probably or definitely related to the recipient's underlying disease(s), prior therapies, or concurrent non-protocol treatments will not be collected or reported.

13.4 Adverse Events - Donors

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

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

13.5 Reporting of SAEs

PI: All serious adverse events will be reported to Dr. Childs, PI of this study

Richard W. Childs, M.D.
Bldg 10, Room 3-5332
Phone: 301-594-8008
Pager: 104-4125-7
E-Mail: childsr@nhlbi.nih.gov

DSMB: All SAEs will be included for review by the DSMB at scheduled DSMB meetings. If the serious adverse event is thought to be due to the experimental component of the protocol, accession to the protocol will be stopped until a full discussion with the IRB has been held. A stopping rule for the primary endpoint was added to this protocol in February, 2001 at the DSMB's request.

14.0 HUMAN SUBJECT PROTECTIONS

As of June 2018, this study is no longer recruiting patients. The study status has been changed to follow-up only.

14.1 Rationale for Subject Selection

All subjects with eligible diseases itemized in section 5.0 will be considered for the protocol. Gender, ethnic background or race will not be taken into consideration.

Strategies for subject recruitment: Hematologists and oncologists throughout the country will be informed of this protocol by letter. Information about the protocol will be posted on the NIH web page. The protocol will also be listed in the physician's data query (PDQ) and clinicaltrials.gov.

Reimbursement for protocol participation, travel, food, and lodging will be consistent with NIH guidelines. In determining reimbursement, the following factors are considered applicable to this protocol: the subjects are diagnosed with a rare disease; the subject population is sick; the protocol offers the potential for direct benefit; the protocol regimen is demanding; and in order to complete accrual in a reasonable timeframe a geographically dispersed participant population is required.

Payment for participation: $0.

14.2 Participation of Children

Participation as a transplant recipient:

We are limiting the protocol to recipients over the age of 8 years as the PI does not have expertise in the care of younger children undergoing allogeneic transplantation. The procedure will be explained to recipients between the ages of 8 -17 years with formal consent being obtained from parents or legal guardians.

Participation as a stem cell donor:

Allogeneic bone marrow (and peripheral blood) transplantation (BMT) provides an option for cure for

subjects accrued to this protocol and is therefore is considered an accepted standard clinical intervention for all diseases accrued to the protocol (ALL, AML, CML, CLL, NHL, MM, SAA, PRCA, PNH, MDS). The donor would be donating stem cells to his/her family member regardless of the objectives of this research protocol.

We are, however, excluding from participation as donors, children who weigh ≤18 kg and are < 2 years of age. 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, (3) need for systemic heparinization because subject is too small to get citrate.

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

19 to 25 kg: A central line is usually required. Donors may or may not need a central line (at the discretion of the Apheresis department). With concurrent magnesium and calcium infusion, children may be safely anti-coagulated with citrate.

For donors less than age 18, a social worker and mental health specialist (psychologist or psychiatrist) will meet with the minor prior to the assent process to determine willingness to participate. Donors who are less than 18 years of age will see a pediatric provider (pediatrician, pediatric nurse practitioner or pediatric physician's assistant) who is separate from the transplant team. This practitioner will serve as the donor's health care provider and advocate during the minor's participation on the clinical trial.

Donors, less than 18 years of age will be included in the discussion of the research when formal consent is obtained We will encourage pediatric donors to ask questions about any of the tests or treatments and to sign an assent document. Donors less than age of assent will be included in the discussion of the research, however they will not sign assent. Rather, the research team will rely on the determination of the mental health specialist as to the young subject's willingness to participate.

As participants in laboratory research studies: Pediatric subjects may participate only in those laboratory studies that the IRB categorizes as "*does not involve greater than minimal risk to children*" provided that adequate provisions are made for soliciting the assent of the children and the permission of their parents or guardians (see section 14.4.5).

14.3 Hazards and Discomforts

14.3.1 The recipient

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 TRM with non-myeloablative PBSC transplantation, the procedure nevertheless carries 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 recipient, donor and/or immediate family. The specific hazards of this study using a non-myeloablative preparative regimen and high PBPC content graft are graft rejection, GVHD and disease relapse. The major discomforts are those of nausea, mucositis, anorexia, diarrhea, fever and malaise, and intolerance of the isolation period.

Related to the common drugs used in BMT:

Antithymocyte Globulin: Febrile reactions, allergic symptoms including rash, wheezing, anaphylaxis, cytopenias, serum sickness, nausea, vomiting, diarrhea, arthralgias, hypotension, tachycardia, bradycardia, fever, chills, myelosuppression. Several cases of a severe lung injury related to ATG treatment have been reported. Although this side effect appears extremely rare, it is serious and can be fatal. There is no information about the mechanism or specific treatment for this condition. A few patients recovered after intensive medical support including use of a breathing machine.

