Synopsis

Title:	HLA-Identical Sibling Renal Transplant Tolerance With Donor Hematopoietic Stem Cells and Campath-1H
Sponsor:	National Institutes of Health (National Institute of Diabetes and Digestive and Kidney Diseases Branch) and the Comprehensive Transplant Center of Northwestern University
Principal Investigator:	Joseph Leventhal, MD, PhD
Accrual Objectives:	30 recipients, 30 donors, 110 control subjects, and up to 60 parents
Study Design:	To induce immunologic tolerance in non-randomized, kidney transplant recipients in a single center study.
Study Duration:	3 years, with clinical and laboratory routine follow-up for at least 10 years.

Primary Study Objectives The primary objectives of this trial are:

1.) To remove all immunosuppressive therapy from recipients of HLA-identical sibling renal transplants within 24 months of transplantation.
2.) To detect and follow cellular (macro) chimerism of donor hematopoietic stem cell (DHSC) lineages and the generation of T-regulatory cells using specialized immunomonitoring assays for these donor/recipient pairs to demonstrate specific immunologic unresponsiveness.
3.) To investigate the safety and efficacy of a treatment regimen consisting of induction therapy with Campath-1H and steroid-free low dose maintenance immunosuppression, consisting of mycophenolate mofetil (MMF)/Myfortic and tacrolimus converted to sirolimus. This is to be followed by complete withdrawal of immunosuppression beginning at one year, at a minimum, post-transplant, in recipients who have also been given four infusions of purified donor hematopoietic CD34+ stem cells (DHSC).

Primary Outcomes The primary endpoints are:

1.) Patient and graft survival measured at the one-year timepoint post-transplant.
2.) The ability to withdraw immunosuppression as above 24 months post-transplant with follow-up to 10 years.

Secondary Outcomes Secondary safety and efficacy endpoints are as follows:

1) Patient and graft survival measured at the three and five year time points post-transplant.

2) Incidence rate of biopsy-proven acute rejection, defined as a renal biopsy demonstrating acute cellular or humoral rejection of Banff Grade IA or greater.
3) Incidence of chronic allograft nephropathy, determined using renal biopsies and laboratory values, including 24 hour urine protein excretion.
4) Incidence of graft versus host disease (GVHD).
5) Incidence of adverse events associated with renal transplantation and immunosuppression, including infections, malignancies, post-transplant lymphoproliferative disease (PTLD), thromboembolic events, hyperlipidemia, leukopenia, thrombocytopenia, GI toxicity, and cytokine release.

ELIGIBILITY

Inclusion Criteria

1. Patient has been fully informed, has signed a dated IRB-approved informed consent form obtained directly by the P.I., Co-P.I., sub-I and/or research nurse and is willing to follow study procedures for the duration of the study (for up to 10 years).
2. Recipient must have a hematocrit of ≥ 33%, or 31% if patient is considered somewhat fluid overloaded.
3. Age 18-65 years.
4. Weight > 40 kg.
5. Primary renal allograft: living related (HLA-identical donor-recipient sibling pairs)
6. Negative B-cell and T-cell cross-match and (<10%) PRA using cytotoxicity or flow cytometry.
7. Women of childbearing potential will be required to have a negative qualitative serum pregnancy test. See also #11 below.
8. Patients are to be studied equivalently as they become available for transplantation using these criteria, without regard to gender, race, or ethnicity.
9. Normal echocardiogram with an ejection fraction >50%.
10. Male participants with reproductive potential must agree to use approved methods of birth control during treatment with Campath-1H and for a minimum of 6 months following their last dose of Campath-1H. Female participants of childbearing potential must agree to use approved methods of birth control for the duration of their participation in the study.
11. Patient must agree to be followed every 2 months after year 3, for up to 10 years.

Exclusion Criteria

1. Patient has previously received or is receiving an organ transplant other than a kidney.
2. Patient is receiving an ABO incompatible donor kidney.
3. Recipient or donor is ELISA positive for human immunodeficiency virus (HIV), antibody positive for hepatitis C, or surface antigen positive for hepatitis B.
4. Recipient or donor is positive for TB (or under treatment for suspected TB), or has had previous exposure to TB (positive Mantoux) who has not undergone an accepted course of treatment.
5. Patient has a current malignancy or a history of malignancy (within the past 5 years), except non-metastatic basal or squamous cell carcinoma of the skin, or carcinoma *in situ* of the cervix that has been treated successfully.

6. Patient has uncontrolled concomitant infections and/or severe diarrhea, vomiting, active upper gastro-intestinal tract malabsorption or an active peptic ulcer or any other unstable medical condition that could interfere with study objectives.
7. Patient is currently receiving an investigational drug or has received an investigational drug within 30 days prior to transplant.
8. Patient is currently receiving any immunosuppressive agent.
9. In the judgment of the investigator, it is anticipated that the patient will be unable to take medications orally or via nasogastric tube by the morning of the second day following completion of the transplant procedure.
10. Concurrent use of warfarin, fluvastatin, astemizole, pimozide, cisapride, terfenadine, or ketoconazole.
11. Patient has a known hypersensitivity to tacrolimus, Campath-1H, Thymoglobulin, sirolimus, MMF or corticosteroids.
12. Patient is pregnant or lactating.
13. Patients with a screening/baseline total white blood cell count <4000/mm^3; platelet count <100,000/mm^3.
14. Patient is deemed unlikely to comply with the visits scheduled in the protocol.
15. Patient has any form of substance abuse, psychiatric disorder or a condition that, in opinion of the investigator, may invalidate communication with the investigator.
16. It is expected that tacrolimus cannot be instituted for longer than 5 days postoperatively.
17. Patients with a cytotoxic PRA value >10% at any time prior to enrollment.
18. Patients with Graves disease will be excluded unless previously adequately treated with radioiodine ablative therapy.
19. EBV negative recipients of an EBV positive donor.
20. History of Idiopathic Thrombocytopenic Purpura (ITP) or Thrombotic Thrombocytopenic Purpura (TTP)

Glossary of Abbreviations

ADCC	Antibody dependent cell-mediated cytotoxicity
ALG	Anti-lymphocyte globulin (rabbit)
ALT(SGPT)	Alanine Aminotransferase
APC	Antigen-presenting cells
AST(SGOT)	Aspartate Aminotransferase
ATG	Antithymocyte Globulin
ATGAM	(horse) Anti-thymocyte globulin induction therapy
BMI	Body Mass Index
BSA	Bovine serum albumin
CAD	Cadaver
cDNA	Complementary DNA
CIOMS	Council for International Organizations of Medical Sciences
CML	Cell-mediated lympholysis

CMV	Cytomegalovirus
CRF	Case Report Form
CRU	Clinical Research Unit
CsA	Cyclosporine
CTC	Common Toxicity Criteria
CTLp	Cytotoxic T Lymphocyte Precursors
D5W	5% Dextrose and Water
DBMC	Donor-specific bone marrow cells
DHSC	Donor hematopoietic stem cells
DSMB	Data and Safety Monitoring Board
DTH	Delayed Type Hypersensitivity
EBV	Epstein-Barr Virus
ECG	Electrocardiogram
EDTA	Ethylenedinitrilo Tetraacetic Acid
ELISPOT	Enzyme-linked Immunospot
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GCSF	Filgrastim (Neupogen)
GFR	Glomerular Filtration Rate
GGT	Gammaglutamyltransferase
GI	Gastrointestinal
GVHD	Graft vs. host disease
HBSAg	Hepatitis B surface antigen
HCG	Human Chorionic Gonadotropin
HDL	High density lipoprotein
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
IV	Intravenously
ICH	International Committee on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
ITN	Immune Tolerance Network
ITT	Intent-to-treat
LDH	Lactic Dehydrogenase
LDL	Low density lipoprotein
LRD	Living-related donor
mAb	Monoclonal antibody

MedDRA	Medical Dictionary for Regulatory Activities
MGH	Massachusetts General Hospital
MLC	Mixed lymphocyte culture
MLDC	Mixed lymphocyte dendritic cell culture
MMF	Mycophenolate Mofetil
mRNA	Messenger RNA
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIDDK	National Institute of Diabetic, Digestive and Kidney Diseases
NMFF	Northwestern Medical Faculty Foundation
NMH	Northwestern Memorial Hospital
NUIRB	Northwestern University Institutional Review Board
NUCRU	Northwestern University General Clinical Research UnitCenter
PBL	Peripheral blood leukocyte
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PP	Per protocol
PRA	Panel Reactive Antibody
PTLD	Post-Transplant Lymphoproliferative Disease
QID	Four times a day
RdD	Recipient-derived donor
RdR	Recipient-derived recipient
SAE	Serious Adverse Event
SAP	Statistical analysis plan
TB	Tuberculosis
TCD	T-Cell Depletion
TMP	Trimethoprim
TSH	Thyroid-Stimulating Hormone

1 Background Information and Scientific Rationale

1.1 Background

1) HLA-Identical renal transplant recipients of sibling donors have a ten-year actual (death-uncensored) graft survival of 65-70% (Cecka JM, UCLA, National Transplant Registry, 1993-1994 to 2003-2004). This 10-year data-point is on a slope that has a (late) downward curve, in all probability reflecting the cumulative effects of immunosuppressive therapy [since close to 20% mortality has occurred by 10 years with a median recipient age at transplant of 40±10.8 (S.D.) years], as well as chronic allograft nephropathy/rejection and (a few) disease recurrences.

Even in this most recent era immunosuppressive therapy continues to be the main cause of early and late morbidity and mortality, in this most favorable group of recipients. These data contradict the ethical argument against immunosuppressive withdrawal **"**because such favorable matches do so well**"**. They override considerations supporting the continuous expense and the greater susceptibility to opportunistic infections, cancer, accelerated cardiovascular disease, diabetes, and blindness, as well as (in over 50% of recipients) the lack of rehabilitation to societal norms, so as to maintain long-term graft survival, which still results in chronic deterioration and loss of life.

2) In preliminary observations, we are now following **7** renal transplant recipients of HLA-identical sibling donors between 12 and 24 months post-operatively, who received the two-dose Campath-1H induction regimen on (steroid-free) maintenance half-dose tacrolimus (Tacro) converted to sirolimus (Siro) at 2-3 months postoperatively together with between 250 and 1,000 mg per day MMF. No rejections or other serious adverse events have occurred.

3) This principal investigator has previously had and is still following patients in an ITN supported ongoing study (University of Miami Medical Center) based on living-related 1-haplotype-matched renal transplant donor/recipient pairs undergoing one DHSC infusion peri-operatively, with a boost infusion three to four months postoperatively using Campath-1H induction and (steroid-free) one-half dose Tacro with conversion to Siro (before the boost infusion), and one-half dose MMF maintenance conditioning before the withdrawal phase. Immunosuppression is being withdrawn over 30 months [beginning at 12 months postoperatively-Siro and at 24 months postoperatively-MMF]. Renal transplant biopsies are being performed at surgery and at 1, 2, and 3 years postoperatively to detect any evidence of cellular or humoral rejection. Such adverse findings would eliminate the recipient from the protocol and cause resumption of immunosuppressive maintenance (see current ITN protocol #022ST). Four patients have been transplanted and infused with DHSC and five controls have been transplanted who have been given the same immunosuppressive regimen without DHSC. (No drug withdrawal was performed in the controls.) Clinical follow-up in the controls have demonstrated 2 of 5 with mild rejection episodes 20 month data on the first of these recipients shows ten-fold more chimerism than seen in our bone marrow infused kidney transplant recipients in previous protocols[1,2] as well as (twofold) higher numbers of CD4+CD25+ T regs and Fox-P3 mRNA copies in CD3+ cells in peripheral blood. For the more recent result update in the DHSC infused patients see Section 2.1.5.

4) Campath-1H is a humanized mAb directed to CD52 determinants on the surface of human leukocytes, of which there are an estimated 400,000 binding sites per cell. Waldmann found that optimal T-cell depletion was attained with the IgG1 isoform (1H).[3] While the precise mechanisms of action are incompletely understood, it is apparent that this antibody prevents T-cell activation via CD45-signaling events and does not interfere with T-cell receptor activation, which might be highly relevant for future tolerance induction protocols. This treatment has proved to be highly effective in bone marrow transplantation for T-cell purging to eliminate graft versus host disease in over 2,000 participants treated in Europe in the treatment of B-cell lymphomas.[3, 4, 5] Efficacy has recently been demonstrated in nonmyeloablative conditioning for stem cell therapy. Conditioning regimens based on Campath, cyclosporine, and donor-marrow stem cell therapy provided durable engraftment in 62 of 64 recipients, with macrochimerism demonstrable in 31 cases.[6, 7] The incidence of graft versus host disease (GVHD) was extremely low in this and other recent series, suggesting that Campath-1H treatment was protective against this complication.[8]
This antibody has been particularly effective in control of autoimmune diseases, including acute vasculitides[9], multiple sclerosis[10], and in autoimmune cytopenias[11, 12]
Although not FDA approved for renal transplantation, this agent has been used in an estimated 2000 renal transplant recipients (off label) over the last 10 years in the United States and elsewhere.

5) It is a well-known phenomenon transcending phylogeny in several inbred experimental animal species enabling defined immune studies (fetal and adult inbred mice, rats, and pigs), that the lesser the immunogenetic disparity, the greater the feasibility of inducing donor-specific immunological tolerance to transplanted organs and tissues. Mixed or total DHSC-derived chimerism has been a basic component of the majority of these most robust tolerance states. There is also less likelihood of GVH reactions, the closer the donor/recipient match in these inbred strains. Both of these (tolerance and lack of GVH) have indeed proven to be the case in the myeloma human renal failure recipients of HLA-identical donor bone marrow and kidneys in the Massachusetts General Hospital series of Spitzer et al.[13]

1.2 Hypothesis:
It is our hypothesis that a four-dose (peri-operative and 3, 6, and 9-month boost) DHSC infusion protocol using two-dose Campath-1H induction combined with transient (conditioning) Tacro/Siro and MMF therapy will result in a high degree of macrochimerism (>10%), and a robust prolonged donor-specific (post-thymic) immunoregulatory condition that will allow renal transplant survival in the absence of permanent immunosuppression.

Innovative mechanistic studies will include a novel HLA-identical chimeric PCR-Flow serial analysis based on non-HLA (cytokine gene) polymorphisms, other marker detection, split tolerance ELISPOT and micro-CML assays, in-vivo delayed type hypersensitivity (DTH), amplified HLA-identical mixed lymphocyte dendritic cell assays and molecular and flow cytometric analysis for T-cell and dendridic cell regulatory markers in peripheral blood and iliac crest marrow performed in our laboratories. These will be complemented with sensitive newer assays for donor-specific and non-specific (HLA and non-HLA) antibody studies in serial long-term follow-up.

