Immune
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Network

ACCEPTOR

Bone Marrow Transplantation and High Dose Post-Transplant Cyclophosphamide for Chimerism Induction and Renal Allograft Tolerance

Protocol ITN054ST
Version 5.0 (March 28, 2016)
IND # 118,975

This clinical study is supported and conducted by the Immune Tolerance Network, which is sponsored by the National Institute of Allergy and Infectious Diseases.

This document is confidential. It is provided for review only to Investigators, potential Investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. It is understood that the contents of this document will not be disclosed to others without written authorization from ITN and NIAID unless it is necessary to obtain informed consent from potential study participants.

Protocol Approval

Trial ID: ITN054ST	**Protocol Version: 5.0**
	Dated: March 28, 2016
IND # 118,975	**Protocol Chair:** [redacted]
Title: Bone Marrow Transplantation and High Dose Post-Transplant Cyclophosphamide for Chimerism Induction and Renal Allograft Tolerance	

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of good clinical practice (GCP) as described in the US Code of Federal Regulations (CFR) - 45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document *Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance* dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements.

As the Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the NIAID.

_____

Protocol Chair **(*Print*)**

_____ _____

Protocol Chair **(*Sign*)** **Date**

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SYNOPSIS

Title	***Bone Marrow Transplantation and High Dose Post Transplant Cyclophosphamide for Chimerism Induction and Renal Allograft Tolerance***
IND Sponsor	NIAID
Conducted by	Immune Tolerance Network
Protocol Chair	[redacted]
Co-PIs	[redacted]
Accrual Objective	The accrual goal for this study is six renal transplant recipient-donor pairs who meet the per-protocol (PP) analysis sample definition.
Study Treatment	**Recipient Induction and Maintenance Therapy** **Study Treatment for Unsensitized Recipients** For unsensitized recipients, antithymocyte globulin (ATG) will be given from Day -9 to Day -7; fludarabine from Day -4 to Day -2 and low-dose cyclophosphamide on Day -4 and Day -3. Participants will undergo hemodialysis 6 to 8 hours after each fludarabine dose. Participants will undergo total body irradiation (TBI) on Day -1. Renal transplantation followed by bone marrow infusion will occur on Day 0. High dose post-transplant cyclophosphamide (PT/Cy) will be administered on Days 3 and 4 with hydration, sodium-2-mercaptoethane sulfonate (MESNA) and anti-emetics. Filgrastim will be administered daily starting on Day 5 until absolute neutrophil count (ANC) recovery. Standard maintenance immunosuppression consisting of tacrolimus, with target trough level of 8-10 ng/ml, and mycophenolate mofetil (MMF) 15 mg/kg three times daily, and prednisone 10mg daily, will be started on Day 5. On Days -9 through -7 ATG will be administered daily at a specialized outpatient Bone Marrow Transplant (BMT) unit. On Day -4 participants will be admitted to the inpatient BMT unit for the three-day course of fludarabine to ensure precise timing of dosing and dialysis as well as monitoring of side effects. Because fludarabine is cleared by the kidneys, these end stage renal disease (ESRD) participants will require daily dialysis to avoid the potentially fatal side effect of neurotoxicity. Participants will remain hospitalized from the first day of fludarabine administration through the kidney and bone marrow transplant. Recipient hospitalization will then continue through post-operative Day 7 under the care of the surgeon and the nephrologist in keeping with the site's standard renal transplantation procedures. Recipients will then be discharged to a specialized outpatient BMT unit on Day 8 and followed per

standard of post-non-myeloablative bone marrow transplant care for 60 days post-transplant.

Study Treatment for Sensitized Recipients

Sensitized participants with detectable DSA at screening who meet inclusion criterion 5b will undergo de-sensitization per Johns Hopkins Hospital (JHH) standard of care (Appendices 8 and 9) prior to receiving the first dose of study therapy. They will receive the same induction and maintenance therapy as unsensitized recipients but with continued de-sensitization therapy and additional monitoring for recurrence of DSA. Please see Appendix 8 for the Study Regimen with De-sensitization Schedule for Sensitized Recipients and Appendix 9 for the De-sensitization Schedule of Events for Sensitized Recipients.

Donor Therapy and Procedures

Donor nephrectomy immediately followed by bone marrow harvest will take place under the same anesthetic on Day 0, with renal transplantation followed by bone marrow infusion into the recipient also on Day 0.

Immuno-suppression Withdrawal

Participants will receive standard maintenance immunosuppression for at least 26 weeks post-transplant. Those participants demonstrating no evidence of rejection will first undergo withdrawal of MMF and prednisone over a 4- to 8-week period. Withdrawal from MMF and prednisone may be initiated no earlier than Week 26 and no later than Week 34 post-transplant. After 8 weeks on stable tacrolimus monotherapy, participants demonstrating no evidence of rejection, as described in section 3.3.2, will undergo withdrawal of tacrolimus. The tacrolimus dose will be reduced in a stepwise fashion over a period of no fewer than 12 weeks and no greater than 16 weeks with the goal of complete discontinuation no later than 66 weeks post-transplant.

Participants who successfully complete immunosuppression withdrawal will then undergo 4 years of follow-up. Participants who fail to initiate or to complete immunosuppression withdrawal will undergo 2 years of safety follow-up.

Study Design

This trial is a phase II, single arm, open-label, single center pilot study to assess a reduced-intensity conditioning regimen, bone marrow transplantation and high dose PT/Cy in six recipients of renal allografts from Human Leukocyte Antigen-haploidentical (HLA-haploidentical) living related donors.

Study Duration

Total study duration will be 361 weeks (6.9 years).

Primary Objective

The primary objective of this trial is to assess the ability of bone marrow transplantation and high dose PT/Cy to induce renal allograft tolerance and thus enable discontinuation of immunosuppressive therapy in haploidentical living related donor renal transplant recipients.

Secondary Objectives

1. To assess the safety of the study regimen.
2. To assess whether maintenance of donor chimerism is required for sustained allograft tolerance.
3. To explore mechanistic assays which might predict successful immunosuppression withdrawal.

Primary Endpoint

The primary endpoint is the proportion of participants who achieve operational tolerance, defined as remaining off all immunosuppression 52 weeks after completion of immunosuppression withdrawal with:

a) no evidence of biopsy-proven allograft rejection and

b) acceptable renal function, defined as a serum creatinine that has increased no more than 25% above baseline (see section 6.5.1 for baseline thresholds) at the primary endpoint visit.

All participants who successfully complete immunosuppression withdrawal will undergo a protocol biopsy at this time point to assess the primary endpoint.

Secondary Endpoints

Safety

1. The incidence, severity and duration of graft versus host disease (GVHD) in transplanted participants.
2. The incidence and duration of engraftment syndrome in transplanted participants.
3. The proportion of transplanted participants who die.
4. The proportion of transplanted participants with acute renal allograft rejection demonstrated by a biopsy or clinically if a biopsy cannot be performed. If participant has allograft dysfunction as defined in section 6.5 and cannot undergo biopsy he or she will be presumed to have rejection without biopsy confirmation.
5. The histological severity of biopsies demonstrating acute rejection as measured by Banff Grade per Banff 2007 Classification Renal Allograft Pathology[1].
6. The proportion of transplanted participants with chronic T cell-mediated or antibody-mediated rejection. This assessment should also include progressive interstitial fibrosis/tubular atrophy (IF/TA), transplant glomerulopathy or chronic obliterative arteriopathy without an alternative, non-rejection-related cause. See Banff 2007 Classification Renal Allograft Pathology for definition of terms[1].
7. Time from transplant to the first episode of acute rejection requiring treatment.

8. The incidence, severity and duration of adverse events including infection, wound complications, post-transplant diabetes, hemorrhagic cystitis and malignancy.
9. The proportion of transplanted participants who develop donor specific antibody:
   a. after initiation of immunosuppression withdrawal
   b. at any time during trial participation
10. The time to absolute neutrophil recovery. This is defined as the interval from the neutrophil nadir to the first day of three consecutive daily neutrophil counts ≥ 500 per μL. The neutrophil nadir is defined as the first day post-transplant on which the absolute neutrophil count (ANC) is below 500 per μL.
11. The time to platelet count recovery. This is defined as the interval from transplant to the first day of a platelet count of 20,000 per μL without a prior platelet transfusion in the preceding seven days.

Efficacy

1. The proportion of transplanted participants who remain off immunosuppression for at least 52 weeks including those in whom the 52 week biopsy was not performed.
2. The proportion of participants who remain free from return to immunosuppression for the duration of the study.

Mechanistic

1. The correlation of operational tolerance with the extent and durability of donor hematopoietic and T cell chimerism as measured by serial short tandem repeat analysis of recipients' peripheral blood mononuclear cells (PBMCs) and T cells.
2. The correlation of operational tolerance with other biomarkers such as cell subsets or gene expression.

The following secondary endpoints pertaining to safety and efficacy will be assessed only in participants who complete tacrolimus withdrawal:

1. Immunosuppression-free duration, defined as time from completion of tacrolimus to end of trial participation or to time of restarting immunosuppression.
2. Time from completion of tacrolimus withdrawal to first episode of acute rejection or presumed acute rejection, defined per Banff 2007 Classification Renal Allograft Pathology[1].
3. Time from completion of tacrolimus withdrawal to first diagnosis of chronic T cell mediated or antibody-mediated rejection. This assessment should also include IF/TA, transplant glomerulopathy or chronic obliterative arteriopathy without an alternative, non-rejection

related cause. See Banff 2007 Classification Renal Allograft Pathology[1].

Inclusion Criteria

Recipient

Recipient participants must meet *all* of the following criteria to be eligible for this study:

1. Recipient of a first renal allograft from an HLA-haploidentical, living related donor. The donor and recipient must be HLA identical for at least one allele (using high resolution DNA based typing) at the following genetic loci: HLA-A, HLA-B, HLA-C and, HLA-DRB1. Fulfillment of this criterion shall be considered sufficient evidence that the donor and recipient share one HLA haplotype.
2. Age 18 to 65 years.
3. Single solid organ recipients (kidney only).
4. ABO compatibility with donor.
5. DSA will be assessed by the local laboratory 30 days or less prior to transplant using solid phase micro particle technology (by Luminex® phenotype panel or Luminex single antigen bead test.) The following criteria apply:
    a. Participants without detectable DSA will be deemed eligible if they meet other entry criteria.
    b. Participants with detectable DSA and a positive flow cytometric crossmatch may undergo de-sensitization per standard of care *if they are cytotoxic crossmatch negative*. Such participants must demonstrate a negative flow cytometric crossmatch by day -9 in order to receive the first dose of study therapy (ATG). Participants who do not demonstrate an acceptable response to de-sensitization by day -9 will be considered screen failures and will be terminated from the study.
    c. Participants with a positive cytotoxicity crossmatch will be excluded.
6. Removed in protocol version 5.0
7. Removed in protocol version 5.0
8. Normal estimated left ventricular ejection fraction and no history of ischemic heart disease requiring revascularization, unless cleared by a cardiologist.
9. Forced expiratory volume (FEV1) and forced vital capacity (FVC) > 40% of predicted at the screening visit.
10. Serological evidence of prior Epstein-Barr virus (EBV) infection as documented by positive IgG and negative IgM antibodies against EBV.

11. For women of childbearing potential, a negative serum or urine pregnancy test with sensitivity less than 50 mIU/m within 72 hours before the start of study medication.
12. Use of two forms of contraception with less than a 5% failure rate or abstinence by all transplanted participants for 18 months after the first dose of study therapy. For the first 60 days post-transplant, recipients should be encouraged to use non-hormonal contraceptives due to the potential adverse effect of hormones on bone marrow engraftment. If using a barrier method, a double barrier method should be used.
13. Ability to receive oral medication.
14. Ability to understand and provide informed consent.
15. All participants must demonstrate a negative QuantiFERON® (QFT) assay result within 52 weeks of transplant regardless of PPD status. Participants with a positive QFT assay will not be eligible for the study unless they have completed treatment for latent TB and have a negative chest x-ray. QFT testing done within 52 weeks before transplant is acceptable as long as there is documentation of the results. Prior recipients of a BCG vaccination are not exempt.