Cyclosporine: CSA is metabolized primarily in liver but the major toxicity is renal. Side effects include renal impairment, reversible renal insufficiency, hemolytic uremic syndrome, elevated bilirubin and transaminases that normalize with continued administration or reduced dose, hypertrichosis, headaches, nausea, gingival hypertrophy, parasthesias (painful hands and feet), hypertension, hypomagnesium, bilirubinemia, hypertrichosis, nausea, tremor, and seizure. An extremely rare complication of cyclosporine is blindness, which may be irreversible. Posterior reversible encephalopathy syndrome (PRES) is an increasingly recognized neurologic disorder seen in 6% of patients on cyclosporine which manifest with acute to subacute hypertension and/or seizures. In the event of hypertension, subjects will be prescribed 1 or more medications to control blood pressure in an effort to decrease the risk of this complication.

Cyclophosphamide: Immediate: tingling and metallic taste, nausea and vomiting, ADH-like effect, cardiotoxic at high doses (>70 mg/kg). Rare: pulmonary toxicity, urticaria and flushing, mucositis Delayed: marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility.

Fludarabine*:* Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, and rarely hemolysis and pulmonary toxicity, hemolytic anemia and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL patients treated with fludarabine. ***Methotrexate:*** Mucositis, nausea, dizziness, neutropenia, thrombocytopenia, malaise, fatigue, fever, melena, headaches, blurred vision, rashes, alopecia, elevated liver functions.

Hematologic: Myelosupression (leukopenia [nadir 7 days] thrombocytopenia, anemia).

Hepatic: Acute (elevated transaminases) and chronic (fibrosis and cirrhosis) hepatic toxicity. Chronic toxicity has generally occurred after prolonged use (generally 2 years or more) and after a total dose of at least 1.5 grams.

Urogenital: Severe nephropathy or renal failure, azotemia, cystitis, hematuria, defective oogenesis or spermatogenesis, transient oligospermia, menstrual dysfunction and vaginal discharge, infertility, abortion, fetal defects. With high doses of methotrexate, close attention to renal function including adequate hydration, and urine alkanization are essential for safe administration.

Gastrointestinal: Gingivitis, pharyngitis, stomatitis, anorexia, nausea, vomiting, diarrhea, hematemesis, melena, gastrointestinal ulceration and bleeding, enteritis. Should be used with extreme caution in the presence of peptic ulcer disease or ulcerative colitis. Therapy may be discontinued if ulcerative stomatitis or other severe GI adverse reactions occur.

Pulmonary: Interstitial pneumonitis deaths have been reported, and chronic interstitial obstructive pulmonary disease has occasionally occurred. Pulmonary symptoms or a nonspecific pneumonitis may be indicative of a potentially dangerous lesion and require interruption of treatment and careful investigation; infection needs to be excluded. This lesion can occur at all dosages.

Skin: Erythematous rashes, pruritis, urticaria, photosensistivty, pigmentary changes, alopecia, ecchymosis, telangiectasia, acne, furunculosis.

Central nervous system: Headaches, drowsiness, blurred vision. There have been reports of leukoencephalopathy following intravenous administration of methotrexate to patients who have craniospinal irradiation. Aphasia, hemiparesis, paresis, and convulsions have also occurred following administration of methotrexate. Following low doses, occasional patients reported transient subtle cognitive dysfunction, mood alteration, or unusual cranial sensations.
Other: Opportunistic infections, arthralgia/myalgia, loss of libido/impotence, diabetes, and osteoporosis. A few cases of anaphylactoid reactions have been reported.

Mycophenolate mofetil (use stopped in 2003): Nausea, diarrhea, leukopenia, headache, weakness, urinary frequency, leg cramps or pain, liver function test abnormalities, infection related to immunosuppression,and skin rash. Hemorrhagic gastritis and pancreatitis have been reported rarely. Malignant neoplasms have been reported in psoriasis patients treated with oral mycophenolic acid, although a definite cause-effect relationship has not been established.

Antimicrobials in general: Allergic reactions, renal impairment (gentamicin, vancomycin, amphotericin, acyclovir), red man" syndrome -vancomycin, hepatic damage (acyclovir, rifampicin).

Risks related to pregnancy and breastfeeding: Pregnant and nursing women are excluded from accrual. Subjects are required to use effective contraceptive measures to prevent pregnancy during protocol participation to eliminate the possibility of drug effects on a developing fetus. Following transplantation, woman of childbearing age will be counseled regarding when it is safe to consider becoming pregnant should they so choose. Female subjects will be advised that should pregnancy occur the research team must be informed immediately. An SAE will be filed on any pregnancy, and this report will be followed up with information on the outcome of the pregnancy and complications of (should there be any).

14.3.2 The donor

Related to filgrastim G-CSF:

The hazard to the donor is low. The discomfort from G-CSF mobilization and leukapheresis for collection of PBPC are probably lower than those associated with marrow harvesting. G-CSF has been given to large numbers of donors without major side effects or long term consequences. The side effects of G-CSF are bone pain, fatigue, insomnia, myalgia, headache insomnia, chills, low grade fever, rash, injection site irritation, exacerbation of preexisting inflammatory conditions, splenomegaly, reversible elevation in uric acid, LDH and leucocyte alkaline phosphatase which are usually mild and self limiting. Reversible thrombocytopenia (mild to moderate decreases in platelet number), with platelet counts falling to the range of 100,000 / mm^3 is common. Two patients have been reported to experience non-fatal splenic rupture after more prolonged treatment with higher doses of G-CSF. One of these two patients had concurrent mononucleosis, a second cause for splenic rupture. Patients with ongoing ischemic heart disease have been reported to have angina seemingly temporally-related to G-CSF administration and apheresis. In addition, a rare occurrence of pulmonary hemorrhage has been reported in a healthy donor who was a cigarette smoker and had underlying pulmonary disease ([24]).