1.3 Description of Investigational Product

1.3.1 Donor Stem Cells

This section briefly describes the collection, processing and delivery of the CD34+ enriched cells, and refers to the included SOPs and other appropriate documents in the Northwestern Memorial Hospital Cell Processing Center. These SOPs, etc., are what will be used for every procedure step, and are included at the end of the entire protocol.
These are numbered RWC 2.2.04, BC GEN 1.2.13, BC GEN 1.2.12, BMT 3.1.02, BMT 3.1.03, BMT 3.1.10, BMT 4.1.01, BMT 5.1.02, BMT 10.1.01, HSCT 3.04 and also include a Table of Contents and Product Certification of Analysis of Reagents, as well as Final Product Certificate of Analysis for Product. Briefly, these stem cell procedures, as mentioned, are divided into three sections: 1. Collection; 2. Processing; 3. Delivery.
1. Collection and 2. Processing of Iliac crest bone marrow cells.
Iliac crest bone marrow or peripheral blood progenitor cells (PBPC) will be harvested and/or processed for the following purposes:

a. Donor BMCs to infuse into the recipient
   Approximately 750 mL of iliac crest bone marrow will be harvested under general anesthesia in a surgical suite from the donor after the donor transplant nephrectomy. A bone marrow mononuclear "buffy coat" will be prepared using the GAMBRO Spectra apheresis instrument equipped with bone marrow buffy coat software set-up. The allogeneic progenitor cells (buffy coat cells) will be further processed to isolate CD34+ progenitors using the Miltenyi CliniMACS system. The isolated CD34+ cells are the investigational drug (cellular product) that will be tested in this protocol.

MILTENYI CLINIMACS CD34$^+$ CELL SELECTION
Both sources of donor stem cells (intraoperative iliac crest bone marrow and HPC-A (leukapheresis) cells) will be positively selected for CD34$^+$ cells prior to patient administration according to manufacturer's instructions using the Miltenyi CliniMACS instrument. Breifly, according to manufacturer instructions for operation of the CliniMACS device:

1. HPC collection(s) will be washed with a phosphate buffered saline solution containing 0.5 percent human serum albumin (HSA) and ethylene diamino-ethyl-tri-acetic acid (EDTA) to remove most of the platelets from the product and to reduce the product volume.

2. Following the platelet wash step, the cells will be incubated with the CD34 monoclonal antibody-super-paramagnetic particle reagent supplied in the kit for 30 minutes and washed to remove unbound CD34 labeling agent.

3. After cell labeling, the cells will be transferred to a sterile transfer pack, connected to the Miltenyi CliniMACS Selection device and an automated CD34 Selection program will be initiated. The selection medium used throughout the procedure will be the manufacturer's PBS/HSA/EDTA medium.

4. At the completion of the selection process, an aliquot of the cells will be analyzed to determine the quantity and purity of the CD34$^+$ cells and to assess the log depletion of

donor $CD3^+$ T-lymphocytes. The desired composition of the donor graft is: $CD34^+$ cell dose $\geq 2 \times 10^6$ $CD34^+$ cells/kg. T-lymphocyte cell dose $\leq 5 \times 10^4$ $CD3^+$ cells/kg.

5. Specific details of the CD34 selection procedures can be found in the standard operating procedures (SOPs) located in Appendix II.

b. Recipient BMCs for rescue
Approximately 500 mL of iliac crest bone marrow will be harvested under general anesthesia in a surgical suite from the recipient *prior to* administration of alemtuzumab. This bone marrow will be minimally manipulated to reduce product volume only and will be frozen following standard cryopreservation methods for bone marrow. This product will serve as a frozen "back-up" rescue source of autologous stem cells in the event that graft vs. host disease occurs in the recipient postoperatively to merit its use.

c. Donor Mobilized HPCs
Approximately 250 mL of peripheral blood progenitor cells (PBPCs) will be harvested from the donor *following* the harvest of the donor bone marrow cells. The PBPCs will be collected using the GAMBRO Spectra apheresis instrument following standard HPC-A collection procedures currently in use at NMH Hematopoietic Stem Cell Transplantation Program. Briefly, a standard volume leukapheresis (~ 12 L) will be performed on two successive days between two to three months postoperatively to obtain sufficient PBPCs to permit isolation of peripheral blood CD34+ cells. (Mobilized by successive daily administration of GCSF). The first day's leukapheresis CD34+ product is to be administered to the recipient the same day (freshly) after the CliniMACS separation. The second day's leukapheresis product, after CliniMACS purification, will be divided into two equal aliquots to be administered after cryopreservation and thawing (at the bedside) at month 6 and 9 respectively. The Miltenyi CliniMACS system is to be used for CD34+ isolation. The isolated CD34+ cells are the investigational drug (cellular product) that will be tested in this protocol.

3. Delivery and Infusion of the Product
These two methods are also described in the accompanying documents (#BMT 10.1.01 and HSCT 3.04) as mentioned at the end of the entire protocol consistent with methodology performed for stem cell patient delivery at Northwestern Memorial Hospital.
In addition, a plan of action for all positive test results during the aforementioned procedures is included in these documents. Please refer to the QAP for a description of the notification of a positive test result following the QAP plan. Adverse event reporting and corrective and preventive action plans will be used to notify the patient's physician or study PI, investigate the incident and take appropriate corrective and preventive actions. The incident (s) is to be reported to appropriate local, state or federal agencies as required. This plan includes a mechanism to notify the PI in the event that a positive result is reported, in the event of positive microbial cultures, notification of manufacturing deviations, or in the event of post transfusion reactions. Specifically, the PI is then to notify the Northwestern Institutional Review Board, the NIDDK sponsor, the Data Safety Monitoring Board and the FDA in an appropriate time sequence defined by the definitions of severe adverse reaction (included previously in this IND application).
These products have been used **7** times in a NIAID, VA Merit Review, ITN and FDA approved study being conducted in 1 Haplotype matched, DHSC infused renal transplant

recipients now in progress in Miami. They have also been used in many other stem cell therapies in hemato-oncology protocols at Northwestern, all without significant adverse events.

2 Summary of Preliminary Work

Over the past decade, we have taken several steps towards understanding the mechanisms of specific immunological tolerance in a series of clinical and *ex vivo* studies in human kidney transplant recipients of DBMC infusions from CAD and LRD. Demographically similar non-infused but non-randomized controls were given equivalent immunosuppression with OKT3 or daclizumab antibody induction and tacrolimus, MMF, and methylprednisolone maintenance. Follow-up included *ex vivo* assays of chimeric cell quantitation and immuno-regulatory function. We also described *in vitro* assays of the general immunoregulatory effect of bone marrow non-T and T cell subsets, stemming from the surprising finding, after bone marrow infusion, of the significant increase in chimeric (donor) cells in the sequestered environment of the recipient marrow compartment, as opposed to peripheral blood. The following summaries therefore also include these published studies, as well as our initial Campath-1H (unpublished) work within the past year.

2.1 Summary of Clinical Experience

2.1.1 Campath-1H and Cyclosporine in Renal Transplantation

Watson, CJ [14] used Campath-1H for induction prophylaxis in 31 participants undergoing renal transplantation. Campath-1H was administered at a dose of 20 mg on Day 0 and repeated on Day 1, and after a 72-hour delay cyclosporin (target trough level 75 to 125 ng/mL) was initiated without glucocorticoids and without any other maintenance drug therapy. At a follow-up of five years, there has been a 22.6% incidence of acute rejection (16.1% at 1 year with 79% graft survival at five years and 88% participant survival at five years) Watson, CJ, Calne R, et al. Am J Transplant 2005;5:1347-53. There was prolonged and profound depletion of circulating lymphocytes with a mean lymphocyte count at 1-year follow-up of approximately 800/mm. Despite this low lymphocyte count, there has not been an increased incidence of infection or malignancy compared to conventional therapy. In this series, Campath-1H did not prevent recurrent disease, 2 participants have had recurrent IgA nephropathy, and 1 participant had recurrent membranous glomerulonephritis. This observation is similar to conventional therapy.

2.1.2 Campath-1H and Sirolimus in Renal Transplantation

Data on the use of Campath-1H in renal transplantation were reported by Kirk et al.[15] This trial was designed to determine whether pretransplant administration of Campath-1H would allow allotransplantation without maintenance immunosuppression. Eight living related-donor renal-allograft recipients were given 3 doses of Campath-1H (0.3 mg/kg), initiated before transplantation. There was rapid clearance of T cells from circulation, which persisted beyond 3 months, and up to 12 months in some cases. Normal renal function was observed in all participants at 2 weeks after transplant. Graft histology was normal at 7 days. By 3 to 4 weeks, all participants had some degree of renal dysfunction, which improved following the initiation of sirolimus. Preliminary data from this study has demonstrated that Campath-1H provided a 2-week window of decreased immune responsiveness. More recent similar steroid avoiding protocols have been described in LRD and CAD renal transplant recipient

by Knechtle, et al. using a two-dose Campath-1H induction regimen and continuous sirolimus therapy.[16]

2.1.3 Campath-1H, Tacrolimus and MMF in Renal Transplantation

At Northwestern University/Northwestern Memorial Hospital, a single intraoperative dose of 30 mg Campath-1H was used in combination with tacrolimus (trough levels of 4-6 ng/mL), and MMF (1 gm daily), beginning after September 2001. In May 2002, 23 consecutive kidney transplant recipients were reported without rejection and all with good function. One participant had a urinary tract infection. Campath-1H was associated with prolonged lymphophenia lasting 4 to 6 months.[17] Kaufman et al recently reported on Northwestern's single center experience with Campath 1H induction in 123 renal transplant recipients [18]. Again a single intraopertive dose of Campath 1H was used with prednisone-free maintenance immunosuppression consisting of tacrolimus and MMF. One year acute rejection rate was 15.4%. Patient and graft survival at 3 years post-transplant were 95.9 and 91.9 %, respectively. Infectious complications in Campath 1H patients were not qualitatively different from an historical cohort of patients receiving a non lymphocyte depleting induction agent (Simulect). Currently, over 1000 renal transplant recipients have been treated with Campath 1H induction at Northwestern.

2.1.4 Campath-1H Experience in Transplantation at the University of Miami

The use of Campath-1H as induction therapy in renal transplantation: preliminary results.[19] In attempt to reduce both initial and long-term (nephrotoxic) calcineurin inhibitor maintenance dosage and totally eliminate maintenance corticosteroids. In Miami, the PI used Alemtuzumab (Campath-1H) as induction therapy in first cadaver and non-HLA identical living donor renal transplantation. Forty-four de novo renal allograft recipients were treated with Campath-1H (0.3 mg/kg) on day 0 and day 4 postoperatively preceded by methylprednisolone boluses. Maintenance target 12-hour Tacrolimus trough levels of 5-7 ng/ml were operational from the outset as well as (reduced) mycophenolate mofetil dosage of 500 mg twice daily. No corticosteroids were planned to be given after the first week postoperatively. With a median follow-up of 9 (range 1-19) months, patient and graft survival were each at 100%. Biopsy-proven acute rejection was diagnosed in 4 patients. A total of 4 patients developed infections that required hospitalization. Thirty-eight recipients remain without the need for long-term corticosteroid therapy. In an early assessment, the combination of Campath-1H, low dosing of tacrolimus and mycophenolate mofetil, and avoidance of maintenance corticosteroid use appears to be safe and effective for kidney transplant recipients. Long-term outcomes will be reported in the future.

A randomized trial of three renal transplant induction antibodies: early comparison of tacrolimus, MMF and steroid dosing, and newer immune-monitoring by the PI.[19,20] In a randomized trial using three different antibody induction agents in 90 first renal transplant recipients from cadaver donors, Group A received Thymoglobulin, Group B received Alemtuzumab, and Group C received Daclizumab. Maintenance immunosuppression included tacrolimus and mycophenolate in all three arms, and methylprednisolone in Groups A and C only (standard clinical institutional practice). The targeted trough level of tacrolimus was between 8 and 10 ng/ml for Groups A and C, respectively, with a targeted mycophenolate dose of 1 gm twice daily. However, in Group B, target tacrolimus trough

level was 4-7 ng/ml to reduce long-term nephrotoxicity, with 500 mg twice-daily doses of mycophenolate, without steroid maintenance. In this 15-month median postoperative interval report, there were no notable differences in demographics and patient and graft survival. Acute rejection rates at one year were equivalent, i.e., 5/30 in all three groups (~16.6%). In Group B, there was slightly lower renal function at one month, but no difference at one year. There was also significantly more leukopenia, but a greater percentage of T regulatory cells and number of Fox-P3 mRNA copies respectively, by flow cytometry and semi-quantitative PCR analysis in Group B. This preliminary analysis has indicated that in Group B 80% of these patients remained steroid-free at 1-year postoperatively, with lower tacrolimus trough levels, and no difference in other adverse events.

Campath-1H does not alter bone marrow cell regulatory function.[20,21] We have previously reported in laboratory volunteers (*in vitro*) and renal transplant recipients (*ex vivo*), that bone marrow cells (BMC) are potent down-regulators of the immune response. Also, the use of alemtuzumab (Campath-1H, C1H) for immunodepletion is associated with the most potent lasting effects yet seen on T cell immunity in renal transplantation. We questioned if the administration of C1H to kidney allograft recipients of donor bone marrow cell (DBMC) infusions would lead to stronger or weaker immunoregulatory effects. *In vitro* Human BMC depleted of T cells (nT-BMC) were either untreated or treated with C1H and rabbit complement, and compared for their ability to down-regulate autologous or allogeneic T cell responses, and to generate T regulatory (T reg) cells. The proliferative responses to anti-CD3 monoclonal antibody of T cells derived from co-cultures with C1H-treated or untreated autologous nT-BMC were equally suppressed, i.e., an equivalent alteration in CD3 complex signaling, not regained by the addition of IL-2. ATP levels were also markedly reduced in T cells both from C1H-treated and untreated nT-BMC co-cultures. The ability of C1H-treated or untreated nT-BMC to suppress autologous T cell cytotoxic function was also equivalent, with a marked, but equivalent, capacity to induce CD4/CD25^{high} T regs from CD3+ cells, which effectively down-regulated cytotoxic T cells. To mimic the clinical infusion of DBMC into (allogeneic) recipients, PBL were also cultured with allogeneic C1H-treated and untreated nT-BMC. T cells derived from these cultures secondarily stimulated with the same donor mature APC, showed suppressed cytotoxicity by 85% and 54%, respectively. These *in vitro* studies suggest that C1H does not abrogate BMC immunoregulation and thus may allow its lympho-depleting effect to be synergistic.