Donor

Donor participants must meet *all* of the following criteria to be eligible for this study:

1. HLA-haploidentical, first-degree relatives or half-siblings of the recipient participant at the allele or allele group. The donor and recipient must be HLA identical for at least one allele (using high resolution DNA based typing) at the following genetic loci: HLA-A, HLA-B, HLA-C, and HLA-DRB1. Fulfillment of this criterion shall be considered sufficient evidence that the donor and recipient share one HLA haplotype.
2. Age 18 to 65 years.
3. Creatinine clearance >80 ml/minute as measured from a 24 hour urine collection within 26 weeks of the screening visit. If a serum creatinine drawn at the screening visit is > 20% higher than the serum creatinine drawn at the time of the 24 hour urine collection, the creatinine clearance must be re-evaluated by a repeat 24 hour urine test. If the new value is ≤80mg/dL the donor will be excluded.
4. Meets institutional selection criteria for organ and bone marrow donation.
5. Ability to understand and provide informed consent for all study procedures including kidney transplant and bone marrow harvest.
6. Serologic evidence of prior EBV infection as documented by positive IgG and negative IgM antibodies against EBV.

Exclusion Criteria

Recipient

Recipient participants who meet *any* of the following criteria will *not* be eligible for this study:

1. Underlying renal disease with a high risk of disease recurrence in the transplanted kidney, including:
   a. focal segmental glomerulosclerosis (FSGS).
   b. type I or II membranoproliferative glomerulonephritis.
   c. hemolytic-uremic syndrome/thrombotic thrombocytopenic purpura.
2. Clinically important genital/urinary tract dysfunction.
3. Body mass index (BMI) > 40.
4. Women who are breastfeeding.
5. History of cancer within the last 5 years, except for nonmelanoma skin cancer, stage 1 renal cell carcinoma, stage 1 prostate cancers cured by local resection and any curatively treated carcinomas in situ.
6. History of positive HIV-1 or HIV-2 serologies or nucleic acid test.
7. Evidence of hepatitis B infection.
   Participants demonstrating any one of the following will be excluded:
   a. positive hepatitis B surface antigen (HBsAg) or
   b. positive anti-HBc IgM.
   c. positive anti-HBc IgG
   d. positive HBV PCR
8. Positive anti-hepatitis C (HCV) antibodies and a positive serum HCV RNA PCR. All positive HCV antibody results must be assessed by an EIA assay and confirmed by a quantitative serum HCV RNA assay. Participants with positive HCV antibodies but undetectable serum HCV RNA may be considered for eligibility. Participants with negative anti-HCV antibodies but unexplained liver enzyme abnormalities must undergo a quantitative serum RNA assay to rule out false negative HCV serologies.
9. History of active tuberculosis (TB).
10. Any active, severe local or systemic infection at the screening visit.
11. Autoimmune disease requiring immunosuppressive drugs for maintenance.
12. Use of investigational drug, other than the study medications specified by the protocol, within 30 days of transplantation.
13. Receipt of a live vaccine within 30 days of receipt of study therapy.
14. The presence of any medical condition that the Investigator deems incompatible with participation in the trial.
15. Positive cytotoxic crossmatch.
16. Calculated PRA greater than 90%.

Donor

Donor participants who meet *any* of the following criteria will *not* be eligible for this study:

1. History of type I or type II diabetes mellitus.

2. History of severe cardiovascular disease, defined as New York Heart Association Class III or IV[2].
3. History of blood product donation to recipient.
4. History of positive HIV-1 or HIV-2 serology or nucleic acid test.
5. Evidence of hepatitis B infection.
   Participants demonstrating any one of the following will be excluded:
   a. positive hepatitis B surface antigen (HBsAg) or
   b. positive anti-HBc IgM.
   c. positive anti-HBc IgG
   d. positive HBV PCR
6. Positive anti-hepatitis C (HCV) antibodies and a positive serum HCV RNA PCR. All positive HCV antibody results must be assessed by an EIA assay and confirmed by a quantitative serum HCV RNA assay. Participants with positive HCV antibodies but undetectable serum HCV RNA may be considered for eligibility. Participants with negative anti-HCV antibodies but unexplained liver enzyme abnormalities must undergo a quantitative serum RNA assay to rule out false negative HCV serologies.
7. Autoimmune disease requiring immunosuppressive drugs for maintenance.
8. The presence of any medical condition that the Investigator deems incompatible with participation in the trial.

LIST OF ABBREVIATIONS

Abs	Antibodies
AKI	acute kidney injury
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
AUC	area under the curve
BMT	bone marrow transplant
CBC	complete blood count
CFR	Code of Federal Regulations
CHF	congestive heart failure
CKBMT	combined kidney and bone marrow transplant
CLS	capillary leak syndrome
CRF	case report form
CMV	Cytomegalovirus
CMVIG	CMV intravenous immune globulin
CRO	contract research organization
CRS	cytokine release syndrome
Cy	Cyclophosphamide
DSA	donor specific antibody
DSMB	Data and Safety Monitoring Board
ESRD	end stage renal disease
FCXM	flow cytometry crossmatch
FDA	US Food and Drug Administration
FCB	for cause biopsy

FSGS	focal segmental glomerulosclerosis
GCP	good clinical practice
GI	Gastrointestinal
HSCT	hematopoietic stem cell transplantation
IARs	infusion associated reactions
IBW	ideal body weight
ICH	International Conference on Harmonization
IRB	institutional review board
IS	Immunosuppression
ISW	immunosuppression withdrawal
ITN	Immune Tolerance Network
JHU	Johns Hopkins University
LDCy	Low dose cyclophosphamide
MedDRA	Medical Dictionary for Regulatory Activities
MESNA	sodium-2-mercaptoethane sulfonate
MHC	major histocompatibility complex
MMF	mycophenolate mofetil
NCI-CTCAE v. 4.0	National Cancer Institute *Common Terminology Criteria for Adverse Events version 4.0*
PP	per protocol
PML	progressive multifocal leukoencephelopathy
PT/Cy, HDCy	post-transplant cyclophosphamide, high dose cyclophosphamide
PTLD	post-transplant lymphoproliferative disease
RB	repeat biopsy
SAE	serious adverse event
SAR	suspected adverse reaction

SIADH	syndrome of inappropriate anti-diuretic hormone
SOT	solid organ transplantation
TBI	total body irradiation
TPE	therapeutic plasma exchange
WHO	World Health Organization

1. BACKGROUND AND RATIONALE

1.1 BACKGROUND

Despite substantial progress in living donor transplantation leading to one year graft survival rates exceeding 95%, the problem of chronic rejection and the complications of long-term pharmacologic immunosuppression remain[3,4]. For these reasons, the achievement of durable transplantation tolerance, as reflected by the maintenance of stable graft function off all immunosuppressive medications, is a paramount objective. Based upon early observations that natural[5] or induced [6,7] hematopoietic chimeras are tolerant of solid organ allografts from the same donor[8], there has been substantial interest in combining solid organ and bone marrow or hematopoietic stem cell transplantation (HSCT) from the same donor to achieve donor hematopoietic chimerism and durable tolerance of the solid organ. Indeed, case reports have shown that patients who develop kidney or liver failure after undergoing allogeneic stem cell transplantation for a hematologic disorder can be successfully transplanted from the same donor and weaned off immunosuppression without experiencing graft rejection[9]. However, the morbidity and mortality of lethal conditioning and allogeneic HSCT have precluded its use specifically for the induction of chimerism and solid organ allograft tolerance.

Study investigators at Johns Hopkins have recently developed non-myeloablative conditioning regimens that permit the sustained engraftment of partially HLA-mismatched (HLA-haploidentical) bone marrow from first-degree relatives with minimal toxicities. To mitigate the risks of graft rejection and graft-versus-host disease (GVHD), the regimen includes the IV administration of high dose post-transplantation cyclophosphamide (PT/Cy) on days 3 and 4 after transplantation to selectively deplete or inactivate proliferating alloreactive cells while permitting immune reconstitution from cyclophosphamide-resistant, non-alloreactive lymphocytes present in the donor graft. They have used this regimen extensively in patients with hematological malignancies and have piloted a similar regimen in sickle cell and thalassemia patients as described in section 1.2.2.

Cyclophosphamide-induced transplantation tolerance: Mechanisms of action

High-dose cyclophosphamide, when administered in a narrow window after transplantation, depletes alloreactive T cells from the donor and host and can inhibit both GVHD and graft rejection[10-13]. As a form of drug-induced immunologic tolerance[14], the strategy of giving high-dose cyclophosphamide after transplantation takes advantage of the heightened cytotoxic sensitivity of proliferating, alloreactive T cells over non-alloreactive, resting T cells to being killed by a DNA-damaging agent[12,15]. Importantly, hematopoietic stem cells are relatively quiescent[16] and express high levels of aldehyde dehydrogenase, which likely confer cellular resistance to cyclophosphamide[17]. These findings explain how high dose PT/Cy can be used to achieve selective depletion of alloreactive T cells without substantially delaying hematologic recovery in the short or long term after transplantation.

Prior experience with combined bone marrow and solid organ transplantation

There has been previous clinical experience with both sequential and combined bone marrow transplant and solid organ transplant (SOT). Several investigators have reported results with combined bone marrow or hematopoietic stem cell and solid organ transplantation from the same donor. A 1998 review of the published literature[18] identified four patients who were treated with allogeneic bone marrow transplantation for a hematologic disorder, developed end-stage renal disease (ESRD), and then received a kidney allograft from the original stem cell donor[19-21]. At the time of reporting, there were no episodes of kidney failure or rejection and all patients were off all immunosuppressive drugs with no evidence of recurrent hematologic disorder or renal dysfunction. These isolated case reports provided the "proof of

principle" that durable donor hematopoietic chimerism permits the safe discontinuation of pharmacologic immunosuppression following transplantation of a kidney from the same donor.

However the considerable toxicities of bone marrow transplant have militated against its use solely as an adjunct to SOT to achieve immunosuppression-free tolerance. Spitzer and colleagues performed combined kidney and bone marrow transplants for seven patients with multiple myeloma and myeloma-related ESRD[22-24]. Interestingly, three patients developed operational tolerance of the transplanted organ and were successfully weaned off all immunosuppression even though donor hematopoietic chimerism was transient. In a subsequent trial sponsored by the ITN, the group at Massachusetts General Hospital treated five ESRD patients with non-myeloablative conditioning and combined kidney and bone marrow transplantation from HLA-haploidentical related donors (ClinicalTrials.gov Identifier: NCT00801632)[25]. Although donor chimerism was lost in all five patients within the first 21 days after combined transplantation, four were successfully weaned off immunosuppression.

These investigators have recently updated the outcomes of these five patients plus an additional five patients receiving treatment in a similar transplantation protocol[26]. Nine of the ten patients developed acute kidney injury (AKI), with or without fever and fluid retention, as part of an idiopathic capillary leak syndrome (CLS) that is similar clinically to engraftment syndrome seen after autologous or allogeneic HSCT[27]. Altogether, seven patients were successfully weaned off immunosuppression with stable renal function. However one successfully weaned patient subsequently was returned to immunosuppression for recurrence of his underlying disease, IgA nephropathy. A second successfully weaned participant was restarted on MMF after 6 years off immunosuppression for chronic humoral rejection, with persistent DSA and c4d+ positive staining on biopsy. Early c4d negative transplant glomerulopathy and low level DSA have been detected in a third patient who has remained off immunosuppression for 6.8 years. Thus as of early 2013, 5 of 10 patients remain off immunosuppression. Two patients have lost their grafts (one due to idiopathic CLS and AKI), and a third had to be placed back on immunosuppression due to an episode of acute rejection following immunosuppression withdrawal[26].

High Dose Post Transplant Cyclophosphamide in Combined Kidney and Hematopoietic Stem cell Transplantation

Investigators at Northwestern have reported successful induction of durable hematopoietic chimerism in highly HLA-mismatched, living donor kidney transplant patients using combined donor hematopoietic stem cell and kidney transplant. The conditioning regimen described: 3 doses of fludarabine (30mg/kg), 200cGy total body irradiation, pre-transplant cyclophosphamide and HSCs, followed by high dose cyclophosphamide 50mg/kg on day 3 and maintenance therapy with MMF and tacrolimus, differs from this trial's planned regimen mainly in the co-administration of proprietary, tolerogenic 'facilitator cells' with the hematopoietic stem cell graft. These investigators initially reported durable, multi-lineage hematopoietic chimerism in 5 of 8 study participants. These five chimeric patients demonstrated immunocompetence and donor-specific hypo-responsiveness and were successfully withdrawn from all immunosuppression 1 year after transplantation without allograft rejection[28]. One participant achieved 100% donor chimerism at month 2 but developed viral sepsis, renal artery thrombosis and subsequent renal allograft loss. Two participants developed transient chimerism and were maintained on immunosuppression. One participant experienced sub-clinical Banff IA rejection and was returned to immunosuppression. Staining for c4d was negative. None of the participants produced DSA, experienced GVHD or engraftment syndrome. The study regimen was well-tolerated, with outpatient management after post-operative day 2[28].