Leukapheresis and apheresis of stem cells:

The apheresis procedures will be performed in accordance with standard apheresis donation policies and procedures operative in the Dept. of Transfusion Medicine and will be in compliance with the Blood Donor Standards of the American Association of Blood Banks and the rules and regulations of the Food and Drug Administration. Adverse reactions to apheresis procedures are rare, but include:

Pain and hematoma at the needle placement site.

Vasovagal episodes, characterized by transient hypotension, dizziness, nausea and rarely, syncope are seen in less than 2% of the procedures. Hypotension secondary to volume depletion is known for the rare potential for a cerebrovascular or cardiovascular event.
Cutaneous or circumoral parasthesias, chills, nausea, heartburn and rarely muscle spasms may result from the use of citrate anticoagulant used to prevent clotting in the extracorporeal circuit. Citrate reactions are usually relieved by slowing the rate of the anticoagulant infusion and by administering oral calcium carbonate tablets or with intravenous calcium gluconate.
Donation of PBPC on three successive days significantly increases the risk of thrombocytopenia (<100,000/ul). However, thrombocytopenia is transient and unlikely to cause clinical sequelae.

Prior to each apheresis, the potential risks associated with the procedure will be explained to the subject and a separate informed consent obtained.

Bone marrow harvest:

In exceptional instances the donor may be required to donate bone marrow. There is no additional risk to the donor giving marrow after PBPC donation (than would normally be associated with bone marrow harvesting). No major risks are involved with bone marrow harvest, other than a small risk of infections, pain, bleeding, and hematoma formation at the site of the aspiration exists with the procedure.

Related to central line placement:

It is estimated that less than 50% of the donors will require intravenous central line placement (femoral vein) 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. Some subjects may experience a vaso-vagal reaction (lightheadedness, or, rarely, fainting due to temporary lowering of blood pressure). These risks are minimized by using only trained experienced MICU staff for the procedure. Central line placement in a minor may be done under conscious sedation. 3 of 10 minor donors (4/15/2009) required femoral lines, one of which required conscious sedation.

14.4 Risks in Relation to Benefit

14.4.1 For adult transplant subjects.

Clinically the approach is ethically acceptable because we are offering adult subjects with debilitating and often lethal hematological diseases incurable with conventional treatments other than allogeneic BMT, an alternative to symptomatic therapy. The protocol aims to decrease the risk of TRM, thus making more patients candidates for potentially curative therapy. The risk of death due to complication from the allogeneic BMT procedure is justified by the anticipated benefit of potentially eradicating the underlying hematological diseases of the subjects, and the relation of the anticipated benefit to the risk is at least as favorable to the subjects as the risk presented by available non-transplantation therapies.

Therefore, for adult transplant recipients on this protocol, the research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102).

14.4.2 For pediatric transplant subjects.

The inclusion of children satisfies the criteria set forth in 45 Code of Federal Regulations 46, Subpart D: 46.405 as follows:

(a) the risk is justified by the anticipated benefit to the subjects: Clinically the approach is ethically acceptable because we are offering a child with debilitating and often lethal hematological diseases incurable with conventional treatments other than allogeneic BMT, an alternative to symptomatic therapy
(b) the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches. The protocol aims to decrease the risk of transplant-related mortality, thus making more patients candidates for potentially curative therapy
(c) the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches. The protocol aims to decrease the risk of transplant-related mortality, thus making more patients candidates for potentially curative therapy
and
(d) adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians, as set forth in 46.408.

Therefore, for pediatric transplant recipients on this protocol, the research involves greater than minimal risk but presenting the prospect of direct benefit to the individual subjects (45 CFR 46.405).

14.4.3 For adult donors

Normal identical or single HLA locus mismatched family members will be co-enrolled into this study as stem cell donors. The stem cell collection aspect of this protocol is not investigational. Despite the risks associated with this procedure (section 14.3.2), potential benefit does exist for family donors. The donor derives psychosocial benefit from donating stem cells both at the time of donation and possibly into the future, especially in view of the reduced life expectancy due to this disease in a family member. Other potential benefits include detection of illnesses, determination of blood cell counts, and evaluation of kidney and liver function in the potential donor at the time of screening.

Therefore, for adults participating as stem cell donors, the research involves greater than minimal risk but presents the prospect of direct benefit to the individual subject (45 CFR 46.102).

14.4.4 For pediatric donors

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

Stem cell transplant is an accepted standard clinical intervention for the diseases under investigation. The donor would be donating stem cells to his/her family member regardless of the objectives of this research protocol. The stem cell collection procedure is not considered part of the research for the donors, the risks of the stem cell collection procedure would not be considered risks of the research for the pediatric donors.

Therefore participation as a stem cell donor on this protocol is considered exempt from the criteria set forth in 45 CFR 46, Subpart D.