2.1.5 DBMC Experience in Transplantation

In the PI's experience at the University of Miami between September 1994 and May 1998, 63 CAD renal transplant recipients of either one or two postoperative vertebral DBMC infusions were prospectively compared with 219 demographically equivalent (non-randomized) non-infused controls given equivalent OKT3, tacrolimus, MMF, and methylprednisolone immunosuppression. There was at least a 1 HLA DR antigen match present between donors and recipients in all cases. Clinical follow-up ranged from 2.9-6.3 years (mean, 4.7 years). Only 2/63 (3.2%) DBMC recipients had biopsy-proven chronic rejection, whereas 41/219 (18.7%) showed chronic rejection in the controls ($p = <0.01$). If death with a functioning graft was excluded, graft survival was 94.1% in the DBMC group and 79.8% in the controls ($p=0.039$). Forty controls had deteriorating renal function compared with 2 in the DBMC group ($p=0.04$). In the DBMC group, chimerism in iliac crest marrow aspirates had increased in sequential yearly PCR-Flow measurements between 1 and 4 years

postoperatively, to average 1.3±0.36% (S.E.), but not in the controls.[1] More recent follow-up at 10 years has confirmed these findings with FoxP3 mRNA copies in the total T cell PBL compartment being five times that of the non-infused controls.(Cirocco et al, Transplantation 2007, in press**)**

Between November 1996 and May 2000, 47 LRD kidney transplants who received iliac crest DBMC in a single infusion 4 days postoperatively were (non-randomly) concomitantly compared with 39 demographically similar non-infused controls. All were given either OKT3 (n=26) or daclizumab (n=21) induction with maintenance tacrolimus, MMF, and methylprednisolone immunosuppression. Clinical follow-up ranged from 19.0-61.6 months (mean: 33.2 months). The incidence of acute rejection did not differ between groups. Immunosuppressive dosaging was somewhat (but not statistically) lower in the DBMC group. Four-year actuarial patient and graft survival for the DBMC-infused group was 98% and 98%, and 98% and 95% in the controls (p=N.S.). DBMC chimerism by PCR-Flow in recipient iliac crest marrow increased more rapidly than might have been predicted from the CAD group, despite four times fewer DBMC infused. [2]

Preliminary Results of ITN Protocol (#022ST) Pilot Study: Four patients are being followed in a protocol of 1-haplotype matched LRD Campath-1H treated donor stem cell infused kidney transplant recipients with the intent to wean immunosuppression over a three year period starting one year post-operatively.

In the first patient, R.O., 1.2 x 10^6 CD34+ donor iliac crest bone marrow-derived purified stem cells were infused five days post-operatively, one day after the second Campath-1H treatment.

At 12 weeks, 2 weeks after conversion to sirolimus from tacrolimus 7 x 10^6 donor CD3+ cells were again infused after five days of Filgastrin CD34+ mobilization, from a peripheral blood leukapheresis product (<.05% T cell contamination).

Table 1 shows the subsets of donor chimeric cells in recipient PBL and iliac crest marrow, compared with those seen at similar intervals in previous DBMC-infused recipients from series generated over the past 7 years (>10-fold higher than previously at similar points in time).
Table 1.

Comparison at 1 year post-transplant of peripheral blood PCR-Flow chimerism of patient "R.O." given DHSC vs. the group of LRD recipients (previous series) given ~50-fold more donor iliac crest whole marrow cells.

			Peripheral Blood	
	N	Time	%[b] Donor CD3+ Cells	%[b] Donor CD34+ Cells
LRD Recipients [a]				
$1.8x10^8 \pm 1.9x10^8$ cells/kg in one infusion perioperatively	33	1 year	.18 ± .08	.10 ± .05
Patient "R.O."				
Infused with $1.6x10^6$ DHSC/kg perioperatively and $7x10^6$ DHSC/kg boost 3 months postoperatively	1	1 year	3.95	9.81

[a] A mean of 1.8×10^8 cells/kg infused ± 1.9×10^8 S.D. in the entire group.
[b] Percent of the total (recipient and donor) CD3+ or CD34+ subset counted.

Patient and Graft Outcome: As a brief summation of that study, similar findings have been present in three other stem cell-infused patients—now all over one-year post-transplant, but one (DHSC Patient #2, a 20 year old Latin female; RO) has had a rapid recurrence of focal segmental glomerulosclerosis (FSGS) that occurred in the native kidney. Although she is not yet on dialysis, she is nearing this point with a serum creatinine of >5.0 mg/dl.

DHSC patient #1, (a 28-year old Latin female) has a serum creatinine of 1.8 mg/dl, due to a recurrence of Type I membranoproliferative glomerulonephritis (MPGN) in the transplant, which has been very slowly progressive but in which allograft rejection has not been present on biopsy. (now 3 year post-transplant).

Patient #3, (a 30-year old Latin male) is now two years after transplantation with a serum creatinine of 1.3 mg/dl. He is about to be totally withdrawn from sirolimus (now on .5 mg/day) to convert solely to MMF.

Patient #4, (a 62-year old Caucasian male) is now 15 months post transplant with a serum creatinine of 1.4 mg/dl and is being slowly withdrawn from sirolimus, with MMF to remain for the additional several months after sirolimus withdrawal as per the ITN protocol.

Patients #3 and #4 have also had no evidence of acute or chronic rejection clinically or on biopsy, but Patient #3 is very susceptible to dehydration, with having had some proteinurea attributed to sirolimus therapy, which has subsided as the drug has been slowly withdrawn.

Treatment Received (Brief Summary): Patients #1, #3 and #4 all received the DHSC infusions and planned immunosuppression of the protocol described in Section 1.1- i.e., two doses of Campath and half dosing of tacrolimus switched to sirolimus. However, Patient #1, because of her recurrence of MPGN, has not had immunosuppression withdrawn and actually has had 4 mg of methylprednisolone added to her therapy.

As mentioned, Patient #2, with rapid recurrence of FSGS, has been on a modified dose of MMF and corticoid steroids as well. She did not receive the second DHSC infusion because FSGS recurred before month 2.

Patients #3 and #4 are on the withdrawal protocol as described.

Chimerism: Patients #1, #3 and #4 have chimerism levels hovering around 1% in iliac crest bone marrow aspirates by PCR-flow analysis, all performed within the last 3 months.

2.2 *Ex vivo* and *in vitro* Correlates of Allogeneic and Autologous Immunoregulatory Mechanisms Evoked by Bone Marrow Cells (*ex vivo* = Cells Derived from the Chimeric Recipient; *in vitro* = Non-chimeric Bone Marrow Cells of Non-transplanted Individuals)

Listed are titles of publications by the P.I. and colleagues (in citations section by number) that apply both to the clinical and mechanistic rationale from the present protocol.

- Donor and recipient chimeric cells present in DBMC-infused renal transplant recipients: potent *ex vivo* regulators of recipient anti-donor immune responses.[21]
- *In vitro* immunogenicity of cadaver donor bone marrow cells.[22]
- Involvement of multiple alloimmune regulatory subpopulations of DBMC.[23]
- Regulation of alloimmune (GVHD) reactions *in vitro* by autologous DBMC.[24]
- DBMC inhibit the generation of autologous EBV-specific CTL, promote TH2 polarization and can cause the transfer of an anergic state between T cells of peripheral blood.[25-28]
- Allogeneic DBMC induce *in vitro* "suppressor T cells" of autologous B cells.[28]
- A novel CML micro-assay for the evaluation of regulatory cells.[29]
- The human bone marrow as an immunoregulatory organ and the immunoregulatory role of chimerism in clinical organ transplantation.[30-32]
- Induction of auto-reactive regulatory T cells by stimulation with immature autologous dendritic cells.[50]
- Killer cell immunoglobulin-like receptor polymorphisms in HLA-identical kidney transplant recipients: lack of 2DL2 and 2DS2 may be associated with poor graft function.[51]
- A Novel Approach to Detect Donor/Recipent Immune Responses Between HLA-Identical Pairs.[52]

2.3 Known and Potential Risks and Benefits to Human Subjects

2.3.1 Potential Benefits

Renal transplantation using Campath-1H induction combined with DHSC infusions from the same HLA identical sibling donor has the potential to allow acceptance of a donor renal allograft without chronic immunosuppression. This treatment is believed to cause a greater degree of tolerance of the transplanted kidney by the body, thus, avoiding rejection and perhaps allowing the partial or even total withdrawal of the need for continuous use of anti-rejection drugs. This therapy has been undertaken in an attempt to offer renal transplantation and hence freedom from dialysis without the complications of long-term immunosuppressive drug administration. Long-term complications of continuous immunosuppression can include cataracts, osteoporosis, diabetes, atherosclerosis, hypertension, malignancy, plus the risk of chronic allograft rejection/nephropathy.

2.3.2 Potential Risks

Participation in this study is associated with certain risks for both the recipient and the donor. These risks are summarized below.

In general, patients with a transplanted organ are treated with immunosuppressive drugs that suppress the immune system. These drugs produce additional adverse events occasionally including over-immunosuppression. This may cause infections, including those caused by viruses, bacteria, and fungi, and by organisms that only produce disease in patients who are immunosuppressed. Over-immunosuppression may also increase the risk for the development of cancer. It is not known if infusion of donor bone marrow will result in an increase or decrease or similar risk of infection or cancers compared to standard therapy.

2.3.2.1 Graft Versus Host Disease

GVHD (graft versus host disease) although considered to be unlikely might occur, with the possibility of the donor bone marrow cells reacting against the recipient. This has been seen in patients who have received bone marrow transplants to treat other diseases, such as leukemia, in which there has been an ablative procedure to totally knock out the recipient's own marrow. However, in this protocol, the patients are not to receive the marrow destroying therapy, which is probably a prerequisite for the development of graft vs. host disease. Nonetheless, one unit of the recipient's own marrow and recipient's white cells will be stored before transplantation to treat GVHD.

T-cell depletion (TCD) of the infused marrow derived cells, a strategy to decrease GVHD, is under investigation in stem cell transplantation for hematologic, oncologic and immunodeficiency diseases. This technique effectively decreases the incidence and severity of acute GVHD, but with increased problems with graft rejection[33-37] and relapse. Methods of graft manipulation may be based upon removing T lymphocytes (TCD) by a variety of antibody mediated approaches or physical methods of separation. Most recently, positively selected CD34+ progenitor cells are commonly used. Transplant related mortality outcome appeared to be predominantly affected by increased GVHD in recipients receiving peripheral blood cell grafts containing more than 2 x 10^5 CD3+ T cells/kg. In patients receiving more than 2 x10^5 CD3+ cells/kg, the incidence of Grade II-IV GVHD was 55%. It is important to note that in all these studies, patients receive myeloablative regimens. Perhaps the largest experience of TCD transplants occurring without preparative chemotherapy is in the setting of severe combined immune deficiency. In this disease, children often receive TCD haploidentical parental marrow. In most cases, GVHD that developed after the administration of TCD depleted marrow was mild (grade I or II) and required no treatment.

2.3.2.2 Campath-1H

Infusion-related events have been associated with the use of Campath. These events include rigors, fever, nausea/vomiting, fatigue, hypotension, shortness of breath, bronchospasm, chills, pruritis, headache, diarrhea, and urticaria and/or rash. In patients using Campath for oncology indications, Campath has been found to induce profound lymphopenia in patients given many-fold higher dosing than in renal transplant recipients in this study. Campath therapy has been associated with the opportunistic viral, bacterial, and fungal infections, some of which were fatal. Leukemia and lymphoma patients receiving Campath have experienced severe, prolonged, and in rare instances fatal myelosuppression. Bone marrow aplasia and hypoplasia have been observed, as well as severe and fatal autoimmune anemia and thrombocytopenia in patients with chronic lymphocytic leukemia.[38]

Although Campath-1H therapy may lead to prolonged suppression of CD4+ cells, this has not been found to be associated with clinical infection in humans who have suppressed CD4+ cells following lymphoid irradiation or bone marrow transplantation. Research participants in the Campath-1H trial in England (31 subjects) have not experienced any serious or life threatening infections following renal transplantation. Two subjects experienced opportunistic infections (systemic cytomegalovirus infection and an abdominal abscess attributed to reactivation of a prior tuberculosis infection) that responded to therapy.[39] Recent reports of an increased risk of Graves disease have only been seen in a single trial of participants with multiple sclerosis, where 1/3 of participants developed the disease following Campath-1H treatment in much higher dosing than used in the present study. At Northwestern University we have not observed any cases of Graves disease in patients receiving Campath 1H as induction for solid organ transplantation.

Patients with Graves disease will be excluded unless previously adequately treated with radioiodine ablative therapy. This trial was unique in that very high doses of Campath-1H were given (100 mg given over 5 days) and Graves disease did not occur until 6 to 18 months later. It has been presumed that this complication may reflect a genetic predisposition in participants with multiple sclerosis, since in controlled trials the complication has not been observed in other participants with chronic leukemias or in participants with vasculitides. Thyroid function tests will be monitored in the current trial.

No long-term animal studies have been performed to establish the carcinogenic or mutagenic potential of Campath-1H or to determine its effects on fertility rates in male or female adults. Campath-1H may cross the placental barrier and cause significant fetal T and B lymphocyte depletion. Male participants with reproductive potential must use approved methods of birth control during treatment with Campath-1H and for a minimum of 6 months following their last dose of Campath-1H. Female participants of childbearing potential must use approved methods of birth control for the duration of their participation in the study.

In some oncology subjects treated with Campath, profound and long-lasting lymphopenia (decreased CD4+ and CD8+ lymphocyte counts) was observed, and total lymphocyte counts did not return to baseline levels even after 1 year post-therapy. CBC and platelet counts will be monitored frequently if worsening anemia, thrombocytopenia, lymphocytopenia, or neutropenia is observed on study.

2.3.2.3 Tacrolimus

Tacrolimus administration will be based on trough blood levels (AM) to provide levels of between 4 and 7 ng/ml during its administration course. These levels will be obtained daily as inpatients and with each clinic visit as outpatients (see calendar). The tacrolimus dose should be decreased in the presence of adverse events as clinically warranted. Risks associated with the administration of tacrolimus include hypertension, hyperkalemia, nephrotoxicity, neurotoxicity, posttransplant insulin-independent diabetes mellitus, myocardial hypertrophy (in most cases reversible upon dose reduction), and increased risk of renal insufficiency in patients with hepatic impairment.