These investigators recently reported long-term follow-up in a total of 15 HLA mismatched kidney recipients who underwent combined hematopoietic stem cell and renal transplant under the regimen described above. Ten of these 15 patients have achieved durable full or mixed hematopoietic chimerism

without GVHD or engraftment syndrome. Eight of these 10 achieved durable, high level (>90%) hematopoietic chimerism. Six of the 8 have successfully completed immunosuppression withdrawn without allograft rejection or graft loss (range of between 10 and 22 months off IS). The two remaining patients with high level chimerism are currently undergoing immunosuppression withdrawal. Two subjects achieved sustained, mixed chimerism. Three participants achieved transient chimerism. Of interest, 2 of these transiently chimeric recipients subsequently experienced subclinical rejection (Banff IA) during immunosuppression withdrawal despite evidence of donor-specific hyporesponsiveness by MLR and CML. None of the participants have produced DSA. The incidence of serious infections has been low. No CMV infections or opportunistic fungal infections have been reported[29]. The ITN 054ST trial does not include use of 'facilitator cells'. Nevertheless, given the similarities in study regimens, these results provide an encouraging backdrop for the current protocol with regard to efficacy and safety.

In summary operational tolerance can be achieved across an HLA barrier following combined, kidney and bone marrow transplantation. Preliminary results suggest that durable, high level donor chimerism may be associated with tolerance of the kidney allograft, though longer term follow-up is required to confirm this finding. This goal must be balanced against the toxicities of bone marrow transplantation, including the risk of GVHD. Operational tolerance is also achievable in the setting of transient, mixed chimerism. However, a subset of patients experienced acute kidney injury, acute rejection and ultimately, graft loss. Thus it remains unclear whether hematopoietic chimerism is required to achieve tolerance and whether the extent or durability of chimerism impacts the likelihood of reaching this objective.

1.2 PRECLINICAL AND CLINICAL EXPERIENCE

1.2.1 Preclinical Studies

For over a decade, the Johns Hopkins investigators have been developing minimally toxic regimens for crossing the HLA barrier in HSCT by incorporating the method of cyclophosphamide-induced immunologic tolerance.

Initial pre-clinical efforts were devoted to using PT/Cy to overcome the HLA barrier in HSCT. A non-myeloablative regimen comprised of pre-transplantation fludarabine, an immunosuppressive purine analog, and 200 cGy total body irradiation and PT/Cy was developed in a mouse model of MHC-mismatched BMT. This regimen was sufficient to induce stable engraftment of donor cells in 100% of transplanted mice. The conditioning regimen did not ablate recipient hematopoiesis, since autologous hematopoietic recovery occurred in mice that received the conditioning and high dose PT/Cy but did not receive an allogeneic bone marrow infusion. Furthermore, high dose PT/Cy given on day 2 post-transplantation mitigated both the incidence and severity of acute GVHD in MHC mismatched donor-recipient pairs[11]. These results provided the pre-clinical rationale to translate this regimen to the clinic for patients with advanced hematologic malignancies[30,31].

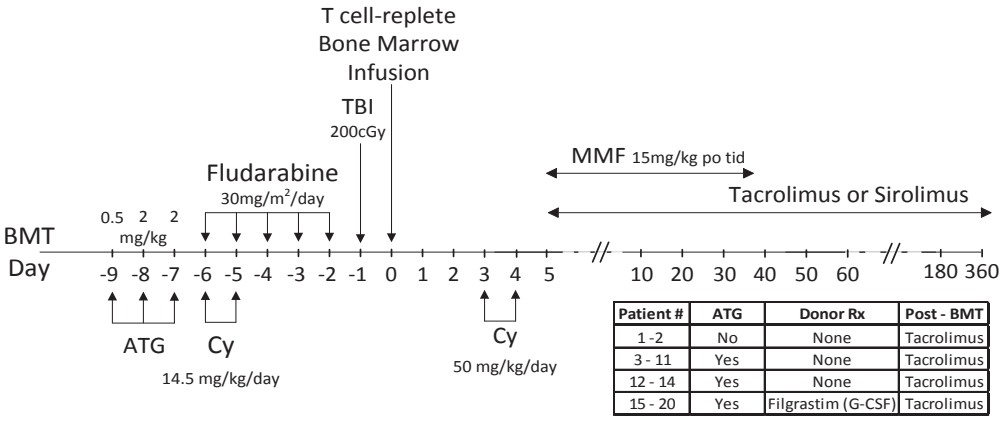
1.2.2 Clinical Experience

The study investigators' extensive experience in performing HLA-haploidentical bone marrow transplantation incorporating high dose PT/Cy is summarized below.

The Hopkins group has now treated over 400 patients with non-myeloablative, HLA-haploidentical bone marrow transplantation and high dose PT/Cy and has recently completed a 16-center phase 2 trial of this approach through the NHLBI-sponsored Blood and Marrow Transplant Clinical Trials Network. Patients (n=50) had either high-risk acute leukemia in remission or relapsed lymphoma and lacked a suitably HLA-matched related or unrelated donor. Of note, 49 out of 50 patients (98%) had stable engraftment of donor cells and there was only one case of primary graft failure. The cumulative incidence of grades II-IV

acute GVHD was 32%, but there were no cases of severe (grades III-IV) GVHD, a 13% cumulative incidence of chronic GVHD, and no GVHD-related mortality. Non-relapse mortality (NRM) at one year after transplantation was 7%, which is comparable to or lower than the incidence of NRM after reduced intensity BMT from HLA-matched donors[32]. These data suggest that high dose PT/Cy achieves selective depletion of alloreactive T cells in vivo, resulting in acceptably low morbidity and mortality in older adults with advanced hematologic malignancies. Further, Johns Hopkins' single center results demonstrating the low toxicity of this platform, including the tolerability of high dose PT/Cy, were easily replicated by multiple centers without a need for a "learning curve."

In light of the low toxicity of HLA-haploidentical transplantation for patients with hematologic malignancies, the Hopkins investigators have opened a protocol of reduced intensity transplantation from HLA-matched or haploidentical first-degree relatives for patients with sickle cell anemia or beta thalassemia and have reported preliminary results[33]. The treatment regimen, illustrated below in Figure 1, is nearly identical to the planned regimen for this study, ITN054ST, with 3 exceptions: a) participants 12 through 26 received sirolimus instead of tacrolimus due to concerns about tacrolimus-associated posterior reversible encephalopathy (PRES) and b) participants 15 through 26 received bone marrow from filgrastim (G-CSF)-treated donors and c) the number of fludarabine doses is being reduced from five to three. Three days of fludarabine dosing is selected because there are no clear data favoring one duration over the other for non-myeloablative conditioning in general; concern exists regarding the potential for neurotoxicity in ESRD patients on dialysis; and recent data from the Northwestern trial show good engraftment results using only three days of fludarabine.



Patient #	ATG	Donor Rx	Post - BMT
1 -2	No	None	Tacrolimus
3 - 11	Yes	None	Tacrolimus
12 - 14	Yes	None	Tacrolimus
15 - 20	Yes	Filgrastim (G-CSF)	Tacrolimus

Figure 1 Schema for reduced intensity allogeneic BMT for hemoglobinopathy

Since initial results were reported in October 2012 seven additional patients have been enrolled in the sickle cell trial for a total of 26 participants. Detailed clinical outcomes for 26 hemoglobinopathy patients transplanted as of May 2014 are provided in the table below:

Table 1. Outcomes of reduced intensity allogeneic BMT for hemoglobinopathy

Pt #	Age at BMT	Hb type	HLA mismatch GVH/HVG	Days to PMNs> 500 µl	Donor G-CSF stim	GVHD	Complications	Off IS	Chimerism Whole Blood/Tcell (BMT day)	Last % HbA (BMT day)	Last Hct
1	33	SC	5/5	14	No	No		Yes	100/ND (739)	52 (245)	38
2	20	SS	5/5	14	No	No	PRES	Yes	0/ND (80)	44 (1579)	18
3	31	SS	4/0	14	No	No	PRES	Yes	100/100 (1083)	56 (795)	42
4	33	SS	Matched	12	No	No		No	85/70 (1601)	53.9 (1601)	42
5	21	SS	0/5	31	No	No		No	0/0 (1019)	44 (755)	33
6	27	SS	2/5	15	No	No	Dental abscess (d-3)	Yes	92/100 (732)	93 (948)	40
7	31	SC	5/4	27	No	No		Yes	100/ND (1483)	55 (1483)	36
8	16	SS	3/3	25	No	No		Yes	0/0 (49)	84 (480)	23
9	18	SS	4/3	23	No	No	CMV reactivation	No	8/13 (1362)	24 (1133)	25
10	25	SS	5/0	17	No	No	PRES	Yes	100/100 (1082)	55 (1082)	33
11	46	SS a-thal	Matched	29	No	Transient Acute Gr I skin		No	89/49 (397)	64 (397)	37
12	42	SS	4/3	41	No	No		Yes	0/0 (367)	92 (1232)	29
13	31	SS	Matched	30	No	No		No	48/40 (1201)	51 (1237)	37
14	28	SS	3/4	29	No	No	CMV reactivation, RSV, TB	Yes	0/0 (68)	95 (61)	30
15	20	SS	5/3	28	Yes	No		No	0/0 (157)	55 (784)	24
16	21	SS	5/4	21	Yes	No		Yes	100/100 (728)	100 (364)	32
17	15	SS	3/3	35	Yes	No	EBV pneumoniits	Yes	100/100 (758)	55 (366)	35
18	26	SS	ND	25	Yes	No		No	43/13 (608)	88 (608)	34
19	26	SS	ND	19	Yes	Mild chronic skin	EBV reactivation	Yes	>95/100 (459)	57 (368)	45
20	38	SS	ND	30	Yes	No	Shingles	No	33/57 (531)	50 (531)	28
21	8	SS	ND	24	Yes	No		No	49/36 (263)	56 (172)	33
22	14	SS	ND	23	Yes	No		No	31/19 (315)	28 (315)	26
23	28	SS	ND	23	Yes	Ext chronic (skin and lung)	DEATH	No	100/100 (181)	75 (71)	25
24	35	SS	ND	28	Yes	No		No	0/0 (194)	91 (56)	26
25	20	SS	ND	21	Yes	Acute Gr III GI		No	100/93 (238)	92 (238)	35
26	12	SS	ND	19	Yes	No		No	0/0 (61)	37 (103)	25

Abbreviations

BMT	Bone marrow transplant	EBV	Epstein-barr virus
Hb	hemoglobin	GVH	graft-versus-host direction
HVG	host-versus-graft direction	PMN	polymorphonuclear leukocyte
GVHD	graft-versus-host disease	IS	immunosuppressive
Hct	hematocrit	PRES	posterior reversible encephalopathy syndrome
CMV	cytomegalovirus	RSV	respiratory syncytial virus
pulm TB	pulmonary tuberculosis	ND	Not done

** patients 4, 11 and 13 are HLA-matched sibling transplants. All other donor-recipient pairs are HLA haploidentical.

Ten recipients of HLA-haploidentical bone marrow (Table 1, patients 1, 3, 6, 7, 10, 16, 17, 19, 23 and 25, in yellow) achieved full or near full donor chimerism. Of these ten, 8 are off immunosuppression. The remaining 2 patients developed GVHD (details below). Nine recipients of HLA-haploidentical bone marrow (patients 2, 5, 8, 9, (blue) 12, 14, 15, 24 and 26, in red) have experienced graft failure, one of whom (patient 12) was re-transplanted unsuccessfully from the same donor. These 9 patients have all experienced return of autologous hematopoiesis. Seven recipients (patients 4, 11, 13, 18, 20, 21 and 22, in green) demonstrate stable mixed hematopoietic chimerism. These 7 patients remain free of sickle cell disease but remain on continued immunosuppression per the choice of the treating physician. Patient 9 (in blue) has stable low level donor chimerism (~5%) over 2 years post-transplantation. She remains on immunosuppression and continues to experience sickle cell crises.