14.4.5 For pediatric donors – healthy volunteers- involved in laboratory research studies

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

(a) The research does not involve greater than minimal risk. Blood and bone marrow specimens for research are obtained concurrently with clinically indicated sampling. Therefore, there is no risk associated with sample collection for research because research will only be performed on material obtained during standard clinical intervention.
(b) Research specimens will be stored in the Dr. Child's laboratory. 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.
(c) Only those laboratory tests approved by the IRB and involving not greater than minimal risk will be conducted (See Appendix C, laboratory studies D1-D6 are determined to be greater than minimal risk). Research will not include genetic testing. Therefore, there is no genetic testing associated risk.
(d) adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians, as set forth in 46.408.

Therefore, for pediatric donors – healthy volunteers- participating in laboratory research studies on this protocol, the research does not involve greater than minimal risk (45 CFR 46.404).

14.5 Informed Consent

The investigational nature and research objectives of this trial, the procedure and its attendant risks and discomforts will be carefully explained to the subject and a signed informed consent document will be obtained prior to entry onto this study. Investigators names marked with an asterisk on the cover sheet of this protocol (Drs. Childs, Srinivasan, Barrett, Gormley or Young) 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 attesting to the fact that the child had given assent.

If the donor is a minor, assent will not be sought until an evaluation by a social worker and mental health specialist (psychologist or psychiatrist) is completed to determine the minor donor's willingness to participate. As detailed above, 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 donor's assent will be obtained. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent.

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

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

Reconsenting for nephritic syndrome testing: Subjects already consented on a stem cell transplant protocol and not available for a NIH clinic visit: The revised consent will be sent to the transplant recipient or donor to be executed by telephone. The investigational nature of the testing will be carefully explained to the subject. The PI, or an AI will lead this discussion (Drs. Childs, Srinivasan, Gormley or nurse Cho, Cook or Ramos). If the subject chooses to participate he/she will be directed to sign and date the revised consent and have someone witness and date his/her signing of the document. The informed consent document will then be mailed to the PI or AI who led the discussion, who will sign and date and mail back a fully executed copy for the subject's records. The informed consent process will be documented on a progress note and a copy of said note and the original informed consent will be filed in the subject's medical record.

(**See Clinical Center Policy and Communication Bulletin M77-2 Dated 30 May 2001. Subject: **Informed Consent, Section IX. Consent from someone not at the clinical center:** "For research protocols or any procedures performed for the purposes of research that involve obtaining consent via technology and/or electronic process, rather than in person, the procedures for obtaining consent, including how information will be transmitted and documented and by whom, shall be detailed in the written protocol. Review and approval must first be obtained from the institute clinical director and the relevant IRB)

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

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

(1) The research involves no more than minimal risk to the subjects.
    a. Analysis of samples and data from this study involves no additional risks to subjects.
(2) The waiver or alteration will not adversely affect the rights and welfare of the subjects.
    a. Retention of these samples or data in secure locations does not affect the welfare of subjects.
(3) The research could not practicably be carried out without the waiver or alteration.
    a. Considering the length of time between a minor's enrollment and their age of majority, it is possible that more than a few subjects may be lost to follow up. A significant reduction in the number of samples analyzed could impact the quality of the research.
(4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
    a. We only plan to request a waiver of reconsent for those subjects who have been lost to follow-up.

14.6 Conflict of Interest and Tech Transfer Agreements

The PI assured that each AI listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation were provided a copy of the document when they were added. Copies of the Conflict of Interest Statement were forwarded to the Clinical Director. No initial or subsequent members of the research team reported a potential conflict of interest.

This protocol has no associated patents, CRADAs, or CTAs.

15.0 PHARMACEUTICALS

15.1 ANTI-THYMOCYTE GLOBULIN, Equine (Atgam, lymphocyte immune globulin, horse [equine] ATG)

Supply: Commercially available.
Product description: Anti-thymocyte globulin (equine) is the purified, concentrated, and sterile gamma globulin, primarily monomeric IgG, from hyperimmune serum of horses immunized with human thymus lymphocytes. It is available in 5 mL (250 mg) ampules containing 50 mg of horse gamma globulin/mL stabilized in 0.3 molar glycine to a pH of approximately 6.8.
Preparation: Dilute anti-thymocyte globulin (ATG) for intravenous infusion in an inverted bottle of sterile vehicle so the undiluted drug does not contact the air inside. Dilute the total daily dose of ATG (equine) in a compatible diluent sterile solution (0.9% sodium chloride injection, 5% dextrose and 0.225% sodium chloride injection, or 5% dextrose and 0.45% sodium chloride injection) to a final concentration that does not exceed 4 mg/mL. Do not dilute anti-thymocyte globulin in dextrose injection (e.g. 5% dextrose injection) as low salt concentrations can cause precipitation.
Storage and stability: Vials of ATG (equine) should be stored in a refrigerator at 2– 8 ^{o}C (36 – 46 ^{o}F). Once diluted for intravenous administration, ATG (equine) is physically and chemically stable for up to 24 hours at concentrations up to 4 mg/mL. It is recommended that diluted ATG (equine) be stored in a refrigerator if it is prepared prior to the time of infusion. Even if it is stored in a refrigerator, the total time in dilution should not exceed 24 hours (including infusion time).
Route of administration: ATG (equine) diluted for intravenous administration should be infused preferentially into a central venous catheter through an in-line filter with a pore size of 0.2 to 1 micron. The use of high-flow veins will minimize the occurrence of phlebitis and thrombosis. Do not infuse a dose of ATG (equine) in less than 4 hours. Follow nursing care SOP guidelines for monitoring during the infusion.