2.3.2.4 Sirolimus

Sirolimus dosage, when instituted (2 to 3 months postoperatively) will be based on AM trough levels of 8 to 12 ng/ml. These levels will be obtained with each clinic visit (see

calendar). The sirolimus dose should be decreased in the presence of adverse events as clinically warranted. Risks associated with the administration of sirolimus include hypertriglyceridemia, hyperlipidemia, leukopenia, thrombocytopenia, anemia, neutropenia, nephrotoxicity, hypertension, anemia, nausea, vomiting, diarrhea, elevated liver enzymes, rash and acne. Sirolimus' effect on the developing fetus is not known and is not recommended for administration to nursing mothers. It is recommended that women of childbearing potential use effective contraception before, during and for at least 4 months following Sirolimus administration.

2.3.2.5 Mycophenolate Mofetil (MMF)

The MMF dose should be decreased in the presence of adverse events as clinically warranted. Risks associated with the administration of MMF include leukopenia, thrombocytopenia, anemia, neutropenia, gastritis, diarrhea, nausea, vomiting or dyspepsia. MMF's effect on the developing fetus is not known and is not recommended for administration to nursing mothers. It is recommended that women of childbearing potential use effective contraception before, during and for at least 4 months following MMF administration.

2.3.2.6 Combination Therapy: DBMC, Campath-1H, Tacrolimus, Sirolimus, and MMF

Other than the experiences cited [40] above, no information to date is available on the combination of DHSC and Campath-1H given together with either tacrolimus, sirolimus, or MMF. Thus, at this time the risk profile of the combination of these therapies are not known. Potential risks may include increases in the incidence or severity of adverse events currently experienced when these agents are administered, in addition to other adverse events that are unforeseeable at this time. Also, as with any immunosuppressive regimen, this combination may result in increased susceptibility to infection and the development of lymphoma and other malignancies.

2.3.2.7 Other Risks

The subject may experience some pain and bleeding at the site of the kidney transplant biopsy, discomfort, and/or blood in the urine following the biopsy. Additional risks of biopsy include bleeding in or around the kidney that can lead to a fall in blood pressure and rise in heart rate. In the event that significant bleeding is suspected, either through clinical observation or a significant decrease in hemoglobin, a blood transfusion will be administered as necessary or the patient will be taken to the operating room for direct control of bleeding.

3 Objectives

3.1 Primary Objectives

The primary objective of this trial is to investigate the safety and efficacy of a treatment regimen consisting of induction therapy with Campath-1H and reduced dose maintenance immunosuppression (consisting of MMF, Tacrolimus, and Sirolimus), followed by complete withdrawal of immunosuppression within 30 months of transplantation, in recipients of living-related (HLA identical) donor kidney transplants who have also been given **four** infusions of purified CD34+ DHSC.

3.2 Secondary Objectives

To assess the utility of various assays and biopsy information as putative tolerance markers in these kidney transplant recipients:

- To use the PCR-Flow assay (modified for cytokine gene polymorphism) and VNTR assays both in recipient peripheral blood on a monthly basis and in iliac crest marrow at 3 months, 6 months and yearly, in order to measure donor cell multi-lineage chimerism;
- To use the newly developed cell-mediated lympholysis (CML) microassay, enzyme-linked immunospot (ELISPOT) assay, and MLDC and *in vivo* DTH assays to demonstrate sequentially increasing donor and recipient chimeric marrow and peripheral blood cell immunohematologic subset regulatory effects;
- To study lymphoid cells derived from yearly kidney biopsies (i.e., before and after planned withdrawal of immunosuppression);
- To use limiting dilution analysis to detect partial or total clonal deletion.
- To follow sensitive alloantibody assays;

4 Study Design

4.1 Donor and Recipient Preparative Regimens

Twenty recipients will be HLA identically matched with their living related donors. There will be occasions in which it will be determined that the standard tissue typing tests may not be enough to define HLA genotypic identity. If this is the case more discriminatory tissue typing will be performed that may include tissue typing of both parents. One month before transplantation (at the time of consent), $1x10^9$ recipient mononuclear cells are to be removed by a 7-liter leukophoresis and cryopreserved in aliquots. Fresh samples of donor and recipient PBMC are to be tested as responding, stimulating, and regulatory cells in MLC and CML reactions at this time. Leukophoresis (Cobe Spectra), without causing recipient anemia, will furnish these (non-regulatory) cryopreserved recipient cells for later comparison.

4.2 Initial Recipient Protocol and Immunosuppression (at and immediately post-surgery)

In the operating room, with induction of general anesthesia, 500 ml of iliac crest bone marrow will be removed from the recipient (inclusion criterion – hematocrit >33), cryopreserved for GVHD rescue (unlikely) and tested by PCR-Flow (baseline absence of chimerism). Some aliquots will be cryopreserved (from 100 ml of this marrow) for comparison in the mechanistic studies. Then 250 mg of hydrocortisone and 50 mg Benadryl® will be administered intravenously (I.V.), followed by an infusion of 0.3 mg/kg of Campath-1H over 2 hours, which will be completed about the time the clamps are released on the renal vascular anastomoses. At clamp release: 500 mg of methylprednisolone is administered. MMF (500 mg) is infused I.V. at surgery and continued enterally at a dose of 500 mg/12 hr (half the usual dose). Tacrolimus is instituted at 0.05-0.1 mg/kg every 12 hr enterally, when good renal function is established (serum creatinine decreases to <4 mg/dl), anticipated to be within 48 hr of surgery, targeting for A.M. trough levels of 5-7 ng/ml.

4.3 Donor Marrow Donation

After the uncomplicated donor nephrectomy is completed, before anesthesia is withdrawn, 750 ml of iliac crest marrow will be removed for CliniMACS stem cell purification, cryopreservation, and infusion 5 days later into the recipient (see below).

4.4 Post-operative Campath-1H

The second infusion of Campath-1H is to be given on post-operative day 4, also at a dose of 0.3 mg/kg. A change in the Campath 1-H dosage is allowed upon the Principial Investigator's judgment to meet specific clinical needs of subjects. This is preceded by 500 mg of hydrocortisone I.V., 50 mg Benadryl® and 650 mg (2 tabs) Tylenol® by mouth to avoid any potential adverse reaction with the agent (package insert). Using this regimen, a Campath-1H functional half-life of ~12 days is anticipated, with prolonged effects up to 1 year. No additional corticosteroids are to be administered.

4.5 Long-term Immunosuppression

At 1 month post-operatively (assuming a normal rejection-free clinical course), tacrolimus will be withdrawn over 2 weeks, overlapping with the prior initiation of sirolimus in doses to achieve target trough levels of 8-15 ng/ml. (usually between 2-5 mg/day), MMF 500 mg twice daily will be maintained as tolerated.
Note: None of these agents are considered investigational. Although Campath-1H is used clinically in organ transplantation (over 2000 patients treated in the United States in the past five years), it is not used routinely for this purpose, but has been indicated to date in the treatment of hematologic malignancies. The decrease in dosing of Tacrolimus and MMF accompanying Campath-1H usage has been routine for those centers using Campath-1H in renal transplantation, as well as the absence of corticosteroids maintenance therapy (over 1000 patients in the United States with more than 1 year of follow-up).

4.6 Donor Stem Cell Infusions (Please see section earlier in the protocol entitled Description of Investigational Product Section 1.3.1)

4.6.1 First Infusion

The first donor purified stem cells will be given on post-operative day 5 extracted from the iliac crest marrow aspirated at donor nephrectomy. After a 10 ml aliquot is taken, together with PBL for PCR-Flow analysis, the marrow will be purified by the CliniMACS® (CD34+ selection) 300i System. From our previous experience in the Diabetes Research Institute at the University of Miami and in this NU/NMH stem cell processing unit in other protocols, approximately 1-2 x 10^6 cells/kg body weight can be expected as an infusion dosage with a CD34+ cell purity of ~75%, with ~0.5% T cell contamination. The cells will be cryo-preserved, thawed, and administered at the recipient bedside (as in previous studies).

4.6.2 Second, Third, and Fourth Infusion

The second infusion of donor peripheral blood-mobilized stem cells, purified similarly, will be administered to the recipient (as a patient in the CRU for <12 hours) 2 to 4 weeks after the initiation of Sirolimus therapy (between 2 to 3 months postoperatively), once sirolimus trough levels have reached 8 ng/ml.
Donors as outpatients will undergo standard 10-12 liter pheresis, at this time using peripheral access whenever possible. Donors will be administered G-CSF (filgrastim) subcutaneously for four consecutive days prior to pheresis with dose according to body weight (10 mcg/kg). A CD34+ cell assay of the product after CliniMACS® selection will be carried out. The donors will then receive one more dose of G-CSF and undergo a second pheresis the following day, and CD34 selection performed. The plan is to administer at least 2x10^6 cells/kg recipient body weight freshly from the first pheresis, after premedication with Benadryl® and hydrocortisone, but without additional Campath-1H treatment.

The product of the second pheresis will be cryopreserved and two aliquots successively thawed and infused into the recipients as above at 6 months and 9 months postoperatively, if clinically stable.

4.7 Withdrawal of MMF/Myfortic

One month after the fourth infusion (after 1 year posttransplant), participants will be evaluated for eligibility for MMF/Myfortic withdrawal. To be eligible for withdrawal of MMF/Myfortic, participants must meet the following criteria:

- Stable renal function, as defined by Glomerular Filtration Rate (GFR) $\geq$ 50 mL/min/1.73 m^2.
- Absence of biopsy-proven or clinically presumed acute rejection episode since transplantation.
- Absence of histologic evidence of acute or chronic rejection, as determined by renal biopsy (acute rejection/Banff Grade IA or higher) obtained within two weeks prior to commencing withdrawal.
- Total lymphocyte count $\geq$ 400/mm^3.
- Completion of all pre-withdrawal tolerance assay studies.

For participants who meet the eligibility criteria MMF/Myfortic will be withdrawn beginning no earlier than 1 year posttransplant**.** MMF/Myfortic will be withdrawn in a stepwise fashion over a minimum withdrawal period of two months at an approximate rate of 50% of the pre-withdrawal dose per month. Clinic visits and frequency are described in the accompanying calendar and its footnotes.

4.7.1 Monitoring During and After MMF/Myfortic Withdrawal

For participants who are withdrawn from MMF/Myfortic, additional laboratory assessments (hematology and chemistries) will be obtained according to the following schedule:

- At the beginning of withdrawal; then
- Weekly until MMF/Myfortic is completely withdrawn; then
- Every other week for the first 6 weeks after complete withdrawal of MMF/Myfortic; then
- Monthly thereafter.

A protocol biopsy will be obtained within 2 weeks prior to commencement of MMF/Myfortic withdrawal. "For-cause" biopsies will also be obtained in the event of a 20% increase in serum creatinine that is unexplained and unresolved within 24 hours. MMF/Myfortic withdrawal will be halted and/or immunosuppressive medications will be reinstituted if any biopsy reveals acute or chronic rejection, or (in the case of protocol biopsies not associated with clinical symptoms or laboratory abnormalities) the presence of tubulitis. If a protocol biopsy reveals normal histology or lymphocytic infiltration without tubulitis, withdrawal and/or continued absence of immunosuppressive medications will be permitted.

4.8 Withdrawal of Sirolimus

At 18 months post-transplant, a second postoperative transplant biopsy will be performed (research) and participants will be evaluated for eligibility for Sirolimus withdrawal. To be eligible for withdrawal of Sirolimus, participants must meet the following criteria:

- Stable renal function, as defined by Glomerular Filtration Rate (GFR) $\geq$ 50 mL/min/1.73 m^2 (24-hour urine collection for creatinine clearance corrected for body surface area, microalbumin, and protein).

- Absence of biopsy-proven or clinically presumed acute rejection episode since transplantation.
- Absence of histologic evidence of acute or chronic rejection, as determined by renal biopsy (acute rejection/Banff Grade IA or higher) obtained within 2 weeks prior to commencing withdrawal.
- Total lymphocyte count ≥ 400/mm^3.
- Completion of all pre-withdrawal tolerance assay studies.

For participants who meet the eligibility criteria, Sirolimus will be withdrawn beginning no earlier than 18 months post-transplant. Sirolimus will be withdrawn in a stepwise fashion over a minimum withdrawal period of 6 months at an approximate rate of 15-20 % of the pre-withdrawal dose per month. Clinic visits and frequency are described in the accompanying calendar and footnotes.

4.8.1 Monitoring During and After Sirolimus Withdrawal

For participants who are withdrawn from Sirolimus, additional laboratory assessments (hematology and chemistries) will be obtained according to the following schedule:

- At the beginning of Sirolimus withdrawal; then
- Every other week until Sirolimus is completely withdrawn; then
- Every other week for the first 4 weeks after complete withdrawal of Sirolimus; then
- Monthly for 3 years post-operatively and every 2 months thereafter for at least 10 years.

Biopsies will be obtained within 2 weeks prior to commencement of Sirolimus withdrawal and at 12 months after completing withdrawal from Sirolimus. "For-cause" biopsies will also be obtained in the event of a 20% increase in serum creatinine that is unexplained and unresolved within 24 hours**.** Sirolimus withdrawal will be halted and/or immunosuppressive medications will be reinstituted if any biopsy reveals acute or chronic rejection, or (in the case of biopsies not associated with clinical symptoms or laboratory abnormalities) the presence of tubulitis. If a biopsy reveals normal histology or lymphocytic infiltration without tubulitis, withdrawal and/or continued absence of immunosuppressive medications will be permitted.

Note: It will be the practice to follow renal transplant recipients with monthly or bimonthly immunologic blood work up to 150 mL and yearly clinic visits (or more frequent if necessary) for the life of the transplant.

4.9 Monitoring of Donors and Healthy Controls

4.9.1 Short and Long-Term Monitoring of Donors

Donors will intermittently provide peripheral blood (up to 60 mL [12 teaspoons]) first with postoperative clinic visits in the first two weeks after surgery and then for renal chemistries and immune assays as outpatients postoperatively at 6 months and yearly intervals for up to 10 years. Donors will also be asked to provide 25 ml (5 teaspoons) of iliac crest bone marrow at 1 or 2 year postoperatively.