With regard to PRES, three cases occurred in the initial cohort (patients 2,3 and 10) but no further incidents of PRES have occurred after sirolimus was substituted for tacrolimus.

With regard to infection, 3 patients (9, 19 and 19) experienced CMV reactivation but not CMV disease. Patient 17 developed EBV pneumonitis. Patient 14 developed RSV and was later found to have *Mycobacterium tuberculosis* on bronchoscopy. Patient 20 developed shingles. Patient 19 experienced EBV viremia that responded to a single dose of rituximab.

Among 26 patients transplanted, there have been up to four incidents of GVHD:

- Patient 11 developed a transient skin rash localized to the forehead which resolved spontaneously and was considered to be questionable Grade 1 cutaneous acute GVHD.
- Patient 19 developed a mild, chronic GVHD skin rash, responsive to treatment.
- Patient 23 developed extensive, severe chronic skin and pulmonary GVHD and ultimately died.
- Patient 25 developed acute Grade III GVHD of the gastrointestinal tract, ongoing.

The first 14 patients received bone marrow from untreated donors whereas the last 12 patients received bone marrow from donors treated with filgrastim for 5 days preceding bone marrow collection. Of the 14 initial patients, five achieved full donor hematopoietic chimerism and one case of GVHD was reported. Of the 12 patients who received marrow from filgrastim-stimulated donors, five patients achieved full donor chimerism but three cases of GVHD were reported, including one case of severe GVHD with multi-organ involvement and death. The single reported case of GVHD in the recipients of untreated marrow occurred in a patient who developed a limited facial rash that was not biopsied and resolved without treatment. This patient receieved a bone marrow graft from an HLA-identical sibling. Though the numbers are small it is possible that filgrastim treatment of donors may be associated with an increased risk of GVHD.

All of the patients treated on this trial had a history of receiving multiple transfusions of red blood cells and were therefore highly alloimmunized. It is therefore notable that durable hematopoietic chimerism has been achieved in roughly 40 percent of evaluable patients. PRES (Posterior Reversible Encephalopathy Syndrome) was seen much more frequently than has been the case in hematologic malignancies patients, likely because of prior neurologic injury associated with sickle cell disease[33].

Figure 2 shows percent donor chimerism early after transplantation among patients who eventually lost their bone marrow grafts.

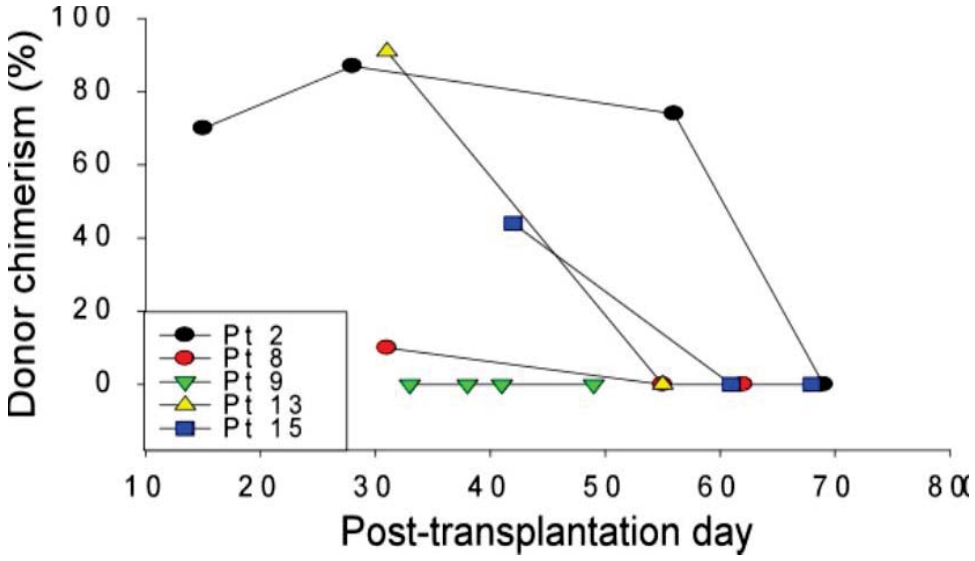
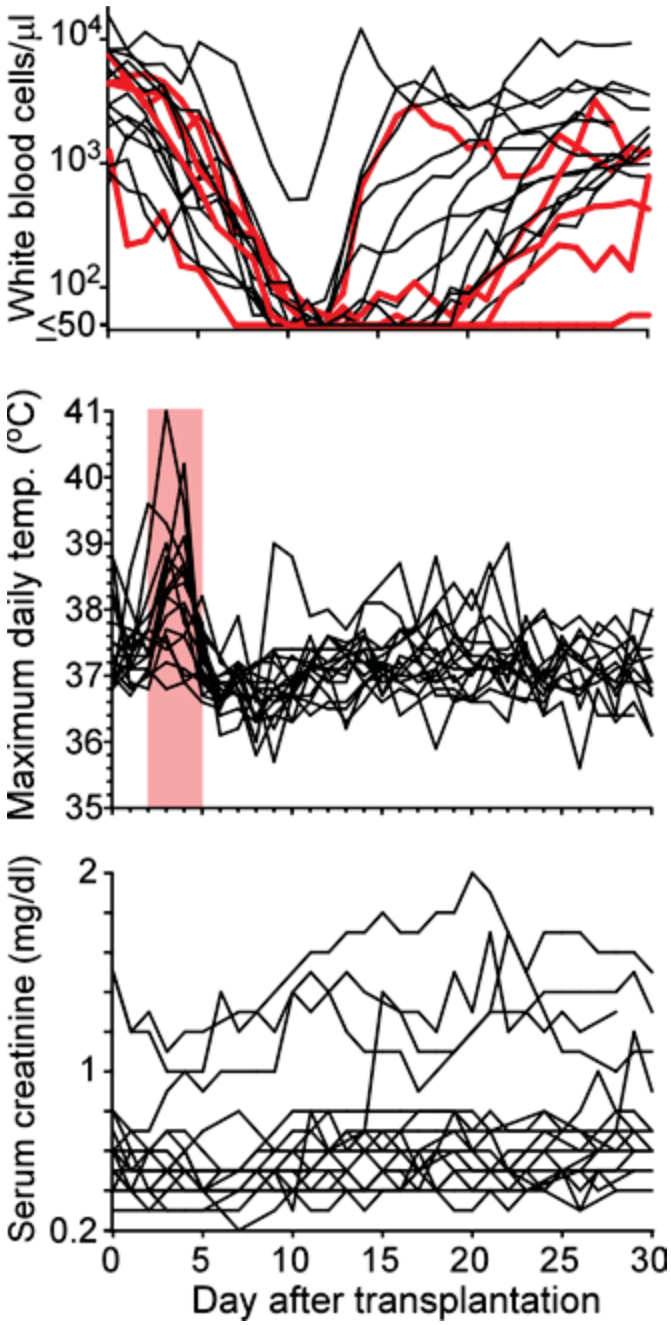


Figure 2: Donor chimerism early after transplantation among patients who experienced bone marrow graft loss

Four of five of these patients still had detectable chimerism by day 30 after transplantation. It will be of interest to determine whether transient hematopoietic chimerism will result in durable renal allograft tolerance or whether sustained chimerism is necessary. The rate of durable hematopoietic chimerism in renal recipients treated with this regimen should be similar or greater to what we observed in the hemoglobinopathy patients, in view of their prior extensive blood product sensitization.

PT/Cy abrogates engraftment syndrome

Figure 3 shows daily white blood cell (WBC) counts, maximum temperatures, and serum creatinines in the first 30 days after transplantation. Interestingly, all but six patients developed fevers>38.0˚C between days 2-4 after transplantation (middle panel, shaded bar), and all fevers resolved within 24 hours following the last dose of PT/Cy. Interestingly, fever did not recur (middle panel) and kidney functioned remained stable (lower panel) during WBC recovery (upper panel), and serum creatinine never exceeded 2.0 mg/dl. These data suggest that PT/Cy may limit or abrogate engraftment syndrome by depleting alloreactive T cells early after transplantation[34].



Red lines indicate patients who experienced graft failure.

Figure 3: Daily WBC counts, maximum temperatures, and serum creatinines in patients undergoing reduced intensity conditioning allogeneic stem cell transplant for hemoglobinopathy

Toxicity of high-dose cyclophosphamide

The feasibility of high-dose cyclophosphamide is based on extensive experience with this approach in a variety of clinical settings, including combined bone marrow and renal

transplant as described above[28]. High-dose cyclophosphamide produces a hypocellular marrow with killing of the differentiated progeny of hematopoietic stem cells (HSCs), but the HSCs themselves survive by virtue of high levels of the enzyme aldehyde dehydrogenase, which detoxifies the active metabolite of cyclophosphamide[13,17,35,36]. High-dose cyclophosphamide can therefore be expected to eliminate mature elements of the immune system, while allowing full bone marrow recovery from the surviving stem cell pool.

Both hypotheses have been substantiated in several human clinical trials conducted at Johns Hopkins University (JHU). Durable remission has been achieved in a high proportion of patients with auto-immune diseases, including aplastic anemia, systemic lupus erythematous, and myasthenia gravis[37-40]. These results were achieved with acceptable toxicity, largely limited to a period of myelosuppression, even in patients with significant pre-existing infectious problems or other co-morbidities. Further favorable toxicity data and additional evidence for the recovery of blood cells after high-dose cyclophosphamide are evident from the results of a recent study of 81 patients in which high-dose cyclophosphamide, without stem cell rescue, was used for the treatment of low-grade and mantle cell non-Hodgkin's lymphoma.[41] (This approach administers significant dose intensification while obviating the problem of disease re-infusion that may result from autologous stem cell rescue). All patients experienced full hematopoietic recovery, with a median time to neutrophil count $\geq$ 500/μL of 15 days. There were no deaths. Treatment was given in an outpatient setting, with fewer than half of the patients ever hospitalized, and when so, primarily for neutropenic fever, which developed in one third. All patients had received several cycles of chemotherapy immediately prior to high-dose cyclophosphamide, and there was no age limit to enrollment. All patients were supported with granulocyte colony stimulating factor (G-CSF), blood product support when needed, antibiotic prophylaxis, effective preventive anti-emetics, and urothelial protection with MESNA.

The Northwestern regimen containing high dose PT/Cy was generally well tolerated in a similar donor-recipient population, with median ANC recovery of 10 days (range 2 to 14) and outpatient management on post-operative day 2 for most patients. One participant developed viral sepsis and hypotension requiring hospitalization and intubation and one participant experienced VZV reactivation. Additional significant infections include histoplasmosis in one subject, BK viremia without nephropathy and 3 episodes of single dermatome herpes zoster[29].

The use of high dose PT/Cy may present specific problems in patients with ESRD in the immediate post-transplant period. Although early renal function is likely after living-related donor transplantation, delayed graft function or renal dysfunction associated with engraftment syndrome is not an obstacle to the use of high-dose cyclophosphamide. The drug is inactive until it has been metabolized by hepatic microsomal enzymes, forming the major intermediates aldophosphamide and 4-hydroxycyclophosphamide. Approximately 15% of the drug is excreted unchanged in the urine, and a variety of inactive metabolites are also excreted through the urine. A large proportion of a cyclophosphamide dose is eliminated by hepatic metabolism. The clinical toxicity of cyclophosphamide, even at high doses, appears to be independent of renal function[42,43]. The available literature is limited[44,45]. The half-life in hours of cyclophosphamide is slightly prolonged in patients with renal failure compared to those with normal renal function (10 versus 7.5 hours). Cyclophosphamide has a protein binding percentage of 14% and a volume of distribution of 0.5-1.0 L/Kg, making it suitable for removal by dialysis if necessary. Recommendations exist for dose reduction of cyclophosphamide by 25% for patients on hemodialysis[46,47]. However, a detailed study of the pharmacokinetics of high-dose cyclophosphamide was performed at Johns Hopkins in one

patient with ESRD and compared to a patient with normal renal function[42,48-51]. The metabolism and AUC achieved in both patients were similar. In the ESRD patient, cyclophosphamide was completely metabolized by the liver and no dose adjustment was required. Therefore additional toxicity or significant problems with the dosing of cyclophosphamide is not anticipated, even in patients with minimal or no renal function and who continue to require hemodialysis in the immediate post-transplant period. It is possible that wound healing could be adversely affected by high-dose cyclophosphamide. This has not been the case among post-transplant FSGS patients at Hopkins treated chronically with cyclophosphamide. Also, because the graft is placed in the retroperitoneum through a small incision, wound problems tend to have less dire consequences, rarely result in an open abdomen, and are quite amenable to wound VAC coverage or local tissue transfer, if needed.