15.2 CYCLOPHOSPHAMIDE (Cytoxan, Neosar)

Supply: Commercially available.
Product description: Cyclophosphamide is available as a lyophilized powder for injection in multiple vial sizes.
Preparation: Cyclophosphamide powder for injection should be reconstituted with sterile water for injection to yield a concentration of 20 mg/mL as described in the product labeling. Once reconstituted, the prescribed dose will be further diluted in 250 mL of 0.9% sodium chloride injection or 5% dextrose in water for intravenous administration over 60 minutes.
Storage and stability: Vials of cyclophosphamide are stored at room temperature. Once reconstituted as directed, solutions of cyclophosphamide are stable for 24 hours at room temperature, or 6 days when refrigerated at 2-8^{o} C.
Route of administration: The prescribed dose of cyclophosphamide will be diluted in an additional 250 mL of 0.9% sodium chloride injection or 5% dextrose in water for intravenous administration over 60 minutes.

15.3 CYCLOSPORINE (Gengraf, Sandimmune, Neoral)

Supply/product description: Cyclosporine will be obtained by the NIH Clinical Center Pharmacy Department from commercial sources and is available in capsules (25 mg and 100 mg), USP [MODIFIED], oral solution (100 mg/ml), USP [MODIFIED], and as a parenteral concentrate for injection (50 mg/ml). When oral capsules are prescribed for this protocol, the cyclosporine capsules, USP [NON-MODIFIED] should NOT be used.
Preparation: For parenteral doses, each milliliter of concentrate (50mg/ml) should be diluted in 20 to 100 ml of dextrose 5% in water or sodium chloride 0.9%. Parenteral doses of cyclosporine will be prepared in non-PVC containers and infused with non-PVC administration sets/tubing. Oral cyclosporine solution may be mixed in orange juice or other beverages, but not milk.
Storage and Stability: Capsules, oral solution, and ampules of parenteral concentrate bear expiration dates and are stored at room temperature and protected from light. Cyclosporine concentrate for injection that has been diluted to a final concentration of approximately 2mg/ml is stable for 24 hours in 5% dextrose or 0.9% sodium chloride injection in glass, PVC or non-PVC plastic containers. To minimize the potential for sorption to PVC plastic bags and tubing as well the leaching of phthalate plasticizer (DEHP) into the solution, only non-PVC plastic bags and intravenous administration sets should be utilized.
Administration: Cyclosporine may be given intravenously or orally.

15.4 FILGRASTIM (G-CSF, Neupogen)

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

15.5 FLUDARABINE PHOSPHATE (Fludara)

Supply: Commercially available.
Product description: Fludarabine phosphate is commercially available as both a lyophilized powder for injection in vials containing 50 mg of fludarabine phosphate with mannitol 50 mg and sodium hydroxide for pH adjustment and a solution for injection in 2 mL vials containing 50 mg of fludarabine phosphate (25 mg/mL of fludarabine) with 25 mg/mL mannitol and sodium hydroxide for pH adjustment.
Preparation: Fludarabine lyophilized powder for injection should be reconstituted with 2 mL of sterile water for injection, USP to a concentration of 25 mg/mL. The prescribed dose of fludarabine should be diluted in 100 mL of either 0.9% sodium chloride or 5% dextrose in water for intravenous administration over 30 minutes.
Storage and stability: Fludarabine vials should be stored under refrigeration between 2-8 ^{o}C (36- 46 ^{o}F). Reconstituted fludarabine phosphate is chemically and physically stable for 24 hours at room temperature or for 48 hours if refrigerated. The manufacturer recommends use of either the reconstituted powder for

injection or the solution for injection (once diluted for administration) within 8 hours because neither product contains an antimicrobial preservative.
Administration: The prescribed dose of fludarabine should be diluted in 100 mL of either 0.9% sodium chloride or 5% dextrose in water for intravenous administration over 30 minutes.

15.6 METHOTREXATE (Methotrexate sodium)

Supply: Commercially available.
Product description: Methotrexate is commercially available as a 25 mg/mL preservative-free isotonic solution for injection.
Preparation: The desired dose will be diluted in 5% dextrose in water or 0.9% sodium chloride.
Storage and stability: Methotrexate should be stored at room temperature and protected from light. Once the prepared dose is diluted for intravenous administration, the solution is stable for 24 hours refrigerated or at room temperature when protected from light.
Administration: The prescribed dose of methotrexate will be given as an intravenous infusion over 15 minutes. Refer to BMT Supportive Care Guidelines for dose adjustments based on serum creatinine and total bilirubin.