4.9.2 Healthy Controls

4.9.2.1 Inclusion Criteria

1. Age to 18 – 65 years old.
2. Male or female of all races and ethnic groups in good health.

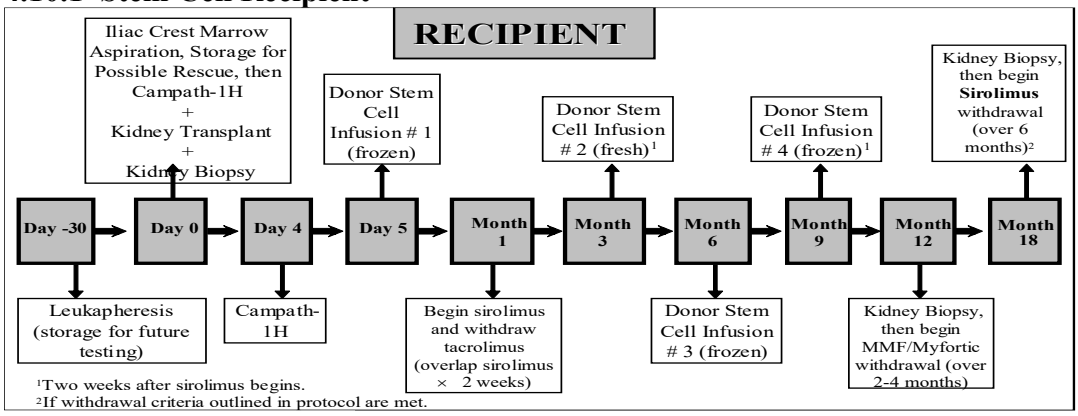
4.9.2.2 Exclusion Criteria

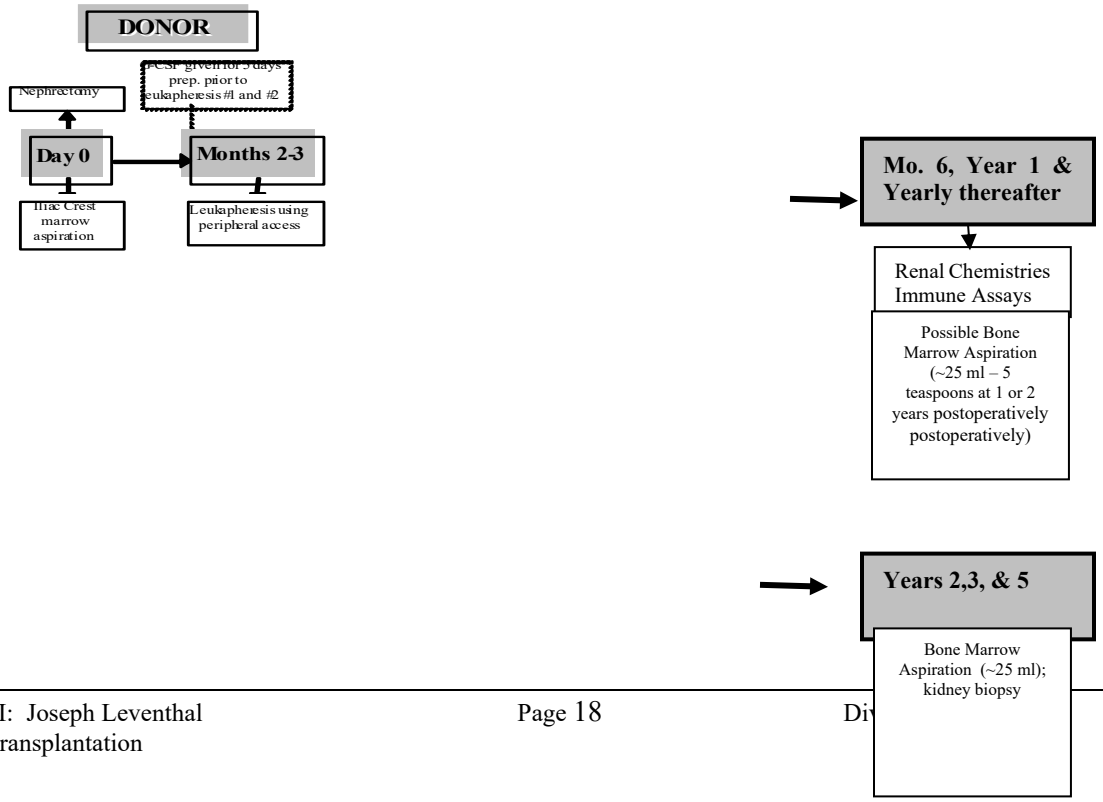
1. History of systemic disease, e.g. autoimmune disease, diabetes, hypertension, infection such as HIV or any disease that requires continuous therapy with prescribed medications.
2. Anyone on current continuous medication including anticoagulants.
3. Pregnancy.

Up to 110 laboratory volunteer controls will be tested at baseline and followed every 6 months using peripheral blood monitoring assays in comparison with the HLA identical sibling donor/recipient renal transplant pairs.
HLA identical kidney transplant recipient (see 10.1-10.5).
Iliac crest bone marrow aspirates (approx. 20-30 mL [4-6 teaspoons] per iliac crest) will similarly be tested with a frequency of one bone marrow aspiration (on one or both iliac crests) per normal control volunteer. The procedure will not be done more often than once on each iliac crest.

4.10 Recipient and Donor Timelines

4.10.1 Stem Cell Recipient





4.11 Study Endpoints

4.11.1 Primary Endpoint

The primary endpoint is patient and graft survival measured at the one-year time point post-transplant.

4.11.2 Secondary Endpoints

Secondary safety and efficacy endpoints are as follows:

- Patient and graft survival measured at the three-year time point post-transplant, and yearly thereafter;
- Patient and graft survival measured at the ten-year time point post-transplant**;**
- Incidence rate of biopsy-proven acute rejection, defined as a renal biopsy demonstrating acute cellular or humoral rejection of Banff Grade IA or greater;
- Incidence of chronic allograft nephropathy, determined using renal biopsies and laboratory values, including 24 urine protein excretion;
- Incidence of GVHD;
- Incidence of adverse events associated with renal transplantation and immunosuppression, including infections, malignancies (including PTLD), thromboembolic events, hyperlipidemia, leukopenia, thrombocytopenia, GI toxicity, and cytokine release.
-

4.11.3 Definition of Acute Rejection

For the purposes of this study, an episode of acute rejection will be considered to have occurred in the event of:

- Biopsy-proven rejection: a renal biopsy demonstrates acute cellular or humoral rejection of Banff Grade IA or greater; or

Presumed rejection: in the absence of biopsy-proven rejection, the participant is treated with augmentation of immunosuppression for an unexplained 20% increase in serum creatinine.

4.12 Study Population

Adult recipients of living-related (HLA identical) donor recipient pairs will be eligible to participate in this study. Because safety has not yet been established, participants under the age of 18 years will not be included in the study. Participants who meet the eligibility criteria may be enrolled with no restrictions due to gender or race.

4.13 Screening and Enrollment

This research study will be explained in lay language to each potential research participant. The participant will sign an informed consent prior to any screening study procedures. All activities (please see study Schedule of Events) performed in conjunction with this study will be performed in an unblinded manner.

4.14 Criteria for Discontinuation of Protocol Regimen

Patients who experience an acute rejection episode or an adverse event related to Campath-1H, tacrolimus, sirolimus, MMF, or a combination of these medications, may, at the

discretion of the Investigator, receive alternate FDA approved immunosuppressive medications and/or dosing regimens appropriate for the participant's clinical condition.

4.15 Criteria for Suspending Enrollment into the Study and/or Modification of Withdrawal of Immunosuppressive Medications

The medical monitor **and/or statistician** will review selected safety and efficacy criteria on an ongoing basis. Study enrollment and withdrawal of immunosuppressive medications will be suspended pending expedited review of all pertinent data if any one of the following occurs:

- Any participant experiences biopsy-proven acute rejection at any time during the study.
- Any participant experiences chronic allograft nephropathy at any time during the study.
- Any participant experiences GVHD at any time during the study.
- Any participant experiences graft loss during the first 12 months of the study.
- Any participant dies during the first 12 months of study, where the death is attributed in any way to treatment received in this study.

In the event of the occurrence of any of the above, pertinent study data will be expeditiously reviewed by the NU Institutional Review Board, and once the protocol is approved by the following groups: the NU/NMH CRU Data Safety Monitoring Board (DSMB) an independent extra institutional DSMB, the FDA, and the NIDDK. After this review, the Investigator and these agencies will jointly make a final determination regarding continuation, modification, or termination of the study.

5 Selection and Withdrawal of Participants

5.1 Inclusion Criteria

1. Patient has been fully informed, has signed a dated IRB approval informed consent form obtained directly by the P.I., Co-P.I., sub-investigator and/or research nurse and is willing to follow study procedures for the extent of the study (3 years).
2. Recipient must have a hematocrit of ≥33%, and a hemoglobin of ≥11.0 g/dL.
3. Age 18-65 years.
4. Weight > 40 kg.
5. Primary renal allograft: living-related (HLA identical donor).
6. Negative B-cell and T-cell cytotoxic and flow cytometry cross-match, and panel-reactive antibodies of < 10%.
7. Women of childbearing potential will be required to have a negative qualitative serum pregnancy test. See also #11, below.
8. Patients are to be studied equivalently as they become available for transplantation using these criteria, without regard for gender, **race, or ethnicity**.
9. Normal echocardiogram with an ejection fraction >50%.
10. Male participants with reproductive potential must agree to use approved methods of birth control during treatment with Campath-1H and for a minimum of 6 months following their last dose of Campath-1H. Female participants of childbearing potential must agree to use approved methods of birth control for the duration of their participation in the study.
11. Patient must agree to be followed every 2 months after year 3, for up to 10 years.

14. Up to 25 laboratory volunteers will be tested at baseline and followed every 6 months using peripheral blood and bone marrow monitoring assays with their HLA identical siblings. Up to 25 other volunteers (without HLA identical siblings) will also be tested once as controls for blood and bone marrow assays. These assays will be similar to those performed on the stem cell infused HLA identical kidney transplant recipient (see 10.1-10.5).

5.2 Exclusion Criteria

1. Patient has previously received or is receiving an organ transplant other than a kidney.
2. Patient is receiving an ABO incompatible donor kidney.
3. Recipient or donor is ELISA positive for human immunodeficiency virus (HIV), antibody positive for hepatitis C, or surface antigen positive for hepatitis B.
4. Recipient or donor is positive for TB (or under treatment for suspected TB), or has had previous exposure to TB (positive Mantoux), who has not undergone and acceptable course of treatment.
5. Patient has a current malignancy or a history of malignancy (within the past 5 years), except non-metastatic basal or squamous cell carcinoma of the skin, or carcinoma *in situ* of the cervix that has been treated successfully.
6. Patient has uncontrolled concomitant infections and/or severe diarrhea, vomiting, active upper gastro-intestinal tract malabsorption or an active peptic ulcer or any other unstable medical condition that could interfere with study objectives.
7. Patient is currently receiving an investigational drug within 30 days prior to transplant.
8. Patient is currently receiving any immunosuppressive agent.
9. In the judgment of the investigator, it is anticipated that the patient will be unable to take medications orally or via nasogastric tube by the morning of the second day following completion of the transplant procedure (i.e., skin closure).
10. Concurrent use of warfarin, fluvastatin, astemizole, pimozide, cisapride, terfenadine, or ketoconazole.
11. Patient has a known hypersensitivity to tacrolimus, Campath-1H, Thymoglobulin, daclizumab (Zenapax®), sirolimus, MMF or corticosteroids.
12. Patient is pregnant or lactating.
13. Patients with a screening/baseline total white blood cell count $<4000/mm^3$; platelet count $<100,000/mm^3$.
14. Patient is deemed unlikely to comply with the visits scheduled in the protocol.
15. Patient has any form of substance abuse, psychiatric disorder or a condition that, in opinion of the investigator, may invalidate communication with the investigator.
16. It is expected that tacrolimus cannot be instituted for longer than 5 days postoperatively.
17. Patients with a PRA value >10% at any time prior to enrollment.
18. Patients with Graves disease will be excluded unless previously adequately treated with radioiodine ablative therapy.
19. EBV negative recipients of a EBV positive donor.
20. Patients with a history of idiopathic thrombocytopenic purpura (ITP) or thrombotic thrombocytopenic purpura (TTP).

5.3 Participant Withdrawal Criteria

5.3.1 Premature Termination from the Study

Participants may be prematurely terminated from the study; however, documentation and follow-up for safety will continue for one year even if an individual participant is terminated from the study. Premature termination of an individual participant will take place should any of the following occur:

1. Informed consent is withdrawn.
2. Adverse events of grade 3 or higher are documented (according to full NIH guidelines in minimizing the risk and in Equipoise considerations).
3. In case of pregnancy.
4. In case of medical non-compliance.
5. In case of other serious intervening illnesses such as infection, GVHD, or posttransplant lymphoma.
6. In case of rejection or other adverse events occur requiring changing to our standard immunosuppressive protocols.

5.3.2 Replacement of Participants who Discontinue Study Treatment or who Prematurely Terminate from the Study

Participants who discontinue study treatment or who prematurely terminate from the study will not be replaced.

6 Study Medication

6.1 Campath-1H: Formulation, Packaging, and Labeling

Although not considered an experimental drug, for the purposes of the study Campath-1H will be supplied by Genzyme Corporation, San Antonio, TX. Campath-1H is supplied as a purified preparation diluted in phosphate buffered saline (PBS) with 0.05 mmol Ethylenedinitrilo Tetraacetic Acid (EDTA). Between 80 is added to a concentration of 0.01%. The final product is a clear, colorless isotonic solution free of visible particulate matter. The ampules will contain 10 mg of antibody in 1mL of sterile PBS at a concentration of 10 mg/mL. Campath-1H should be stored, protected from light, in a refrigerator between 2° and 8°C.

Intravenous Campath-1H will be diluted in 100 cc of 0.9% normal saline or 5% Dextrose and water (D5W) and administered intravenously over 2 hours. Campath-1H must be filtered with a sterile, low-protein binding, 5-micron filter prior to dilution.

6.2 Prophylactic Medications

6.2.1 Pneumocystis carinii (PCP)

Trimethoprim / sulfamethoxazole (Septra/Bactrim) one single-strength tablet will be administered daily for the first 6 weeks, then 3 times a week for the duration of the study, as tolerated. If unable to tolerate or if allergic, dapsone will be substituted after a Glucose –6-Phosphate Dehydrogenase hemolysis (G-6-PD) blood test has been shown to be normal.