1.3 SUMMARY OF KNOWN AND POTENTIAL RISKS FOR HUMAN PARTICIPANTS

1.3.1 Risks Associated with Study Procedures for the Recipient

1.3.1.1 Bone Marrow Transfusion

The donor bone marrow will be transfused into the recipient through an intravenous catheter in the chest or neck. The risks of transfusion include chills, fever, headache, nausea, vomiting and dyspnea. These side effects can be ameliorated by administration of antihistamine and analgesic pre-medications.

1.3.1.2 Total Body Irradiation

Recipients will receive a single dose of 200 cGY total body irradiation on Day -1. This is considered a low dose of radiation and thus is not usually associated with side effects common at higher doses such as nausea and vomiting, alopecia and mouth sores. Low dose total body irradiation is associated with minimal to no short term side effects with the exception of possible edema in the radiation field and transient cytopenias. Long term side effects of total body irradiation include infertility. In addition there is an increased risk of lung fibrosis, pericarditis, thyroid disease, and cataracts. The additional risk of secondary malignancy due to TBI is detailed below.

1.3.1.2.1 Secondary malignancy as a long-term risk of TBI-containing conditioning.

Data regarding subsequent malignancies after TBI-containing conditioning regimens are almost all in the setting of malignant disease and reflect the overall risk attributable to the cumulative chemotherapy and radiation therapy used to treat the malignancy, as well as any contribution from the conditioning regimen.

A retrospective study of 1626 pediatric patients treated with autologous bone marrow transplantation showed a secondary malignancy cumulative incidence of 2.6% at 10 years, with no significant difference between TBI-containing and non-TBI containing conditioning regimens[52]. Leukemia is very uncommon after allogeneic transplantation, and most of the malignancies have been solid tumors or EBV-related lymphoproliferative disorders. A study of 2062 patients who had undergone allogeneic hematopoietic stem cell transplantation in Japan showed a cumulative incidence of solid tumors of 2.4% at 10 years. There was no

difference between TBI-containing and non-TBI containing regimens[53]. A study of 1036 long-term bone marrow transplant survivors conducted by the European Cooperative Group for Blood and Marrow Transplantation, Late Effects Project Group, found a cumulative second malignancy incidence of 3.5% at 10 years, with no difference between TBI-containing and non-TBI containing regimens. Increasing donor or recipient age, a female donor, and the use of immunosuppressive drugs to manage graft versus host disease were all identified as factors related to increased risk[54]. A study of 926 patients who had undergone allogeneic hematopoietic stem cell transplantation in British Columbia found a 10 year cumulative incidence of secondary malignancy of 2.3%, all solid tumors. Basal cell skin cancer and carcinoma-in-situ were excluded. The relative risk compared to the age-matched general population was 1.85. Approximately half the patients had received TBI, dosed at < 1200 cGy. No difference in secondary malignancy incidence was evident between the group that had and the group that had not received TBI[55]. Earlier studies have related the risk of secondary malignancies to TBI dose, particularly to doses >1000 cGy, more than 5 times the low-dose TBI (200 cGy) used in this protocol[54,56,57].

In summary the short term risk of TBI are less commonly seen with the relatively low dose of radiation used in this regimen. The risk of secondary malignancy is approximately 2.5% at 10 years in participants who undergo hematopoietic stem cell transplantation, but does not appear increased by TBI. Other long term risks of TBI such as infertility, pulmonary fibrosis etc. have been described; these risks are above and beyond the risks associated with standard renal transplant.

1.3.1.3 Renal Biopsies

Renal biopsies will be obtained in the event of suspected allograft dysfunction. In addition, surveillance protocol renal biopsies will be performed at pre-specified time points to monitor the status of the graft, including before initiation of immunosuppression withdrawal, to exclude the presence of subclinical rejection. These biopsies will also be used for performing mechanistic evaluations.

Obtaining biopsy samples using ultrasound guidance and automated needles had an incidence of complications requiring intervention of 0.2% (3/1302) while minor complications (i.e., self-limited hematuria, small hematoma, non-significant arteriovenous fistula) occurred in 6.8% (89/1302) of patients in two series comprised of adults and children. There were no allograft losses or patient deaths.

Significant bleeding following renal biopsy may require blood component therapy for participants with significant anemia, coagulopathy, or thrombocytopenia.

1.3.1.4 Risk Associated with Immunosuppression Withdrawal

Inherent in the withdrawal of immunosuppression is the increased risk of precipitating graft rejection. This risk will be mitigated by the study design, which includes careful patient selection and close follow-up during and after drug withdrawal. Rejection may occur with a higher frequency or with greater severity than it does with conventional immunosuppression.

The protocol provides for frequent clinical evaluation of participants during the period of active administration of this novel immunosuppressive combination and for continued follow-up monitoring through approximately 5 years after enrollment.

1.3.2 Risks Associated with Study Procedures for the Donor

1.3.2.1 Bone Marrow Harvest

Bone marrow harvest will occur under local or general anesthesia. Bone marrow stem cells will be obtained by using a large needle to draw cells from the bone marrow in the iliac crests, the hip bone. The risks associated with bone marrow harvest include pain and bleeding at the harvest site, adverse reactions to anesthesia and infection. Rarely, mechanical injury to the underlying bone, nerves, or soft tissue can occur. Factors associated with more complications are use of regional anesthesia, longer duration of harvesting, female gender and older age.

1.3.3 Risks Associated with Study Medication for the Recipient

1.3.3.1 Fludarabine

Clinical toxicities of fludarabine monophosphate include: myelosuppression, primarily lymphopenia and granulocytopenia, alopecia, rash, dermatitis, nausea, vomiting, anorexia, mucositis, stomatitis, diarrhea, somnolence, fatigue, peripheral neuropathy, mental status changes, cortical blindness, hepatocellular toxicity with elevation in serum transaminases, and interstitial pneumonitis. These effects may be reversible when the drug is discontinued. The use of fludarabine has been associated with an increased risk of secondary malignancy as described in section 1.3.3.

For additional information on the risk associated with fludarabine, refer to the fludarabine drug label at the following website:
http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=6f1094e3-4c28-40ae-a82f-37cdfa865f49

1.3.3.2 Cyclophosphamide

The clinical toxicities associated with cyclophosphamide are more severe at higher doses such as those used in this study regimen. The most common side effects of high dose cyclophosphamide include nausea and vomiting, transient alopecia and neutropenic fever. Treatment is associated with leukopenia, including neutropenia, which can be prolonged at high doses, resulting in increased rates of infection. Anemia and thrombocytopenia have been reported but are less common. Cyclophosphamide is associated with headaches and dizziness, elevated liver function tests, hemorrhagic cystitis, cardiotoxicity and syndrome of inappropriate anti-diuretic hormone (SIADH). Long term risks include infertility, malignancy and interstitial pulmonary fibrosis. Cyclophosphamide may interfere with normal wound healing. Cyclophosphamide can cause fetal harm when administered to pregnant women and has been associated with birth abnormalities.

For additional information on the risk associated with cyclophosphamide, refer to the cyclophosphamide drug label at the following website:
http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=591d9955-3d9c-4cdc-a308-2f1288376b9f

1.3.3.3 Antithymocyte Globulin

Risks associated with rabbit antithymocyte globulin (ATG) (Thymoglobulin®, Sanofi) include immune-mediated reactions, infections, reactivation of infection, malaise, dizziness, sepsis,

thrombocytopenia, leukopenia, and increased incidence of malignancies. Occasional reactions are observed at the infusion site including pain, swelling and erythema.

Immune-mediated reactions

Serious immune-mediated reactions have been reported with the use of thymoglobulin and consist of anaphylaxis or severe cytokine release syndrome (CRS). Fatal anaphylaxis has been reported. Severe, acute infusion-associated reactions (IARs) are consistent with CRS which is attributed to the release of cytokines by activated monocytes and lymphocytes. Severe acute CRS can cause serious cardiorespiratory events and/or death.

Infection

ATG is routinely used in combination with other immunosuppressive agents. Infections (bacterial, fungal, viral and protozoan), reactivation of infection (particularly cytomegalovirus (CMV)), and sepsis have been reported after ATG administration in combination with multiple immunosuppressive agents. Severe acute infections can be fatal.

Malignancy

Use of immunosuppressive agents, including ATG, may increase the incidence of malignancies, including lymphoma or post-transplant lymphoproliferative disease (PTLD).

Carcinogenesis, Mutagenesis, Impairment of Fertility

The carcinogenic and mutagenic potential of thymoglobulin and its potential to impair fertility have not been studied.

In 82 kidney-transplant recipients receiving ATG 1.5 mg/kg/day for 7–14 days, the principal adverse events were fever (52%) and chills (47%) associated with the infusions, leucopenia (47%) and thrombocytopenia (30%). CMV infection occurred in 13% of the recipients and PTLD in 2% of the recipients. Neutropenia has been described; anaphylaxis has been reported rarely.

ATG is contraindicated in patients with a history of allergy or anaphylaxis to rabbit proteins or to any product excipients, or who have active acute or chronic infections that contraindicate any additional immunosuppression.

For additional information on the risk associated with rabbit antithymocyte globulin, refer to the rabbit antithymocyte globulin drug label at the following website:

http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=bbd8ab99-552e-4b81-aca4-6b0c7af8b9ae

1.3.3.4 Filgrastim (G-CSF)

The most common side effects of filgrastim injection are bone pain, muscle pain and headaches. These symptoms can usually be relieved by non-aspirin analgesics such as acetaminophen. Nausea, fatigue and low grade fevers have been reported. Some patients experience swelling, erythema or itching at the injection site. Rare but serious adverse events associated with filgrastim include splenic rupture and acute respiratory syndrome, both associated with the rapid proliferation of neutrophils. Filgrastim has not been studied in pregnant women and its effect on the developing fetus is unknown.

For further information on the risks associated with filgrastim, please refer to filgrastim drug label at the following website: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=3bc802bd-76b4-4f45-8571-a436ec26228e

1.3.3.5 Mycophenolate Mofetil/Mycophenolic Acid

The following risks are associated with the use of mycophenolate mofetil (MMF). The risks associated with mycophenolic acid are identical; therefore only those associated with MMF are listed below:

- Increased risk of developing lymphomas, lymphoproliferative diseases and other malignancies especially of the skin in patients receiving MMF as part of an immunosuppressive regimen. The risk appears to be related to the intensity and duration of immunosuppression rather than to the use of any specific agent.
- Increased susceptibility to infection, including opportunistic infections, fatal infections and sepsis.
- Increased risk of first trimester pregnancy loss and congenital malformations, especially of the external ear, face, limbs, heart esophagus and kidneys.
- Increased risk of neutropenia and leukopenia.
- Increased risk of pure red cell aplasia
- Increased risk of gastrointestinal bleeding.
- Immunosuppressed patients are at increased risk for opportunistic infections, including activation of latent viral infections. These include cases of progressive multifocal leukoencephalopathy (PML) and BK virus-associated nephropathy which have been observed in patients receiving immunosuppressants, including mycophenolate mofetil.

Common side effects associated with use of MMF include diarrhea, abdominal pain, nausea and vomiting, headache and drug induced fever.

For further information about the risks of MMF, please refer to the MMF drug label at the following website: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=209efe01-7908-46e4-a964-0665078c0acd#druglabelcontent.

1.3.3.6 Prednisone

Side effects of prednisone include convulsions, headache, vertigo, mood swings, psychosis, congestive heart failure, hypertension, salt and water retention, increased potassium excretion, Cushing syndrome, menstrual irregularities, hyperglycemia, GI irritation, peptic ulcer, weight gain. Dermatologic effects may include thin skin, petechiae, ecchymosis, facial erythema, poor wound healing, hirsutism and urticaria. Muscle weakness, loss of muscle mass and osteoporosis may also occur. Ophthalmologic complications may include increased intraocular pressure, glaucoma, exophthalmos and cataracts. Other complications may include immunosuppression and increased susceptibility to infection.

For further information about the risks associated with prednisone, please refer to prednisone drug label at the following website: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=3115aef0-fd50-4ec8-a064-3effb695f3f2#druglabelcontent .