15.7 MYCOPHENOLATE MOFETIL (MMF)—not used after February 2003

Generic*:* Mycophenolate mofetil.
Other*:* CellCept, Roche Laboratories.
Classification*:* Immunosuppressive agent.
Dose*:* 1 gram p.o. twice daily given to some recipients with steroid resistant acute GVHD or steroid dependent chronic GVHD.
Action*:* Mycophenolic acid produces potent, noncompetitive inhibition of inosine monophosphate dehydrogenase, thus blocking de novo synthesis of guanosine nucleotides; as lymphocytes depend upon the de novo pathway for purine synthesis, lymphocyte proliferation is inhibited. In vitro and in vivo studies have demonstrated the ability of mycophenolic acid to block proliferative responses of T and B lymphocytes, and inhibit antibody formation and the generation of cytotoxic T-cells.
Metabolism*:* Mycophenolate mofetil is rapidly converted to its active metabolite, mycophenolic acid in the liver. Mycophenolic acid is subsequently metabolized to mycophenolic acid glucuronide which is inactive and is excreted via the urine and bile. Enterohepatic recirculation of mycophenolic acid may occur. The elimination half-life of mycophenolic acid is approximately 11 to 18 hours.

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APPENDIX A PREPARATIVE REGIMEN

HLA IDENTICAL SIBLING REGIMEN

Day	Hour	Regimen
-7	0 (midnight)	Hydrate: NS 2.6ml/kg/hr 10 mEq/l KCl (starting 12 hours pre-cyclophosphamide and continue hydration until 24 hours after last cyclophosphamide infusion)
	11	Ondansetron 8 mg IV q12 hrs (or equivalent serotonin antagonist) + decadron 8 mg PO q12hrs x 1 day Mesna (per Supportive Care Guidelines) Furosemide 20 mg IV
	12	Cyclophosphamide 60 mg/kg IV in 250 ml D_5 in 1 hr Mesna (per Supportive Care Guidelines)
-6	11	Ondansetron 8 mg IV q12 hrs (or equivalent serotonin antagonist)+ decadron 8 mg PO q12 hrs x 1 day Furosemide 20 mg IV
	12	Cyclophosphamide 60 mg/kg I.V. in 250 ml D_5 I.V. over 1 hr
	13	Mesna (per supportive care guidelines)
Day -5		Stop IV hydration (24 hours after last cyclophosphamide dose) Monitor electrolytes and fluid balance while receiving hydration for cyclophosphamide
Day -5 to Day -1	9	Fludarabine 25 mg/m^2 IVBP over 30 minutes for 5 days

SINGLE HLA ANTIGEN MIS MATCH REGIMEN

Same as HLA identical sibling regimen except some subjects will receive ATG at a dose of 40 mg/kg/d for 4 consecutive days on day -5 to day –2 (see section 7.5 preparative regimen).

APPENDIX B: Donor stem cell mobilization and apheresis.

I. Donor stem cell mobilization with G-CSF and donor stem cell apheresis

After medical evaluation and clearance for suitability as an allogeneic donor, each donor will undergo mobilization with G-CSF as an outpatient. The donor will receive 10 mcg /kg G-CSF subcutaneously daily starting day –4 before apheresis. On day 0, a large volume leukapheresis of 15- 25 liters will be performed. The volume of the leukapheresis procedure will be targeted to obtain a progenitor cell dose of $5x10^6$ CD34+ cells/kg. Obtaining a dose of $> 3x10^6$ CD34+ cells/kg will be considered adequate and no further apheresis will be performed. If after the first day of apheresis, the product contains $< 3x10^6$/kg CD34+ cells, a decision regarding further apheresis will be made at the discretion of the PI, in consultation with Department of Transfusion Medicine. The minimum acceptable CD34+ cell dose to proceed with the protocol is $2x10^6$/kg.

Predictable side effects of G-CSF, including headache, bone pain, and myalgia, will be treated with acetaminophen or ibuprofen. Prophylactic treatment of these side effects may also be carried out with the same medications. Other side effects will be evaluated and treated accordingly.

Leukapheresis procedures will be carried out in the Apheresis Unit, Department of Transfusion Medicine. These procedures will be done via a 2-armed approach or by temporary central venous catheter in the femoral vein, using the Baxter CS3000Plus instrument or the Cobe Spectra. These procedures will use ACD-A anticoagulant, routinely used in normal donors. If the donor is very small or relatively intolerant to ACD-A, and the adverse effects cannot be controlled by usual means, consideration will be given to using partial anticoagulation with heparin.

II. Leukapheresis PBPC product

The processing goals for this protocol are to provide a product with a maximum dose of CD34+ cells. ABO or other erythrocyte incompatibility is not an issue because the resulting number of contaminating erythrocytes is well below 1 ml using this system. The final cell suspension will be prepared for infusion by transferring the cells into a 50-150 ml volume of an infusible solution (Plasmalyte A with 1% human serum albumin), suitable for intravenous infusion over 15 minutes to 1 hour, depending on clinical circumstances.