6.2.2 Cytomegalovirus (CMV)

Valganciclovir (Valcyte®) 450mg will be administered daily as tolerated for the first three months posttransplant. The valgancyclovir dosing will be based on the participant's calculated creatinine clearance. Subsequent dose reductions may be made at the investigator

discretion. Valtrex® will be given, as tolerated, if patient does not tolerate Valcyte® or becomes leukopenic.

6.2.3 Fungal
Nystatin or Clotrimozole will be administered for fungal prophylaxis for the first 3 months posttransplant.

7 Study Procedures
A detailed schedule of events (Up to year 3) for this study is provided in Appendix 1.

Year 4-10 follow-up: will be conducted by annual EMR review of Standard of Care (SOC) laboratory and clinical follow up on the subject's transplant anniversary date. The study staff will also contact the subjects once annually (around patients' anniversary date) to review adverse events. The annual subjects findings will be communicated and signed off by the PI regularly.

7.1 Protocol and For-cause Biopsies
Protocol biopsies will be obtained 2 weeks prior to commencement of MMF withdrawal, 2 weeks prior to Sirolimus withdrawal, and 1, 2, and 5 years after Sirolimus withdrawal. "For-cause" biopsies will also be obtained in the event of a 20% increase in serum creatinine that is unexplained and unresolvefd within 24 hours. Withdrawal will be halted and/or immunosuppressive medications will be reinstituted if any biopsy reveals acute or chronic rejection, or (in the case of protocol biopsies not associated with clinical symptoms or laboratory abnormalities) the presence of tubulitis. If a protocol biopsy reveals normal histology or lymphocytic infiltration without tubulitis, withdrawal and/or continued absence of immunosuppressive medications will be permitted.

7.1.1 Protocol Bone Marrow Aspirations
Protocol bone marrow aspirations will be obtained at 5 years after Sirolimus withdrawal.

7.2 Treatment of Acute Rejection
Mild (Banff IA): 3 daily I.V. pulses of 500 mg of methylprednisolone, followed by an oral taper over approximately 2-3 weeks. Moderate (Banff IB) or severe (Banff IIA or greater): Thymoglobulin® (1 mg/kg per day for 5-10 days). Treatment for acute rejection may be modified by the Investigator or as clinically indicated.

7.3 Guidelines for Treating Neutropenia
If a patient's absolute neutrophil count (ANC) is <700 cells/μL, the following guidelines are recommended:
- Reduce the prophylactic dose of valganciclovir.
- Stop administration of Septra SS and replace with inhaled pentamidine.

If the participant's ANC is <500 cells/μL, the following guidelines are recommended:
- Consider discontinuation of valganciclovir.
- Obtain a CMV PCR.
- If fever is associated with neutropenia obtain a hematology and infectious disease treatment consultation and initiate appropriate antibiotics and antifungal agents as per consultation.

- Consider fluoroquinolones.
- Consider GCSF (neupogen).
- If currently taking sirolimus, obtain a new whole-blood sirolimus trough level (and also refer to the most recent available sirolimus trough level to avoid delay in patient management), and if the sirolimus levels are >12 ng/mL, then consider holding 1 to 2 doses (based on clinical judgment). If the sirolimus levels are <12 ng/mL, then use clinical discretion in continuing on sirolimus.
- Consider restarting sirolimus as soon as ANC count is >500 cells/μL.

The participant's safety is of utmost importance. Local clinical treatment decisions take precedence over recommended guidelines.

7.4 Diagnosis and Treatment of Graft Versus Host Disease

In the current Campath-1H induction protocol, by the very use of the antibody and its half-life of 12 days with effects felt up to one year, it is conceptually highly unlikely that in the recipient non-myeloablated environment that contaminating donor post-thymic T cells can do this. Furthermore, it is perhaps more likely that a mixed (mutually tolerant) chimeric state could ensue if macrochimerism of >10% donor immunohematologic cells would occur. GVHD would be especially unlikely under recipient continuous immunosuppressive therapy, essentially for 2 years after the second stem cell infusion. Additionally, by purifying CD34+ cells post-thymic, T cells in the inoculum are minimized.

Nonetheless, as a safeguard, it is planned to store one unit of iliac crest recipient bone marrow taken at transplant surgery before Campath-1H is started, so that it can be re-infused any time during the long observation period in order to reverse GVHD if needed. In addition, our collaborator Dr. Richard Burt, Director of Immunotherapy at Northwestern, will be following these patients and has diagnostic and therapeutic protocols in place that incorporate the following.

7.4.1 Diagnosis of Acute GVHD

Acute GVHD generally develops within the first three months after transplantation and appears as a characteristic dermatitis often accompanied by hepatic cholestasis and enteritis. The clinical appearance of GVHD may be mimicked by drug reactions. Therefore, documentation by skin biopsy will be performed. Liver biopsy can be helpful but often cannot be done because of clinical contraindications such as thrombocytopenia.

7.4.2 Treatment of GVHD

Grade I – II GVHD will be treated with Solumedrol (2 mg/kg) and Prograf. In cases of moderate to severe GVHD, Zenapax and anti-TNF antibody (Remicade) will be utilized.

8 Assessments of Efficacy and Safety

8.1 Safety and Efficacy Parameters

In the absence of medical contraindications, all acute rejection episodes will be confirmed by biopsy. Biopsies will be histologically evaluated at the Northwestern Memorial Hospital, utilizing the Banff criteria.

For the purposes of this study, an episode of acute rejection will be considered to have occurred in the event of:
Biopsy-proven rejection: a renal biopsy demonstrates acute cellular or humoral rejection of Banff Grade IA or greater; or
Presumed rejection: in the absence of biopsy-proven rejection, the participant is treated with augmentation of immunosuppression for an unexplained 20% increase in serum creatinine.
Graft loss is defined as the institution of chronic dialysis (at least 6 consecutive weeks, excluding participants with delayed graft function), transplant nephrectomy, or retransplantation.

For this study, severe acute rejection is defined as that which is histologically evaluated as Type IIA or greater utilizing the Banff criteria.

Renal function will be evaluated through the measurement of serum creatinine monthly posttransplant.

Adverse events to include infections and malignancies and participant information to include participant weight and resting blood pressure will be collected on study case report forms throughout the study. For purposes of this protocol, rejection, graft loss, and status of graft function will not be recorded on the adverse event case report form. These events will be collected on specialized case report forms.

9 Adverse Event Collection and Reporting

Adverse events that are classified as serious, according to the definition of regulatory authorities, must be rapidly and adequately reported to the regulatory authorities mentioned above. This section provides definition of the types of adverse events and outlines a process for the appropriate reporting and follow-up procedures. Information in this section was obtained from the ICH guideline for Good Clinical Practice (mentioned previously), ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, and the Common Toxicity Criteria Manual Version 3.0 (August 9, 2006).

9.1 Definitions

9.1.1 Adverse Event

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom (including an abnormal laboratory finding), or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Throughout the study-defined collection period, the Investigator must record all adverse events on the appropriate adverse event form, regardless of the severity or relationship to study medication or procedure. The Investigator should treat participants with adverse events appropriately and observe them at suitable intervals until the events resolve or stabilize.
Adverse events may be discovered through:

- Observation of the participant;
- Questioning the participant;
- Unsolicited complaint by the participant; or
- Discovery of abnormal clinical laboratory values or abnormal results of other evaluations (radiographs, ultrasound, ECG, etc.).

In questioning the participant the questioning should be conducted in an objective manner.

In the event of an abnormal laboratory value, the test should be repeated until it returns to normal or can be explained and the participant's safety is not at risk. Clinically significant laboratory abnormalities as determined by the Investigator must be recorded as adverse events, as well as recorded on the appropriate laboratory evaluation form(s).
As indicated in the Appendix I, AEs will be collected from the start of the study through Month 36. AEs that are unresolved at the time that the participant completes this period of the study will be followed until they resolve or for a maximum of 30 days.

9.1.2 Serious Adverse Event

A serious adverse event (SAE) or reaction is defined as any adverse event occurring at any dose that suggests a significant hazard, contraindication, side effect or precaution. This includes, but may not be limited to any of the following events:

- Death: A death occurring during the study or which comes to the attention of the Investigator during the protocol-defined follow-up after the completion of therapy, whether or not considered treatment-related, must be reported;
- Life-threatening: Any adverse therapy experience that places the patient or participant, in the view of the Investigator, at immediate risk of death from the reaction as it occurred;
- Inpatient hospitalization or prolongation of existing hospitalization;
- Persistent or significant disability/incapacity;
- Congenital anomaly/birth defect;
- Other conditions as specified in the protocol;
- An event that required intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

SAEs will be collected from the time informed consent is obtained, to 30 days after study completion or for a minimum of one year after a participant prematurely withdraws from the study or for the duration of the funding period.

However, patients will be followed for collection of events such as death, graft survival, resumption of immunosuppressive medications, the development of life-threatening infections, and the development of cancer during a 10-year long-term follow-up.
Regardless of the relationship of the adverse event to study drug, the event must be reported as a serious adverse event if it meets any of the above criteria.

9.1.3 Unexpected Adverse Event

An adverse event is considered unexpected when the nature (specificity) or severity of the event is not consistent with applicable product information, such as safety information provided in the package insert, investigational plan, investigator's brochure, protocol or informed consent document.

9.2 Grading of Adverse Events

9.2.1 Toxicity Grading of Adverse Events

Toxicity grades are assigned by the study site to indicate the severity of adverse events occurring in study participants. These have been adopted from the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) for application in adverse event reporting. The purpose of using the NCI-CTC system is to provide a standard language to describe toxicities, to facilitate tabulation and analysis of the data, and to facilitate the assessment of the clinical significance of all adverse events. The NCI-CTC provides the following grades and descriptions in the NCI-CTC Manual (Version 3.0, dated 8/9/06). Adverse events should be recorded and graded 1 to 5 according to the NCI-CTC grades provided below:
Grade 1 = Mild adverse event.
Grade 2 = Moderate adverse event.
Grade 3 = Severe and undesirable adverse event.
Grade 4 = Life-threatening or disabling adverse event.
Grade 5 = Death.
Note: In contrast to the CTC guidelines provided in the NCI-CTC Manual (Version 3.0) all adverse events are to be reported and graded, whether or not related to disease progression or treatment.

9.2.2 Relationship to Study Drug

The relationship or attribution between an adverse event and an investigational product is determined by the site Investigator or sub-Investigator and recorded on the appropriate Case Report Form and/or SAE Reporting Form. The NCI-CTC provides the following descriptors and definitions (one category classified as unrelated [Code 1] and 4 categories classified as related [Codes 2-5]) for assigning an attribution to each adverse event as described below:

ATTRIBUTION OF ADVERSE EVENTS		
Code	Descriptor	Definition
"Unrelated" Category Code (1 Category):		
1	Unrelated	The adverse event is clearly not related to the investigational agent(s)
"Related" Category Codes (4 Categories):		
2	Unlikely	The adverse event is doubtfully related to the investigational agent(s)
3	Possible	The adverse event may be related to the investigational agent(s)
4	Probable	The adverse event is likely related to the investigational agent(s)
5	Definite	The adverse event is clearly related to the investigational agent(s)

The Investigator's determination of drug-relatedness (attribution) for each adverse event should be recorded in the source documentation.
For additional information, consult the Common Toxicity Criteria Manual and the Common Toxicity Criteria Document at the following URL: http://ctep.cancer.gov/reporting/ctc.html.

9.3 Serious Adverse Event Reporting

The following process for reporting a serious adverse event will ensure appropriate reporting compliance with the ICH guidelines.

9.3.1 Serious Adverse Event Identification and Determination of Reporting Timeline

When an Investigator identifies a serious adverse event (as defined above), the Investigator must notify the NU IRB and/or other Reporting Centers (the NUCRU, FDA, DSMB**,** and NIDDK) of the serious adverse event within 24 hours of discovery. In addition, these events must be entered on the Serious Adverse Event Form and the Adverse Event CRF.
Three possible reporting scenarios (to the appropriate health authorities) could arise after assessment of the event:
No requirement to report. This would occur if the adverse event is deemed not serious by protocol definition.

Standard reporting is required (report in IND annual report). This would occur if the adverse event were classified as one of the following: (a) serious, expected and drug related; (b) serious, expected and not drug related; or (c) serious, unexpected and not drug related.
Expedited reporting is required. This would occur if the adverse event is considered serious, unexpected and drug related. These events must be reported by the Sponsor to the appropriate health authorities within 15 days unless the event is fatal or life- threatening, the latter must be reported within 7 days.

The PI **in any event,** must report the serious adverse event to the NU IRB as mandated by the NU IRB.

9.3.2 Reporting Serious Adverse Events to the Data and Safety Monitoring Board

A Transplant Data and Safety Monitoring Board (DSMB) will be provided listings of all SAEs on an ongoing basis. Further, the DSMB will be informed of expedited SAEs at the same time as regulatory authorities.

9.4 Pregnancy (SAE Reporting Requirements)

Any pregnancy that occurs during a clinical study with an investigational drug must be reported as an SAE for tracking purposes only. All pregnancies that are identified during this study need to be followed to conclusion and the outcome reported. Female participants should immediately inform the Investigator of any pregnancies and should be instructed by the Investigator to stop taking study medication. The Investigator should report all pregnancies within 24 hours (as described above in SAE Reporting) using the SAE form. The Investigator should counsel the participants and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the participants should continue until the conclusion of the pregnancy, and a follow-up SAE Reporting Form should be submitted detailing the outcome.

9.5 Updating Source Documentation

Documents describing the safety profile of a drug, such as the investigator's brochure, should be amended, as needed, to ensure that the description of safety information adequately reflects any new clinical findings. Until these documents are updated, expedited reporting is required for additional occurrences of the reaction.

10 Tolerance Assay Studies

A series of assays of chimerism analyses and tolerance mechanism are to be performed.

10.1 Cytokyne Gene Polymorphism (CGP)-PCR-Flow Measurements of DHSC Chimerism

Hypothesis: We propose that macrochimerism brought about by DBMC stem cell infusions, together with Campath-1H treatment for making "space" in recipient central and peripheral immune compartments, will be a reflection of the development of donor-specific unresponsiveness. Thus, after treatment with Campath-1H, these assays (and other measurements of DBMC chimerism) should demonstrate significantly higher levels of chimerism (macrochimerism) than in our previous DBMC protocols[1,2,41], especially since CD34+ cells are not sensitive to Campath-1H lysis.