1.3.3.7 Tacrolimus

The following risks are associated with the administration of tacrolimus:

- Hypertension.
- Hyperkalemia.
- Nephrotoxicity.
- Neurotoxicity.
- Post-transplant insulin-independent diabetes mellitus.
- Myocardial hypertrophy (in most cases reversible upon dose reduction).
- Increased risk of renal insufficiency in patients with hepatic impairment.
- Increased susceptibility to infection.

Posterior reversible encephalopathy syndrome (PRES)[58] when used in the setting of bone marrow or solid organ transplantation.For further information on the risks of tacrolimus, please refer to the tacrolimus drug label at the following website: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=bd447ffa-9196-4c3c-accf-5adf29b84665#druglabelcontent

1.3.3.8 Sirolimus

The following risks are associated with use of sirolimus:

- Delayed or poor wound healing
- cytopenias
- Lymphocoele
- Peripheral edema
- Angioedema
- Hyperlipidemia,
- Proteinuria
- Mouth ulcers
- Severe gastrointestinal adverse effects
- Interstitial pneumonitis
- Increased susceptibility to infection

- Increased risk of lymphoma

For additional information about the risks of sirolimus, refer to the Rapamune® package insert at the following website: http://labeling.pfizer.com/showlabeling.aspx?id=139.

1.3.4 Risks Associated with Study Regimen for the Recipient

This section describes risks to the recipients associated with the combined use of study drugs and study procedures specified in this trial. These risks extend beyond the risks associated with standard kidney transplantation.

1.3.4.1 Neutropenic Fever

The median time to neutrophil recovery (ANC> 500 cells/uL) was 24 days (range 0 to 35 days) in 17 patients with sickle cell disease who received this study regimen[33]. Recipients may experience fevers during this period of neutrophil nadir and recovery. These episodes will be managed with supportive care and may require hospitalization of the recipient.

1.3.4.2 Infection

Recipients will be at increased risk of infection due to immunosuppression associated with the study regimen and TBI. Patients will receive appropriate viral, fungal and PCP prophylaxis as described in section 5.6. Asymptomatic viral reactivation (CMV, EBV) without active disease has been reported in sickle cell and malignancy patients treated with this regimen[30,32]. One case of *M. tuberculosis* reactivation was observed in the sickle cell cohort. Invasive *Aspergillus* infections occurred in 5 of 68 (7%) malignancy patients treated with this regimen, including 2 infections resulting in death[31].

1.3.4.3 Graft versus Host Disease

Study recipients may be at increased risk of both acute and chronic GVHD as a result of undergoing the conditioning regimen and bone marrow transplantation. However the exact risk of GVHD in this study population is unclear. A lower rate of GVHD (4 cases in 26 patients, 15%) was observed in the sickle cell patients who received this regimen compared to the rate of GVHD in patients with hematological malignancies (27%). Among 26 sickle cell patients:

- One patient developed a transient skin rash localized to the forehead which resolved without treatment and was considered to be questionable Grade 1 cutaneous acute GVHD.
- One patient developed a mild, chronic GVHD skin rash, responsive to treatment.
- One patient developed extensive, severe chronic GVHD involving the skin and lungs, and ultimately died.
- One patient developed Grade III acute GVHD involving the gastrointestinal tract.

In contrast, the incidence of acute GVHD in 210 patients with hematological malignancies treated with this regimen was 27%, with a 5 % incidence of severe (Grade III-IV) GVHD. The incidence of chronic GVHD in these patients was 13%[59]. The first renal transplant recipient in the ITN054ST trial experienced a grade 2 GVHD limited to the skin and responsive to treatment. We hypothesize that the incidence of GVHD in renal transplant recipients who are

chemotherapy-naïve will be closer to the low incidence in sickle cell patients than the malignancy patients, but this is unknown.

1.3.4.4 Carcinogenicity

The conditioning regimen, consisting of fludarabine, cyclophosphamide, and total body irradiation (TBI) has the potential for causing malignancies at a later date, including hematologic malignancies such as myelodysplastic syndrome and leukemia, and also solid tumors. Standard renal transplantation and the immunosuppressive drugs typically used, including antithymocyte globulin (ATG) , tacrolimus, and mycophenolate (MMF) also carries the risk of subsequent malignancies, including lymphomas, Kaposi's sarcoma, and squamous carcinomas.

The largest study of malignancies following allogeneic stem cell transplantation to date, involving 28,874 patients[60] found that patients developed new solid cancers at about twice the frequency expected in the general population. The risk was three-fold at 15 years or more following transplantation. Analysis of risk following TBI showed a strong relationship with age, with patients $\geq$ 30 years showing little elevation in risk (RR 1.08), but patients <30 years showing a 9 to 10 fold increase in risk. A separate study of secondary malignancies occurring after fludarabine-based non-myeloablative conditioning[61] showed a 10 year cumulative incidence of 5.6%, twice the rate expected in a normal population, with a related 10 year mortality rate of 2.4%.

Standard renal transplantation and immunosuppression to prevent rejection has been associated with an age-related increased risk in malignancy compared to the general population of 15-20 times greater risk in the pediatric population, 1.8-5 times greater risk in patients 35 to 65 years old, and 2 times the risk in patients over the age of 65[62].

1.3.4.5 Reproductive Risk

Fertility

The effect of the non-myeloablative conditioning regimen and post-transplant high-dose cyclophosphamide on subsequent male and female fertility is not well defined. It is clear that fertility can be preserved after such therapy[63], but the rate of infertility is not clearly determined. Female infertility after high dose cyclophosphamide alone appears to be related to age, with younger patients being more likely to preserve fertility. Male patients considering this protocol will be advised to bank sperm; female patients will be offered consultation in the fertility clinic to explore possible interventions that may reduce the risk of infertility.

Teratogenicity

Cyclophosphamide, TBI and MMF all have the potential for fetal toxicity. Effective contraception is required for both male and female participants in the trial.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective of this trial is to assess the ability of bone marrow transplantation and high dose PT/Cy to induce renal allograft tolerance and thus enable discontinuation of immunosuppressive therapy in haploidentical living related donor renal transplant recipients.

2.2 SECONDARY OBJECTIVES

1. To assess the safety of the study regimen.
2. To assess whether maintenance of donor chimerism is required for sustained allograft tolerance.
3. To explore mechanistic assays which might predict successful immunosuppression withdrawal.

3. STUDY DESIGN

3.1 DESCRIPTION

This trial is a phase II, single arm, open-label, single center pilot study to assess a reduced-intensity conditioning regimen, bone marrow transplantation and high dose PT/Cy in six recipients of renal allografts from HLA-haploidentical living related donors. The proposed trial design is depicted in Figure 4**.**

The proposed treatment regimen is described in detail in section 1.1 and is depicted in Figure 5: Study regimen for unsensitized recipients. Briefly, it consists of a non-myeloablative pre-transplant conditioning regimen followed by bone marrow and kidney transplantation as described in section 3.2.1 and Figure 5: Study regimen for unsensitized recipients. Recipients will receive high dose PT/Cy and maintenance immunosuppression. This will be followed by immunosuppression withdrawal at six months in eligible participants with completion of withdrawal at 12 months post-transplant. The primary endpoint is assessed at 52 weeks after completion of immunosuppression withdrawal.

3.1.1 Amendment to Allow Sensitized Participants

3.1.1.1 Rationale

Version 5.0 of the protocol allows the inclusion of sensitized recipients of a first renal transplant (see section 4). The rationale for this change was as follows: 1) Broadening the entry criteria may improve recruitment. 2) Allowing sensitized patients will improve the equipoise of the study. Such sensitized patients have less favorable outcomes with standard transplantation and therefore would benefit more from undergoing the study regimen than an unsensitized patient. 3) Inclusion of such patients does not affect the safety of the study regimen.[64] 4) The site (JHH) has extensive experience with de-sensitization protocols and has demonstrated that it can be performed safely in selected candidates.[65]

3.1.1.2 Study Flow for Sensitized Participants

Sensitized participants will undergo screening per protocol. Once it is confirmed that they have met all entry criteria (section 4) these participants will undergo de-sensitization per standard of care at JHH (see Appendix 8. Study Regimen with De-sensitization Schedule for Sensitized Recipients and Appendix 9. De-sensitization Schedule of Events per JHH Standard of Care). Adequacy of the response to de-sensitization will be assessed prior to the first dose of study therapy (ATG). Participants who fail to achieve an adequate response to de-sensitization, defined as a negative flow cytometric crossmatch by day -9, will be considered screen failures and will be terminated from the study. There is no upper limit on screen

failures. The Johns Hopkins Hospital (JHH) standard-of-care de-sensitization algorithms are described in Appendices 8-9.

3.1.2 Accrual

The accrual goal for this study is six renal transplant recipient-donor pairs who fulfill the per protocol (PP) analysis sample definition (see section 9.1). Enrollment is defined as the signing of informed consent. Enrolled participants who do not fulfill eligibility criteria will be considered screen failures and will be terminated from the study. There is no upper limit on screen failures. A sufficient number of recipient-donor pairs will be enrolled in order to achieve the accrual goal.

Enrolled participants who receive any form of study therapy but who do not fulfill the PP analysis sample definition will remain in the study and will undergo safety follow-up per Appendix 5. Safety follow-up: Recipients. These participants will be included in the safety analysis but will not count towards the accrual goal. A minimum of 60 days must elapse between transplantation of each subsequent participant.

3.1.3 Informed Consent for Participation

Informed consent will be obtained from each study participant at two points during the study:

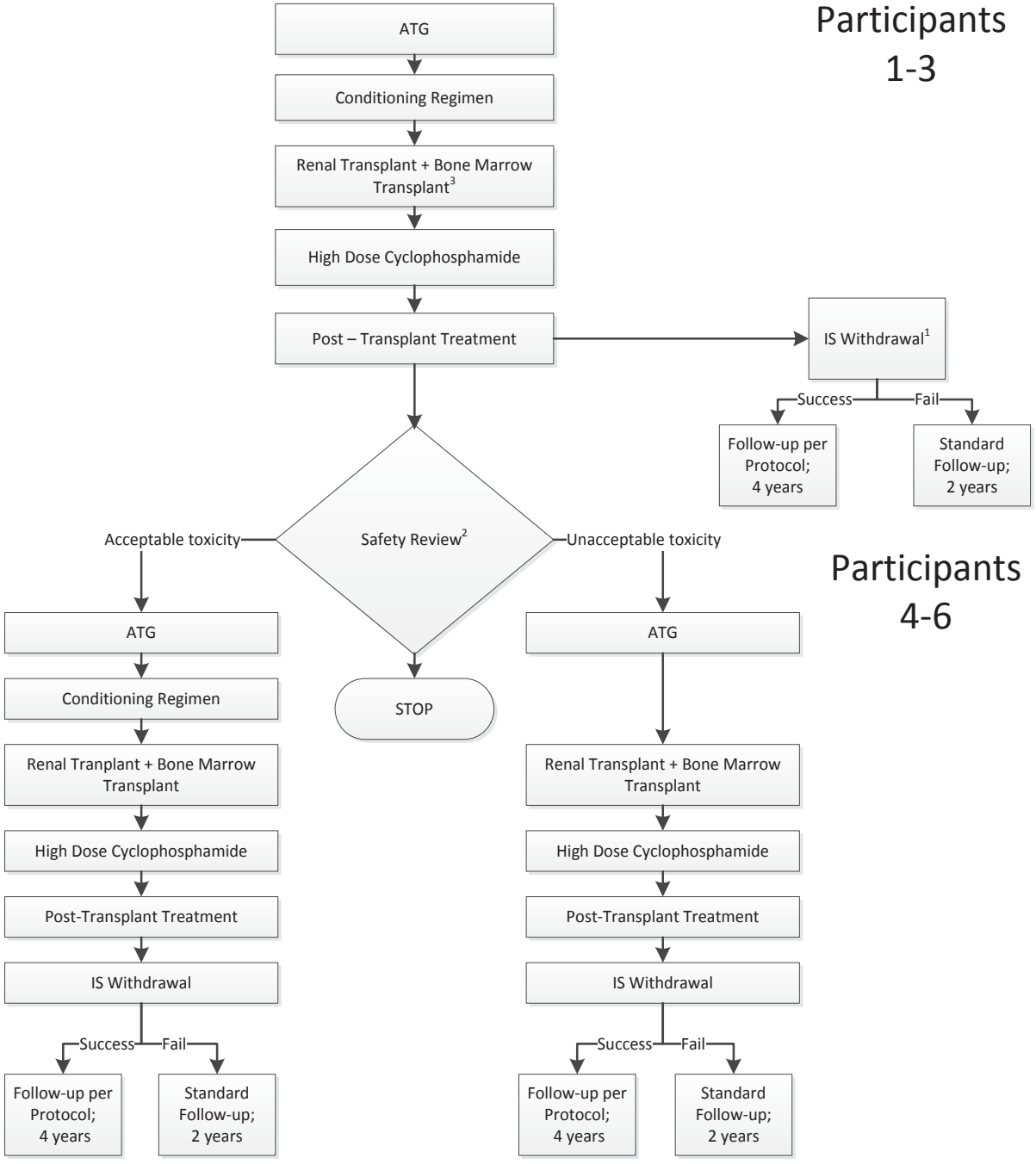
- Study enrollment: A consent addressing all aspects of the study will be obtained before any protocol-directed procedures are performed.
- Before initiation of tacarolimus withdrawal: Study participants will reaffirm consent prior to withdrawal of tacrolimus.