APPENDIX C NHLBI LABORATORY RESEARCH STUDIES

IRB APPROVED NHLBI LABORATORY RESEARCH STUDIES v. 2.5.13

	DESCRIPTION OF LABORATORY STUDY BY BRANCH SECTION	Does this test pose a greater than minimal risk to pediatric subjects per 45 CFR 46.404?	Does this test pose a greater than minimal risk to healthy pediatric donors per 45 CFR 46.404?
A	**Stem Cell Allotransplantation Section (Dr. A. John Barrett) (No longer active)**		
B	**Molecular Hematopoiesis Section (Dr. Cynthia Dunbar)**		
B.1	Flow cytometric analysis of cell surface and cytoplasmic proteins, including cell adhesion molecules, putative retroviral receptors, and markers of differentiation, using bone marrow and mobilized peripheral blood cells.	No	No
B.2	Hematopoietic progenitor-derived colony ascertainment in vitro (as described above), and engraftment of immunodeficient mice for detection of human stem cell number and function.	No	No
B.3	Testing ability of hematopoietic progenitor cells to be transduced with retroviral, lentiviral, and novel gene transfer vectors in vitro.	No	No
B.4	Reprogramming of adult mature cells, including skin fibroblasts and blood cells, into induced pluripotent stem cells in vitro.	No	No
C	**Cell Biology Section (Dr. Neal Young)**		
C.1	Studies of blood and bone marrow hematopoietic progenitor numbers, including early and late erythroid progenitors, myelomonocytic progenitors, and multi-potential progenitor cells. In addition, bone marrow may be placed in long-term bone marrow culture to assess the function of stroma and stem cells and to assay more primitive progenitors, as well as organelle culture. Whole or selected bone marrow populations are cultured short-term for CD34 cell expansion.	No	No
C.2	Assays of apoptosis in hematopoietic cells and their progeny, using flow cytometric methods such as annexin and caspase-3 staining, propidium iodide uptake, and mitochondrial permeability tests.	No	No
C.3	Separation and functional study of cell populations characteristic of paroxysmal nocturnal hemoglobinuria, identified by absence of glycosylphosphatidylinositol anchored proteins.	No	No
C.4	Studies of mutation rates in hematopoietic cells and in buccal mucosa cells, using conventional hypoxanthine phosphoribosyltransferase activity functional assays, sequencing of mitochondrial DNA after specific gene amplification, and measurement of GPI-anchored deficient cells in blood and bone marrow.	No	No

C.5	Assays of immune function of T-cells, including intracellular cytokine staining, ELISPOT, semiquantitative gene amplification for gamma-interferon, tumor necrosis factor, interleukin-2, and other cytokines, and functional assessment in co-culture using specific neutralizing monoclonal antibodies. In addition, peripheral blood lymphocytes are subjected to spectratyping for CDR3 size distribution as well as nucleotide sequence of CDR3 peaks obtained.	No	No
C.6	Studies of engraftment of human normal and diseased bone marrow and peripheral blood in immunodeficient mice in order to determine the presence of hematopoietic repopulating stem cells as well as functional differences among selected populations.	No	No
C.7	Flow cytometric analysis of blood and bone marrow for lymphocyte phenotype, especially for evidence of activation of lymphocytes, for markers of apoptosis, and for antigens associated with primitive and mature hematopoietic cell populations.	No	No
C.8	Flow cytometric analysis of blood and bone marrow for hematopoietic stem cell progenitors and CD34 positive cells.	No	No
C.9	Studies of chromosomal instability in myelopdysplastic syndromes including BM cell and CD34 cell response to PAS crosslinking and examination of the cytotoxic effect of lymphocytes to the abnormal clone of cells.	No	No
C.10	Surface Enhanced Laser/Desorption Ionization (SELDI) time-of-flight mass spectrometry (Ciphergen) (proteomics methodology).	No	No
C.11	Mitochondrial DNA (mtDNA) sequence heterogeneity.	No	No
C.12	Measurement of EBV viral load.	No	No
C.13	Measurement of EBV LMP-1 via RT-PCR for LMP-1 RNA or flow cytometry for LMP-1.	No	No
C.14	Outgrowth assay of EBV transformed B cells.	No	No
C.15	Quantification of serumchemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12).	No	No
C.16	Quantification of EBV cytotoxic T cells (tetramerstaining).	No	No
C.17	Telomere length measurement by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA	No	No
C.18	Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: *DKC1* ,*TERC*, *TERT*, *SBDS*, *NOp10* , *NHP2*.	No	No
C.19	Analysis of inflammatory markers and/or bacterial, viral, fungal or protozoal elements in plasma or serum using molecular, colorimetric, enzymatic, flow cytometric or other assays in subjects receiving immunosuppressive therapy, chemotherapy and/or bone marrow transplantation.	No	No
C.20	Confocal microscopic imaging of bone marrow.	No	No
C.21	Characterization of intracellular signaling proteins by cell permeabilization and flow cytometry, and quantitative immunoblots.	No	No
C.22	Assays for chromosomal aneuploidy by florescence in situ hybridization (FISH) and other molecular techniques.	No	No
C.23	Conversion of human dermal fibroblasts into hematopoietic progenitors using Oct4 transfection.	No	No
C.24	Quantification of gene expression with RNA-seq	No	No
C.25	Characterization of chromatin and promoter/enhancer landscapes with ATAC-seq	No	No
C.26	Measurement of protein markers with SomaLogic's SOMAscan assay	No	No
D	**Virus Discovery Section (Dr. Neal Young) THESE ASSAYS WILL NOT BE PERFORMED ON SAMPLES FROM HEALTHY PEDIATRIC DONORS**		