Plan: The CGP-PCR-Flow assays[42,43] (using cytokine polymorphism) as well as VNTR assays of PBMC subsets will be used to sequentially measure donor immunohematopoietic cell chimerism in peripheral blood and iliac crest marrow, i.e., of donor and recipient CD3+, CD4+, CD8+, CD34+, CD19+, CD16/56+, and CD14+ cells, according to the following time-table:

1. Intraoperatively (baseline control values), before Campath-1H administration, but after anesthesia induction in: (a) donor peripheral blood and donor iliac crest marrow, and (b) recipient peripheral blood and recipient iliac crest marrow.
2. In (a) recipient peripheral blood on post-operative days 4, 6 (1 day post-DBMC infusion), 21 and 28, then monthly for months 2-12 and every six months thereafter, and (b) recipient iliac crest marrow at 6 months, 1-year and yearly thereafter.
3.

It will be of interest to determine with the CGP-PCR flow assay whether or not the relatively more rapid return of certain recipient immunohematopoietic subsets, i.e., relatively Campath-1H resistant CD14+ and CD16/56+ cells, influences the presence of specific donor chimeric subsets, i.e., CD34+ cells or CD3+ cells, as they regenerate (compared with DBMC-infused recipients tested in previous studies).

Alternatively: If there is no difference using CGP-PCR-Flow analysis between the DHSC-infused Campath-1H group and patients in previous studies, there may well be a greater difference in *the ratio of CD34+ to CD3+ donor chimeric cells in the stem cell-infused group*.[1,2] This in itself will be of interest for possible future protocol design and other mechanistic *ex vivo* assays.

Alternatively: If significant macrochimerism (>10%, *vide supra*) does not develop under Campath-1H treatment, the assays and clinical studies described in the succeeding sections may well show differences between this stem cell-infused group and those previously reported, so at to test whether immunosuppressive withdrawal is still be possible.

10.2 Sequential Measurement of Regulatory Cells and their Products

Ex vivo immune assessment: Such cells in the peripheral blood and (bone marrow) at 6, (12), 18, (24), 30, (36), 42, (48), 54, and (60) months postoperatively. [( ) = iliac crest marrow samples]

Hypothesis: Regulatory cells of donor and recipient phenotypes that develop as a result of chimerism in the DBMC-infused group of renal transplant recipients are causative agents of specific hyporesponsiveness to donor alloantigens and can be quantitated sequentially as a means of assessing the effect of immunosuppressive withdrawal. We will use ELISPOT assays, flow cytometric analysis, and the micro-CML that requires fewer cellular reactants. We will monitor the renal transplant recipients from the DBMC-infused groups for their anti-donor immune status and T cell regulatory function. Greater immunoregulatory activity is expected in the Campath-1H donor stem cell infusion group than tested in previous DBMC-infused patients. Studies by Heeger and colleagues have suggested that IFN-□ or Granzyme-B producing cells, as detected by ELISPOT in allograft recipients, provide an *ex vivo* reflection of the evolving *in vivo* donor reactive immune response.[44] The antigen specific production of these cytokines in this short-term assay represents the fingerprint of a previously primed T cells of the CD45RO+ and CD45RA- memory phenotype, i.e., a putative escape from the regulatory or clonal deletion effects of the tolerance protocol. However, production of IL-4, IL-10 and other TH2 cytokines may represent the presence of regulatory cells.

10.2.1 ELISPOT

Plan: Ninety-six-well ELISPOT plates (Cellular Technology Ltd, Cleveland, OH) are coated with 100 □l per well of capture antibodies for IFN-□, Granzyme-B, IL-4 or IL-10 (BD Sciences) in PBS overnight at 4ºC. The plates are then blocked with 150 □l of PBS-1% BSA (Sigma) per well and washed three times with PBS. Usually 300,000 recipient responder cells per well are stimulated with 300,000 (donor) stimulator cells per well. Phytohemagglutinin is added to selected wells as a positive control. Final volume for all assays is 200 □l per well. Control wells contain responder cells or stimulators plus medium alone. After incubation at 37ºC for 24 hr, the plates are washed three times with PBS, then four times with PBS-Tween (0.025%). Next, 100 □l of biotinylated detection antibodies for IFN-□, Granzyme-B, IL-4 or IL-10 (BD Sciences) in PBS-Tween-1% BSA are added to the wells overnight at 4ºC. On the following day, the plates are washed three times with PBS-Tween. Streptavidin-HRP (PharMingen) is plated at optimal dilution in PBS-Tween-BSA for 90 min at room temperature. The spots are developed using 800 □l of 3-amino-9-ethylcarbazole (Pierce; 10 mg/mL in N, N-dimethylformamide) freshly diluted into 24 mL 0.1M sodium acetate (pH 5.0), filtered through a 0.45-mm filter and mixed with 12 □l of H_2O_2 (200 □l/well). After the plates are dried, the resulting spots are counted on a computer-assisted ImmunoSpot image analyzer in use in our laboratories (Cellular Technology Ltd),that has 100% reproducibility when repeatedly counting the same well using single, defined criteria. Results are presented as mean values of ELISPOT detected in triplicate wells containing responder PBL plus mitogen or donor stimulator cells, after subtracting the response of wells with responder cells or donor cells alone (<15 spots per 300,000 cells in each case).

10.2.2 Micro-CML Assays

These will be performed using recipient responding PBL against both donor and 2DR mismatched indifferent (third party) cells of a selected laboratory volunteer.[30]
Inhibition in Micro-CML or in MLR by RdD cells will be indicative of regulatory activity by the donor chimeric cells present in the recipient and, by RdR cells, will be suggestive of the presence of "infectious tolerance". The presence of this regulatory activity will be confirmed

with the use of cryopreserved recipient pre-transplant PBL as responders and posttransplant RdD and RdR cells as well as pre-transplant recipient cells as modulators. Inhibition under these conditions will indicate that chimerism can drive the recipient to develop regulatory cells that act on autologous anti-donor immune responses.

Alternatively: In recipients who show micro-CML and MLC unresponsiveness to the donor, the presence of possible split or linked tolerance will be evaluated.[45] The recipient PBL will be stimulated with irradiated PBL from laboratory volunteers who have common donor HLA antigens and at least 1 indifferent HLA antigen (from both donor and recipient), compared with volunteers with totally different HLA genes, in the presence of graded numbers of purified RdD and RdR cells. Inhibition of these semi-allogeneic vs. fully allogeneic responses will be indicative of linked suppression.

For each of these assays, 40 ml of recipient blood and 20 ml of bone marrow are needed at each testing interval. They will be performed at the time of the PCR-Flow assays when bone marrow is obtained (5 ml of marrow).

10.2.3 Treg and Immunophenotyping Assays

We are now using the Treg MLR functionally in invitro assays testing peripheral blood from our recipients as well as immunophenotyping for Tregs.

10.3 Donor Reactive Cells or Regulatory Cells in the Kidney Allograft

Hypothesis: Either donor reactive or regulatory cells may be sequestered in the allograft itself. Regulatory reactivity can be assessed with more frequency in the peripheral circulation (or bone marrow), using the approaches described above, and (less frequently) in the allograft by monitoring infiltrating cells in biopsies. With the sequential (yearly) renal transplant biopsies assessing the effect of immunosuppressive withdrawal, it will be possible to assay this graft-associated lymphoid compartment for such reactivity.

Plan: Protocol renal transplant biopsies will be taken at yearly intervals, i.e., before, during and after planned immunosuppressive therapy withdrawal. In addition to histopathological examination, a portion of the biopsies will be placed in culture in medium containing 50 U/ml rIL-2 and 50% MLR supernatant.[46] During rejection, lymphocytes usually grow out of the biopsy within 48 hours.[46] We will keep the biopsy cultures for at least 1 week, and characterize them for donor vs. recipient HLA genomic DNA by PCR (or even by cellular PCR-Flow) at 48 hr, 72 hr, or 7 days after the initiation of the biopsy culture. If sufficient numbers of cells are available, their ability to kill donor cells or regulate killing in a modified micro-CML assay and to produce Granzyme-B, IFN-□, IL-4 and IL-10 in the ELISPOT assay will be monitored.

For PCR amplification and reverse probe hybridization of CGP profiles using the Innogenetic LIPA (Line Probe Assay), approximately 100 cells will be needed for the genomic DNA extraction and purification. If a mixed genotype is resolved, a quantitation analysis will be accomplished by Sequence Specific Priming (SSP) of mismatched donor/recipient alleles. The recipient amplified product will be compared to a standard to ascertain the quantity (%) of recipient versus donor alleles. From as few as 1000 of these cells, the profile of messenger RNA (mRNA) for Granzyme-B, IFN-□, IL-4, and IL-10 will be delineated using a real

time PCR quantitation with cytokine primers.[47,48] The absence of CTL or Granzyme-B and IFN-□ producing cells and/or a high ratio of donor to recipient genomic MHC DNA activity growing out from the biopsies will be considered indicative of donor specific unresponsiveness, and the presence of IL-4 or IL-10 producing cells will be indicative of regulatory cells.

Alternatively: If no cells grow out (and no histopathology is present), this will be an even clearer sign of alloimmune non-reactivity.

10.4 Absence of Recipient Donor-reactive Cytotoxic T cell Precursors and/or the Presence of Regulatory Cells in the Peripheral Circulation

If the recipient demonstrates donor-specific unresponsiveness in the ELISPOT and micro-CML assays, limiting dilution analysis for the enumeration of CTLp will be performed. A precursor frequency of less than 10 donor reactive CTLp in $1x10^6$ responder PBL will be taken as unresponsiveness. Additionally, the shape of the limiting dilution analysis curve would also indicate the presence of regulatory cells in the responding PBL.

10.5 Mixed Lymphocyte (Dendritic) Cell Culture (ML(D)C), Treg MLR, and *In-vivo* DTH Reactions

These assays are to be performed on the recipient to detect unresponsiveness and regulatory cells in iliac crest marrow aspirates and peripheral blood.

10.6 Sensitive Alloantibody Assays

Since, the presence of antibodies to donor MHC antigens has been associated with rejection and graft destruction in organ transplant recipients[49] and, the absence of such donor reactive antibodies is a prerequisite for allograft acceptance, we propose that monitoring serial serum samples even from HLA identical recipients for expanded HLA specificities, and non-HLA epitopes and RBC and endothelial cell line using the Flow technique should help delineate this alloimmunoreactive predisposition. This will also be performed in collaboration with laboratories of Dr. Paul Terasaki in Los Angeles, California.

11.0 Additional genomic studies on paraffin embedded transplant biopsy samples

These samples will have been obtained at the time of the transplant biopsies and will be processed and sent to our collaborators at the Scripps Institute in La Jolla, California for genomic assays on biopsy material already obtained.

The purpose of the additional genetic studies on paraffin embedded transplant biopsy samples is to obtain a biomarker that is indicative of kidney tolerance compared to kidney rejection in renal transplant patients.

12.0 Statistical Considerations and Analytical Plan

12.1 Study Objectives

12.1.1 Primary Objective

The primary objective of this trial is to investigate the safety and efficacy of a treatment regimen consisting of induction therapy with Campath-1H and steroid-free low dose maintenance immunosuppression (consisting of methylprednisolone, MMF, tacrolimus, and sirolimus), followed by complete withdrawal of immunosuppression beginning at one year,

at a minimum, posttransplant, in recipients of living-related (HLA identically matched) donor kidney transplants who have also been given infusions of purified DBMCs.

12.1.2 Secondary Objectives

To assess the utility of various assays and biopsy information as putative tolerance markers in these kidney transplant recipients:

- To use the short tandem repeat (STR) assay both in recipient peripheral blood on a monthly basis and in iliac crest marrow at 6 months and yearly, in order to measure donor cell multi-lineage chimerism;
- To use the newly developed cell-mediated lympholysis (CML) microassay and enzyme-linked immunospot (ELISPOT) assay to demonstrate sequentially increasing donor and recipient chimeric marrow and peripheral blood cell immunohematologic subset regulatory effects;
- To study lymphoid cells derived from yearly kidney biopsies (i.e., before and after planned withdrawal of immunosuppression)
- To follow sensitive alloantibody assays;

.

12.2 Endpoints

12.2.1 Primary Endpoint

The primary endpoint is patient and graft survival measured at the one-year timepoint post-transplant.

12.2.2 Secondary Endpoints

Secondary endpoints to be evaluated include those parameters outlined and defined in Section 4.10.2 Secondary Endpoints.

12.3 Sample Size

Ad described above.

12.4 Randomization and Blinding - none.

12.5 Statistical Analysis

12.5.1 Analysis Samples

The following analysis samples will be utilized for safety and efficacy analyses:
Safety sample: All participants who receive any form of study therapy will be included in all safety analyses.
Intent-to-treat (ITT) sample: All participants will comprise the ITT sample. All efficacy endpoints will be analysis on an ITT basis.
Per Protocol (PP) sample: All participants without major protocol violations who are not replaced due to technical reasons and who have adequate efficacy assessment data will comprise the PP sample. The PP sample will be further defined in the statistical analysis plan (SAP) before database lock. Major efficacy endpoints will be analyzed with the PP sample in addition to the ITT sample.

12.5.2 Description of Baseline Characteristics and Demographics
Summary descriptive statistics for demographic and baseline characteristics will be provided for all enrolled participants. Demographic characteristics will include age, race, sex, body weight, and height. Continuous demographic and baseline data (e.g., age, body weight, and height) will be summarized in the electronic medical record.

12.5.3 Medical History
Medications will be listed in the subject's electronic medical record.

11.5.4 Use of Medications
All medications used will be coded using the WHO drug dictionary. The number and percentage of participants receiving concomitant medications/therapies will be presented.

12.5.5 Study Completion
The percent of participants who fail to complete the study, losses to follow-up, times to lost to follow up, and reasons for discontinuation (adverse events, other) will be presented.

12.5.6 Efficacy
All efficacy endpoints will be evaluated on an ITT basis. Major efficacy endpoints will also be analyzed using the PP sample to provide supportive or exploratory information.

12.5.7 Primary Efficacy Analysis
The primary endpoint, namely, patient and graft survival at one year posttransplant, will be descriptively summarized with 95% confidence intervals.

12.5.8 Secondary Efficacy Analyses
The secondary efficacy endpoints will be descriptively summarized with 95% confidence intervals (as appropriate).