Informed consent will also be obtained from the donor and will be completed prior to performance of donor screening procedures.

3.1.4 Interim Safety Review

Accrual of six participants will occur in two stages with a pre-specified interim safety analysis. Treatment will consist of the regimen described in section 3.2 for at least the first three participants. An interim safety review will be performed after the third participant reaches three months post-transplant and the first and second participants are at least six months post-transplant. Immunosuppression withdrawal may proceed in eligible participants before completion of the safety review. Further enrollment will halt until the safety review is completed. The decision to proceed with further accrual will be evaluated by the NIAID Transplant Data and Safety Monitoring Board (DSMB). The acceptability of the regimen will be evaluated based on the stopping guidelines listed in section 3.6 as well as the DSMB's discretion.

If one or more stopping guideline is met or the DSMB deems that the toxicity of the regimen is unacceptable, enrollment of the second cohort of three participants will proceed but the pre-transplant conditioning regimen consisting of fludarabine, low-dose cyclophosphamide and total body irradiation will be omitted. This decision would also be strongly bolstered by the observation of allograft tolerance after transient chimerism in the initial patient group. Otherwise the accrual of the next three participants will resume.



1. IS withdrawal may proceed while the Safety Review is ongoing.
2. The Safety review will occur after 3rd participant completes 3 months and 1st and 2nd participants are at least 6 months post-transplant.
3. A minimum of 60 days must elapse between transplantation of each subsequent participant.

Figure 4: Schematic of trial design with decision points for safety review

3.2 STUDY REGIMEN

3.2.1 Recipient Induction and Maintenance Therapy

3.2.1.1 Unsensitized Recipients

The treatment regimen for unsensitized participants is outlined in Figure 5**.** Antithymocyte globulin (ATG) will be given from Day -9 to Day -7; fludarabine from Days -4 to Day -2 and low-dose cyclophosphamide on Day -4 and Day -3. Participants will undergo hemodialysis 6 to 8 hours after each fludarabine dose. Participants will undergo TBI on Day -1. Renal transplantation followed by bone marrow infusion will occur on Day 0. High dose PT/Cy will be administered on Days 3 and 4 with hydration, MESNA and anti-emetics. Filgrastim will be administered daily starting on Day 5 until absolute neutrophil count (ANC) recovery. Standard maintenance immunosuppression consisting of tacrolimus, with target trough level of 8-10 ng/ml, and MMF 15 mg/kg three times daily and prednisone 10mg daily will be started on Day 5.

On Days -9 through -7 ATG will be administered daily at a specialized outpatient BMT unit. On Day -4 participants will be admitted to the inpatient BMT unit for the three-day course of fludarabine to ensure precise timing of dosing and dialysis as well as monitoring of side effects. Because fludarabine is cleared by the kidneys, these ESRD participants will require daily dialysis to avoid the potentially fatal side effect of neurotoxicity. Participants will remain hospitalized from the first day of fludarabine administration through the kidney and bone marrow transplant. Recipient hospitalization will then continue through post-operative Day 7 under the care of the surgeon and the nephrologist in keeping with the site's standard renal transplantation procedures. Recipients will then be discharged to a specialized outpatient BMT unit on Day 8 and followed per standard of post-non-myeloablative bone marrow transplant care for 60 days post-transplant.

3.2.1.2 Sensitized Recipients

Sensitized participants with detectable DSA at screening who meet inclusion criterion 5b will undergo de-sensitization per JHH standard of care (Appendices 8 and 9) prior to receiving the first dose of study therapy. They will receive the same induction and maintenance therapy as unsensitized recipients but with continued de-sensitization therapy and additional monitoring for recurrence of DSA. Please see Appendix 8 for the Study Regimen with De-sensitization Schedule for Sensitized Recipients and Appendix 9 for the De-sensitization Schedule of Events for Sensitized Recipients.

3.2.1.3 Reinitiating conditioning regimen

If the recipient receives any part of the conditioning regimen but is unable to undergo combined kidney and bone marrow transplant (CKBMT) due to unanticipated issues with the tissue and organ harvest from the donor, he/she will be allowed the option of undergoing a second course of conditioning in order to proceed with the combined bone marrow and kidney transplant (CKBMT) per protocol. The recipient may undergo CKBMT using the same donor or a different donor. Please see Appendices 1C, 1D and 10 for further details.

Recipients may reinitiate the conditioning regimen no more than once. Recipients who are unable to undergo CKBMT after reinitiating conditioning will move to Safety Follow up (Appendix 5).

3.2.2 Donor Therapy and Procedures

Donor nephrectomy immediately followed by bone marrow harvest will take place under the same anesthetic on Day 0, with renal transplantation followed by bone marrow infusion into the recipient also on Day 0.

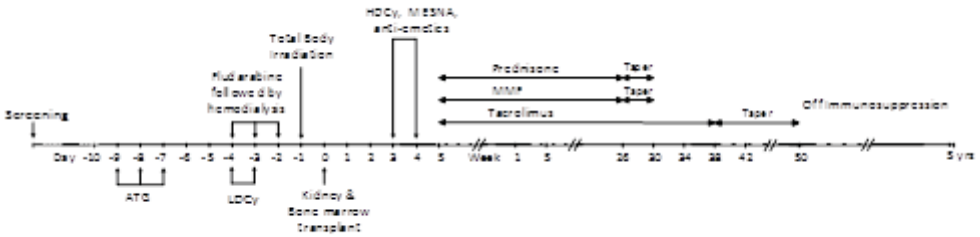


Figure 5: Study regimen for unsensitized recipients

3.3 IMMUNOSUPPRESSION WITHDRAWAL

Participants will receive standard maintenance immunosuppression for at least 26 weeks post-transplant. Those participants demonstrating no evidence of rejection will first undergo withdrawal of MMF and prednisone over a 4- to 8-week period. Withdrawal from MMF and prednisone may be initiated no earlier than Week 26 and no later than Week 34 post-transplant. After 8 weeks on stable tacrolimus monotherapy, participants demonstrating no evidence of rejection, as described in section 3.3.2, will undergo withdrawal of tacrolimus. The tacrolimus dose will be reduced in a stepwise fashion over a period of no fewer than 12 weeks and no greater than 16 weeks with the goal of complete discontinuation no later than 66 weeks post-transplant.

Participants who successfully complete immunosuppression withdrawal will then undergo 4 years of follow-up. Participants who fail to initiate or to complete immunosuppression withdrawal will undergo 2 years of safety follow-up.

3.3.1 Withdrawal of MMF and Prednisone

3.3.1.1 Eligibility

After at least 22 weeks on tacrolimus, MMF and prednisone triple therapy, participants may be evaluated for eligibility for withdrawal of MMF and prednisone. MMF and prednisone withdrawal may be initiated as early as week 26 in eligible participants. To be eligible for MMF and prednisone withdrawal participants must meet the following criteria:

1. A renal biopsy that demonstrates the absence of rejection of any type including acute humoral rejection, acute cellular rejection and chronic rejection as defined in section 6.5.4.1 within the 4 weeks prior to initiation of withdrawal.
2. No history of biopsy-proven rejection or clinically presumed rejection since transplantation.
3. No history of biopsy-confirmed indeterminate rejection (less than Banff Grade IA) on the withdrawal eligibility biopsy that has not resolved, as assessed by repeat biopsy performed within 1 month of diagnosis.
4. A history of stable renal function. Stable renal function is defined as no unexplained increases in serum creatinine of greater than 25% relative to baseline for a 72 hour time interval. Baseline is defined as the mean of the three lowest creatinines during Weeks 2 to 4 post-transplant, excluding days on dialysis.
5. An estimated GFR > 40 mL/min/1.73m^2 as calculated by CKD-EPI equation.
6. Must demonstrate absence of donor-specific antibody (DSA) as determined by solid phase micro particle technology (by Luminex® phenotype panel or Luminex single antigen bead test) performed within 4 weeks prior to initiating withdrawal; as assessed by local laboratories.
7. No evidence of active GVHD at the time of initiation of withdrawal.

3.3.1.2 Initiation and Completion of Withdrawal of MMF and Prednisone

MMF and prednisone withdrawal must be initiated between Week 26 and Week 34 post-transplant (see Appendix 3. Immunosuppression Withdrawal (Initiated Between 26 and 3 Weeks Post-Transplant)).

MMF and prednisone withdrawal will occur simultaneously according to a standard algorithm. Withdrawal must occur over a minimum of 4 weeks and a maximum of 8 weeks, with scheduled dose reductions every 2 weeks (QOW) as follows:

- The daily dose of prednisone will be reduced by 2.5 mg every 2 weeks until off.
- The daily dose of MMF will be reduced by 500 mg every 2 weeks until off. This dose reduction will be achieved by a 250 mg reduction per BID dose**.** Similarly, the daily dose of mycophenolic acid will be reduced by 320 mg every 2 weeks until off. This dose reduction will be achieved by a 180 mg reduction per BID dose.
- No more than one drug should be completely discontinued on the same day.

3.3.1.3 Failure to Start or to Complete Withdrawal of MMF and Prednisone

Participants who do not start MMF and prednisone withdrawal by the end of week 34 post-transplant will continue on study medication. At Week 34, these participants will move to Appendix 5 and complete all safety follow-up visits per the schedule of events.

Participants who experience rejection as described in section 6.5 will be ineligible for continuation of MMF and prednisone withdrawal. These participants will convert to site-specific immunosuppression and will undergo safety follow-up per Appendix 5.

Participants who attempt, but fail to complete MMF and prednisone withdrawal for any reason, including rejection, will convert to site-specific immunosuppression and will complete all safety follow-up visits per the schedule of events in Appendix 5.

Participants who successfully complete immunosuppression withdrawal but subsequently return to maintenance immunosuppression for any reason (i.e., rejection, recurrent disease) will move to Appendix 5 and complete all safety follow-up visits per the schedule of events.

Withdrawal from MMF and prednisone may not be reattempted.

3.3.2 Withdrawal of Tacrolimus

3.3.2.1 Eligibility

After at least 34 weeks on tacrolimus therapy including at least 4 weeks on tacrolimus monotherapy, participants will be evaluated for eligibility for tacrolimus withdrawal. Withdrawal from tacrolimus may be initiated no earlier than week 38 and no later than week 50 post-transplant. To be eligible for withdrawal of tacrolimus, participants must meet the following criteria:

1. A minimum of 34 weeks on tacrolimus therapy at the time of the eligibility evaluation.
2. A renal biopsy that demonstrates the absence of rejection of any type, including acute humoral rejection, acute cellular rejection, and chronic rejection as defined in section 6.5.4.1, within the 4 weeks prior to initiation of withdrawal.
3. No history of biopsy-proven rejection or clinically presumed acute rejection without biopsy confirmation since transplantation.
4. No history of biopsy-confirmed indeterminate rejection (less than Banff Grade IA) on the withdrawal eligibility biopsy that has not resolved, as assessed by RB performed within 4 weeks of diagnosis.
5. History of stable renal function. Stable renal function is defined as no unexplained increases in serum creatinine of greater than 25% relative to baseline sustained over a 72-hour time interval. Baseline is defined as the mean of the three lowest creatinines during Weeks 2 to 4 post-transplant, excluding days on dialysis.
6. An estimated GFR > 40 mL/min/1.73m^2 as calculated by CK-EPI equation.
7. Eligible participants must demonstrate absence of donor-specific antibody as determined by solid phase micro particle technology (by Luminex® phenotype panel or Luminex single antigen bead test) performed within 4 weeks prior to initiating withdrawal, as assessed by local laboratories.
8. No evidence of active GVHD at the time of initiation of withdrawal.
9. Participant informed consent for withdrawal of tacrolimus.