D.1	Assays of serum, blood cells, and bone marrow cells for B19 parvovirus and possible B19 variants using gene amplification, cell culture, and hematopoietic colony inhibition assays.	No	N/A
D.2	Assays of blood, bone marrow, liver, and other tissues for potentially novel viruses, using a variety of techniques including RNA and DNA assays, differential display, gene amplification with conserved and random primers, cell culture assays, immunohistochemical methods, and inocculation of mice, rabbits, and monkeys, as well as antibody measurements.	No	N/A
D.3	Assays of blood, bone marrow, and liver for known viruses, including herpesviruses such as cytomegalovirus, human herpesviruses 6, 7, and 8, enteric viruses such as A-6, circiviruses, and parvoviruses, using assays as in (2).	No	N/A
D.4	Spectra-typing of blood cells to determine response to known or putative viral infections.	No	N/A
D.5	HLA typing or subtyping to determine risk factors/determinants for hepatitis-AA studies.	No	N/A
D.6	Cytotoxic lymphocyte assays with intracellular cytokine measurement for determining anti-viral response and lymphocyte cloning to obtain clones with specific antiviral activity.	No	N/A
E	**Solid Tumor Section (Dr. Richard Childs)**		
E.1	Cr51 cytotoxicity assay to evaluating killing of patient tumor cells by patient NK cell clones and T-cells.	No	No
E.2	ELISA for IL-12 maturity of DC's made from subjects monocytes.	No	No
E.3	ELISA for IFN ã to evaluate specificity of CTL clones.	No	No
E.4	H thymidine uptake to evaluate proliferation potential of antigen specific T-cells.	No	No
E.5	PCR of STR to assess chimerism status of cellular subsets grown in-vitro or retrieved from subjects post-transplant.	No	No
E.6	Flow sorting of PBL and/or tissue samples to evaluate chimerism of different subsets.	No	No
E.7	Surface marker analysis of peripheral blood mononuclear cells using flow cytometry.	No	No
E.8	cDNA expression arrays to evaluate T-cells expression/gene patterns in subjects with GVHD and a GVT effect.	No	No
E.9	Geno typing of tumor or tissue samples by high density cDNA arrays.	No	No
E.10	VHL mutation analysis on kidney cancer tissue.	No	No
E.11	Transduction of dendritic and tissue cells with tumor antigens using plasmids, viral vectors and hybrid fusions.	No	No
E.12	Lasar capture microdisection of cells from tumor biopsies and tissue samples to determine origin (donor vs patient).	No	No
E.13	Quantification of polyoma virus BK exposure by serology and PCR in stem cell transplant donors and recipients from blood and urine samples.	No	No
E.14	Quantification of polyoma virus BK specific T cells in stem cell transplant donors and recipients from peripheral blood samples.	No	No
E.15	Determination of origin of neovasculature endothelial cells in tumor and tissue samples obtained from subjects post transplant.	No	No
E.16	Quantification of lymphocyte subsets CD34 progenitors and endovasculator progenitors in G-CSF mobilized peripheral cell allografts.	No	No
E.17	Testing for polyoma virus BK latency in CD34 progenitors, B cells and T cells in the G-CSF mobilized peripheral cell allografts.	No	No
E.18	Determination of etiology of membraneous nephropathy using serum from subjects.	No	No

E.19	Serum Proteomic patterns analysis to diagnose complications related to allogeneic transplantation.	No	No
E.20	Determine cell origin (donor vs patient) of tissue samples using IHC, IF, sorting, and FISH.	No	No
F	**Lymphoid Malignancies Section (Dr. Adrian Wiestner)**		
F.1	Culture of cells from research subjects to investigate molecular disease mechanisms, model host tumor interactions, and to test effect of drugs on cell survival and cellular functions.	No	No
F.2	Generation of stable cell lines for the study of hematologic malignancies.	No	No
F.3	Modifications of cells using standard expression systems or biologic molecules, e.g. interfering RNA, to investigate the effects of candidate genes on cellular functions.		
F.4	Identification and monitoring of B or T cell populations as identified by flow cytometry and by their B cell or T cell receptor expression.	No	No
F.5	Measurement of gene expression in cells or tissues. Techniques frequently used include gene expression profiling on microarrays, quantitative RT-PCR, Western blotting, flow cytometry and ELISA assays.	No	No
F.6	Analysis of chromosomal abnormalities or mutations in malignant cells and non-malignant cells including FISH technology and DNA sequencing.	No	No
F.7	Assays of immune function of B-cells and T-cells, including intracellular cytokine staining, ELISPOT, quantitative RT-PCR for cytokines or other immune regulatory genes.	No	No
F.8	Analysis of antibody specificities in serum and antigen specificity of the B-cell receptor on cells. Techniques may include expression of antibodies in phage display systems, generation of antibodies in cell culture systems and use of such antibodies to screen for cognate antigens.	No	No
F.9	Transplantation of human cells into mice (xenograft model) to study disease biology and to investigate the effect of experimental therapy.	No	No
F.10	Measurements of drug concentrations, biologic molecules and disease markers in blood, serum, and plasma.	No	No