12.5.9 Safety
Participants comprising the Safety sample will be included in all safety analyses.
All adverse events, including posttransplant infections, malignancies, thromboembolic events, morbidity, and side effects associated with the low dose steroid-free maintenance immunosuppression, will be classified by body system and preferred term according to the Medical Dictionary for Regulatory Activities (MedDRA).

Frequency tables by category of event (e.g., serious, related to study therapy, causing the discontinuation of study therapy) and by NCI CTC grade will be presented by treatment group. The safety displays will include summaries of the occurrence and incidence of posttransplant infections, malignancies (including PTLD), thromboembolic events, hyperlipidemia, leukopenia, thrombocytopenia, GI toxicity, and cytokine release by treatment group. Descriptive statistics (mean, standard deviation, mean change from baseline) of laboratory values and vital signs will be presented by treatment group. Laboratory values will also be summarized by mean, standard deviation, and change from baseline by treatment group.

12.6 Planned Interim Analyses

Enrollment in the trial will be suspended for safety reasons i.e. unexplained adverse events. The DSMB will review any events as requested by the Protocol Chair or the medical monitor. They will review listings of all adverse events and laboratory findings periodically.

12.7 Procedures for Reporting Deviations from the Original Plan

Any changes in these principal features would require a protocol or SAP amendment, which would then be subject to review by the independent Data and Safety Monitoring Board (DSMB), study sponsor(s), and regulatory agencies. These changes will be described in the final report as appropriate.

13 Access to Source Data / Documents

The investigational site participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants and donors participating in this clinical trial. Medical and research records should be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (obscuring any personally identifying information). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. The investigational site will normally be notified in advance of auditing visits.

14 Quality Control and Quality Assurance

The Investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The Investigator is required to ensure that all case report forms are legibly completed for every participant entered in the trial.

The sponsor is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency and accuracy of all documented data.

To ensure the reliability of the data recorded in the database, double data entry will be used for all fields on the CRF. The data will be verified by a series of computerized edit checks, and all relevant data queries will be resolved on an ongoing basis. When the CRFs are complete, they will be reviewed and signed by the Investigator. All data from the original signed CRF will be entered in the database, and a comparison program will be run again. All discrepancies will be reviewed, and any resulting queries will be resolved with the Investigator and amended on the database. All elements of data entry (i.e., time, date, verbatim text and the person performing the data entry) are recorded in an electronic audit trail to allow all data changes in the database to be monitored and maintained in accordance with federal regulations.

15 Ethical Considerations and Compliance with Good Clinical Practice

15.1 Statement of Compliance

This trial will be conducted in compliance with the protocol, current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate ethics review committee or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are implemented.

15.2 Informed Consent

The informed consent form is a means of providing information regarding the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign and date a consent form prior to participation in the study, taking study drug and/or undergoing any study-specific procedures. If a participant does not speak and read English, the consent materials must be translated into a language understandable to the subject.

The informed consent form must be updated or revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect a patients' participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The Principal Investigator, Co-Principal Investigator, Sub-Investigator and/or research nurse, will review the consent and answer questions. The participant will be informed that their participation is voluntary and they may withdraw from the study at any time, for any reason, without prejudice to their ongoing care.

15.3 Privacy and Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts will be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

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*additional relevant immune assays conducted by the PI that may be applicable but have not been described in detail in the text.

Appendix 1: Schedule of Events for Years 1 through 3

HLA Identical Sibling Renal Transplant Tolerance with Donor Hematopoietic Stem Cells and Campath-1H. Schedule of Events																					
Test	**Day**			**Week**				**Month**													
Timing of Study Participation	**00**	**0(Tx)**	**1**	**1**	**3**	**4**	**6**	**2**	**3**	**5**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**	**33**	**36**
Visit	**00**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16.1.**	**16.1.**	**16.1.**	**16.1.**
GENERAL ASSESSMENTS																					
History & Physical, Inclusion/Exclusion criteria, Informed consent	X																				
Chest X-ray and ECG	X																				
Physical Exam[!]	X					X		X	X		X	X	X	X	X		X		X		X
Vital signs[!]	X		X			X		X	X		X	X	X	X	X		X		X		X
QuantiFeron Gold	X																				
Adverse Event Assessment			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Graft Survival			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Kidney Biopsy		X											x		X[1]		X[2]				X
LABORATORY ASSESSMENTS																					
Blood type/screen	X																				
HLA Typing	X																				
PRA	X	X																			
Lymphocytoxic cross match		X																			
Pregnancy (serum b-HCG for females)	X	X																			

[!] During the drug withdrawal period, as per Sections 4.7.1 and 4.8.1, these tests are to be performed weekly and recorded both on case report forms, as well as in the patients' charts.

HLA Identical Sibling Renal Transplant Tolerance with Donor Hematopoietic Stem Cells and Campath-1H. Schedule of Events																					
Test	**Day**			**Week**				**Month**													
Timing of Study Participation	**00**	**0(Tx)**	**1**	**1**	**3**	**4**	**6**	**2**	**3**	**5**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**	**33**	**36**
Visit	**00**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16.1.**	**16.1.**	**16.1.**	**16.1.**
CBC (including differential)[!]	X		X			X		X	X		X	X	X[3]	X	X	X	X[4]	X	X	X	X
Chemistries[!]	X		X			X		X	X		X	X	X[3]	X	X	X	X[4]	X	X	X	X
Lipids (Chol, Trig, HDL, LDL)									X				x								
Urinalysis[!]				X		X			X		X		X								
Serology-CMV, EBV, Hep B, Hep C, & HIV (participant and donor)	X																				
Quantitative PCR for CMV reactivation (if needed for safety)						X		X	X		X		X								
GFR													X				X				
CD52 (T cells)	X		X[5]	X[6]		X[7]	X[7]	X[7]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]
Tacrolimus trough blood levels*			X	X	X	X	X	X	X												
Sirolimus trough blood levels**								X	X	X	X	X	X	X	X						

[!] During the drug withdrawal period, as per Sections 4.7.1 and 4.8.1, these tests are to be performed weekly and recorded both on case report forms, as well as in the patients' charts.

HLA Identical Sibling Renal Transplant Tolerance with Donor Hematopoietic Stem Cells and Campath-1H. Schedule of Events																					
Test	**Day**			**Week**				**Month**													
Timing of Study Participation	**00**	**0(Tx)**	**1**	**1**	**3**	**4**	**6**	**2**	**3**	**5**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**	**33**	**36**
Visit	**00**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16.1.**	**16.1.**	**16.1.**	**16.1.**
Donor Renal Chemistries and Immune Assays	X						X						X				X				**X**
MEDICATIONS AND DHSC INFUSIONS																					
Campath-1H		X		Day 4																	
DHSC infusion #1 (frozen)				Day 5																	
DHSC infusion #2 (fresh)								Month 2-3													
DHSC infusion #3 (frozen)***											X										
DHSC infusion #4 (frozen)***												X									
Tacrolimus			X	X	X	X	X	X?													
Sirolimus								X	X	X	X	X	X	X	X	X	X				
MMF		X	X	X	X	X	X	X	X	X	X	X	X	X	X						
Valganciclovir			X	X	X	X	X	X	X	X	X	X	X								
TMP/Sulfa			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				

[!] During the drug withdrawal period, as per Sections 4.7.1 and 4.8.1, these tests are to be performed weekly and recorded both on case report forms, as well as in the patients' charts.

HLA Identical Sibling Renal Transplant Tolerance with Donor Hematopoietic Stem Cells and Campath-1H. Schedule of Events																					
Test	**Day**			**Week**				**Month**													
Timing of Study Participation	**00**	**0(Tx)**	**1**	**1**	**3**	**4**	**6**	**2**	**3**	**5**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**	**33**	**36**
Visit	**00**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16.1.**	**16.1.**	**16.1.**	**16.1.**
Clotrimazole or oral nystatin			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
*Tacrolimus blood levels are actually to be performed weekly and recorded in the electronic medical record with each clinic visit. This will include the visits as per our standard of care until the drug is discontinued. **Sirolimus blood levels are actually to be performed weekly and recorded in the electronic medical record until month 5 and then monthly as is our standard of care until the drug is discontinued. ***Note: Infusions #3 and #4 will follow similar CRF format.																					
TOLERANCE ASSAY STUDIES (SITE PERFORMED)																					
Sequential measurement of regulatory cells and their products[11, 12, 13, 14]											X		X		X		X		X		X

[1] Two weeks prior to sirolimus withdrawal.
[2] Two weeks prior to MMF withdrawal.
[3] CBC and blood chemistries will occur weekly until sirolimus is completely withdrawn, then every other week for the first 6 weeks after complete withdrawal of sirolimus, then monthly thereafter.
[4] CBC and blood chemistries will occur weekly until MMF is completely withdrawn, then every other week for the first 6 weeks after complete withdrawal of MMF, then monthly thereafter.
[5] CD52 QD x 7 days.
[6] CD52 2x/week x 30 days.
[7] CD52 Q/week x 60 days.
[8] CD52 Q2/week x 60 days.

[!] During the drug withdrawal period, as per Sections 4.7.1 and 4.8.1, these tests are to be performed weekly and recorded both on case report forms, as well as in the patients' charts.

[11] *Ex vivo* immune assessment will occur for peripheral blood at 6, 18, 30, 36, (visits 42, and 54 months will be captured at the site level not on CRF's).

[12] *Ex vivo* immune assessment will occur for iliac crest bone marrow at 12, 24, 36, (visits 48, and 60 months will be captured at the site level not on CRF's).

[13] Protocol biopsies will be obtained 2 weeks prior to commencement of sirolimus withdrawal, at 2 weeks prior to MMF withdrawal and 6 months after completion of MMF withdrawal. "For-cause" biopsies will also be obtained in the event of a 20% increase in serum creatinine that is unexplained and unresolved within 24 hours.

[14] If the recipient demonstrates donor-specific unresponsiveness in the ELISPOT and micro-CML assays, limiting dilution analysis for the enumeration of CTLp will be performed. This also will include trans-vivo DTH reactions and specialized donor specific antibody assays.Note: All adverse events (X) are to be recorded on case report forms (clinically significant or non-significant).

[!] During the drug withdrawal period, as per Sections 4.7.1 and 4.8.1, these tests are to be performed weekly and recorded both on case report forms, as well as in the patients' charts.

APPENDIX II

16.1.6 Isolation of CD34-Positive HPC

Growth factor mobilized (G-CSF) donor peripheral blood progenitor cell (PBPC) products will be collected by apheresis. Depending upon the success of the mobilization, a second PBPC collection may be required to obtain sufficient PBPCs to perform the $CD34^+$ cell selection step to recover sufficient donor $CD34^+$ target cells.

$CD34^+$ cell selection of the mobilized PBPC collection or iliac crest bone marrow will be performed on the Miltenyi Biotec CliniMACS selection device. The CliniMACS device features an automated, computer-driven program utilizing a closed system (sterile pathway) with a sterile, disposable plastic processing set and sterile buffer solutions for ease of cell processing. The CliniMACS has been approved for clinical trials. Approved materials and reagents that will be used with the CliniMACS device are indicated in Table 1 below. This cell enrichment method results in greater than 60% $CD34^+$ cell recovery and approximately 80-90% $CD34^+$ cell purity with a $\geq$ 3 log depletion of T-cells. The PBPC products will be processed according to the Operator's Manual for the CliniMACS selection device. Refer to the standard operating procedure (SOP): CTL 2.6 HCT/P Manufacturing: CD34 Positive Cell Selection using CliniMACS. The following steps will be performed to select the $CD34^+$ cells and deplete the $CD3^+$ cells:

1. Donor PBPCs will be washed by centrifugation with incubation buffer to remove interfering platelets from the PBPC product

2. PBPCs will be incubated with the CD34 monoclonal antibody/super-paramagnetic particle conjugate to label the $CD34^+$ cells. Labeled cells will be incubated for 20 minutes at room temperature on a rocking platform to facilitate labeling of cells

3. Labeled cells will be washed twice by addition of 10-20 times labeling volume of phosphate buffered saline supplemented with 0.5% HSA and 2mM EDTA. The mixture will be centrifuged at 300 x *g* for 10 minutes at room temperature using a COBE 2991 Cell Washer. The supernatant will be removed and discarded

4. Selection of $CD34^+$ cells is accomplished by immobilizing the $CD34^+$ cell/antibody/bead mixture in a magnetic field and, washing the unbound (by-pass cells) $CD3^+$ cells, and other cellular elements, from the column containing the magnetically bound $CD34^+$ cells.

5. After the unbound $CD3^+$ T-cells and other unwanted cells have been removed, the $CD34^+$ cells will be released from the column by removing the magnetic field surrounding the column.

6. The resulting enriched $CD34^+$ cell product should contain a minimum of 1-2 x 10^6 $CD34^+$ cells/kg recipient weight with $\leq$ 5 x 10^4 $CD3^+$ T-cells/kg for bone marrow selected products, and > 2 x 10^6 $CD34^+$ cells/kg recipient weight with $\leq$ 5 x 10^4 $CD3^+$ T-cells/kg depending upon the unfractionated $CD34^+$ cell content of the HPC-A product.

7. After completion of the $CD34^+$ cell selection step, the $CD34^+$ enriched product will either be administered immediately following $CD34^+$ cell analysis or it will be cryopreserved in 10%

[!] During the drug withdrawal period, as per Sections 4.7.1 and 4.8.1, these tests are to be performed weekly and recorded both on case report forms, as well as in the patients' charts.

dimethyl sulfoxide (DMSO) and Normal Saline solution containing a minimum of 20% autologous plasma, if storage is required prior to transplantation.

8. The cryopreserved CD34-enriched HPC-A product will be stored in the vapor phase of a liquid nitrogen (LN_2) freezer until the recipient has received the preparative regimen. Following administration of the conditioning regimen, the CD34-enriched product will be rapidly thawed in a 37ºC water-bath and the cells will be infused without further manipulation according to Cell Therapy Program policies.

Table 1
Miltenyi CliniMACS Approved Materials & Reagents

Materials:	CliniMACS Tubing Set (Ref. No. 161-01), or Large Scale Tubing Set (Ref. No. 162-01)
Reagents:	CliniMACS PBS/EDTA Buffer, 1 L bags (Ref. No. 700-25) CliniMACS CD34 Reagent Kit (Ref. No. 171-01)

[!] During the drug withdrawal period, as per Sections 4.7.1 and 4.8.1, these tests are to be performed weekly and recorded both on case report forms, as well as in the patients' charts.