3.3.2.2 Initiation and Completion of Tacrolimus Withdrawal

Tacrolimus will be withdrawn in a stepwise fashion at an approximate rate of 25% of the pre-withdrawal dose every 3 weeks. The dose of tacrolimus may not be reduced by more than 50% of the starting dose per dose reduction. Participants will be allowed a window of an additional 1 week per 3-week interval before proceeding to the next dose reduction, especially if increases in creatinine are observed.

- **Initiation of tacrolimus withdrawal is defined as** the day of first dose reduction made with intent to withdraw.
- **Completion of tacrolimus withdrawal is defined as** the first day on which tacrolimus is intentionally no longer administered after initiation of withdrawal.

Tacrolimus withdrawal:

- May not be initiated earlier than Week 38 or later than Week 50 post-transplant.
- Must be completed in no fewer than 12 weeks and no more than 16 weeks after initiation.
- Will be discontinued if the participant experiences acute rejection as defined in section 6.5.4.

May not be reattempted if discontinuation of withdrawal was due to renal allograft rejection. All participants who meet the eligibility criteria for tacrolimus withdrawal will undergo study visits as specified in Appendix 2.

3.3.2.3 Failure to Start or to Complete Tacrolimus Withdrawal

Participants who do not attempt to start tacrolimus withdrawal by Week 50 post-transplant will continue on study medication and will complete all study visits for safety follow-up per the schedule of events in Appendix 5.

Participants who experience rejection will be ineligible for continuation of tacrolimus withdrawal. They will convert to site-specific immunosuppression and will undergo safety follow-up per Appendix 5.

Participants who attempt but fail to complete tacrolimus withdrawal for any reason including rejection will convert to site-specific immunosuppression and will complete all study visits for safety follow-up per the schedule of events in Appendix 5.

3.4 STUDY DURATION

Total study duration will be approximately 361 weeks (6.9 years).

- The enrollment phase will be 79 weeks (1.5 years) and includes enrollment activity of 71 weeks plus an allowance for the safety review between the enrollment of the third and fourth participants of as much as eight weeks.
- The duration of the study for an individual participant may range from 271 to 383 weeks depending on the length of time required for successful completion of immunosuppression withdrawal and includes 216 weeks of post-withdrawal follow-up.

- The study primary endpoint, measured 52 weeks after the last participant's completion of immunosuppression withdrawal, could be achieved as early as 181 weeks (3.5 years), or as late as 197 weeks (3.8 years) after enrollment of the first participant.

3.5 STUDY ENDPOINTS

3.5.1 Primary Endpoint

The primary endpoint is the proportion of participants who achieve operational tolerance, defined as remaining off all immunosuppression 52 weeks after completion of immunosuppression withdrawal with:

a) no evidence of biopsy-proven allograft rejection and
b) acceptable renal function, defined as a serum creatinine that has increased no more than 25% above baseline (see section 6.5.1 for baseline thresholds) at the primary endpoint visit.

All participants who successfully complete immunosuppression withdrawal will undergo a protocol biopsy at this time point to assess the primary endpoint.

3.5.2 Secondary Endpoints

Safety

1. The incidence, severity and duration of GVHD in transplanted participants.
2. The incidence and duration of engraftment syndrome in transplanted participants.
3. The proportion of transplanted participants who die.
4. The proportion of transplanted participants with acute renal allograft rejection demonstrated by a biopsy or clinically if a biopsy cannot be performed. If participant has allograft dysfunction as defined in section 6.5 and cannot undergo biopsy he or she will be presumed to have rejection without biopsy confirmation.
5. The histological severity of biopsies demonstrating acute rejection as measured by Banff Grade per Banff 2007 Classification Renal Allograft Pathology[1].
6. The proportion of transplanted participants with chronic T cell-mediated or antibody-mediated rejection. This assessment should also include progressive interstitial fibrosis/tubular atrophy (IF/TA), transplant glomerulopathy or chronic obliterative arteriopathy without an alternative, non-rejection-related cause. See Banff 2007 Classification Renal Allograft Pathology for definition of terms[1].
7. Time from transplant to the first episode of acute rejection requiring treatment.
8. The incidence, severity and duration of adverse events including infection, wound complications, post-transplant diabetes, hemorrhagic cystitis and malignancy.
9. The proportion of transplanted participants who develop donor specific antibody:
    a. after initiation of immunosuppression withdrawal
    b. at any time during trial participation

10. The time to absolute neutrophil recovery. This is defined as the interval from the neutrophil nadir to the first day of three consecutive daily neutrophil counts ≥ 500 per μL. The neutrophil nadir is defined as the first day post-transplant on which the absolute neutrophil count (ANC) is below 500 per μL.
11. The time to platelet count recovery. This is defined as the interval from transplant to the first day of a platelet count of 20,000 per μLwithout a prior platelet transfusion in the preceding seven days.

Efficacy

1. The proportion of transplanted participants who remain off immunosuppression for at least 52 weeks including those in whom the 52 week biopsy was not performed.
2. The proportion of participants who remain free from return to immunosuppression for the duration of the study.

Mechanistic

1. The correlation of operational tolerance with the extent and durability of donor hematopoietic and T cell chimerism as measured by serial short tandem repeat analysis of recipient peripheral blood mononuclear cells (PBMCs) and T cells.
2. The correlation of operational tolerance with other biomarkers such as cell subsets or gene expression.

The following secondary endpoints pertaining to safety and efficacy will be assessed only in participants who complete tacrolimus withdrawal:

1. Immunosuppression-free duration, defined as time from completion of tacrolimus to end of trial participation or to time of restarting immunosuppression.
2. Time from completion of tacrolimus withdrawal to first episode of acute rejection or presumed acute rejection, defined per Banff 2007 Classification Renal Allograft Pathology[1].
3. Time from completion of tacrolimus withdrawal to first diagnosis of chronic T cell mediated or antibody-mediated rejection. This assessment should also include progressive interstitial fibrosis/tubular atrophy (IF/TA), transplant glomerulopathy or chronic obliterative arteriopathy without an alternative, non-rejection related cause. See Banff 2007 Classification Renal Allograft Pathology[1].

3.6 STOPPING GUIDELINES

If *any one* of the criteria listed below is met, study enrollment will be suspended and participants will be maintained on their current immunosuppressive treatment regimens (i.e., further reduction of immunosuppressive agents will be suspended) pending expedited review of all pertinent data by the NIAID Transplant DSMB, NIAID DAIT and the ITN.

3.6.1 General Stopping Guidelines

General stopping criteria include any of the following observed in any study participant:

- Death.
- Grade 4 infection.
- Grade 3 or 4 acute GVHD or steroid refractory grade 2 acute GVHD (see Table 4 for grading criteria for acute GVHD). Steroid-refractory acute GVHD is defined as acute GVHD that progresses despite 48 hours of treatment with methylprednisolone (≥2 mg/kg/day) or that does not improve despite 4 days of methylprednisolone (≥2 mg/kg/day).
- Any occurrence of severe chronic GVHD (see Table 5 for grading criteria for chronic GVHD).
- Renal graft loss defined as any of the following:
    - Institution of chronic dialysis (at least 6 consecutive weeks, excluding participants with delayed graft function).
    - Transplant nephrectomy.
    - Re-transplantation.
- PTLD.
- Progressive multifocal encephalopathy.
- Failure to achieve neutrophil count recovery; recovery is defined as an ANC ≥ 500 per μL, by day 30 following the post-transplant neutrophil nadir. The neutrophil nadir is defined as the first day post-transplant on which the absolute neutrophil count (ANC) is below 500 per μL.
- Irreversible grade 4 organ toxicity.

3.6.2 Stopping Guidelines for Rejection

Stopping criteria for rejection include:

- The occurrence of rejection of the renal allograft in more than one participant prior to initiation of immunosuppression withdrawal.
- The occurrence of rejection of the renal allograft in more than two participants at any time in the trial.

For the purposes of assessing the stopping rule, renal allograft rejection will be defined to include: acute humoral rejection, acute cellular rejection, and chronic rejection as defined in section 6.5.4.1 as well as presumed rejection without biopsy confirmation as defined in section 6.5.4.2). See section 6.5 for guidelines for use of kidney biopsies to determine rates of acute rejection.

3.6.3 Ongoing Review

The Protocol Chair, the ITN Clinical Trial Physician, the NIAID Medical Monitor and the NIAID Transplant Data and Safety Monitoring Board (DSMB) will periodically review safety data. Enrollment of participants in the trial and further immunosuppression withdrawal for current trial participants will be suspended at any time if any of these reviews concludes that there are significant safety concerns.

4. ELIGIBILITY

4.1 INCLUSION CRITERIA

4.1.1 Recipient

Recipient participants must meet *all* of the following criteria to be eligible for this study:

1. Recipient of a first renal allograft from an HLA-haploidentical, living related donor. The donor and recipient must be HLA identical for at least one allele (using high resolution DNA based typing) at the following genetic loci: HLA-A, HLA-B, HLA-C, and HLA-DRB1. Fulfillment of this criterion shall be considered sufficient evidence that the donor and recipient share one HLA haplotype.
2. Age 18 to 65 years.
3. Single solid organ recipients (kidney only).
4. ABO compatibility with donor.
5. DSA will be assessed by the local laboratory 30 days or less prior to transplant using solid phase micro particle technology (by Luminex® phenotype panel or Luminex single antigen bead test.) The following criteria apply:
   a. Participants without detectable DSA will be deemed eligible if they meet other entry criteria.
   b. Participants with detectable DSA and a positive flow cytometric crossmatch may undergo de-sensitization per standard of care *if they are cytotoxic crossmatch negative*. Such participants must demonstrate a negative flow cytometric crossmatch by day -9 in order to receive the first dose of study therapy (ATG). Participants who do not demonstrate an acceptable response to de-sensitization by day -9 will be considered screen failures and will be terminated from the study.
   c. Participants with a positive cytotoxicity crossmatch will be excluded.
6. Removed in protocol version 5.0.
7. Removed in protocol verision 5.0.
8. Normal estimated left ventricular ejection fraction and no history of ischemic heart disease requiring revascularization, unless cleared by a cardiologist.
9. FEV1 and FVC > 40% of predicted at the screening visit.
10. Serological evidence of prior EBV infection as documented by positive IgG and negative IgM antibodies against EBV.
11. For women of childbearing potential, a negative serum or urine pregnancy test with sensitivity less than 50 mIU/m within 72 hours before the start of study medication.
12. Use of two forms of contraception with less than a 5% failure rate or abstinence by all transplanted participants for 18 months after the first dose of study therapy. For the first 60 days post-transplant, recipients should be encouraged to use non-hormonal

contraceptives due to the potential adverse effect of hormones on bone marrow engraftment. If using a barrier method, a double barrier method should be used.

13. Ability to receive oral medication.
14. Ability to understand and provide informed consent.
15. All participants must demonstrate a negative QuantiFERON® (QFT) assay result within 52 weeks of transplant regardless of PPD status. Participants with a positive QFT assay must complete treatment for latent TB and have a negative chest x-ray. QFT testing done within 52 weeks before transplant is acceptable as long as there is documentation of the results. Prior recipients of a BCG vaccination are not exempt.

4.1.2 Donor

Donor participants must meet *all* of the following criteria to be eligible for this study:

1. HLA-haploidentical, first-degree relatives or half-siblings of the recipient participant at the allele or allele group. The donor and recipient must be HLA identical for at least one allele (using high resolution DNA based typing) at the following genetic loci: HLA-A, HLA-B, HLA-C and HLA-DRB1. Fulfillment of this criterion shall be considered sufficient evidence that the donor and recipient share one HLA haplotype.
2. Age 18 to 65 years.
3. Creatinine clearance >80 ml/minute as measured from a 24 hour urine collection within 26 weeks of the screening visit. If a serum creatinine drawn at the screening visit is > 20% higher than the serum creatinine drawn at the time of the 24 hour urine collection, the creatinine clearance must be re-evaluated by a repeat 24 hour urine test. If the new value is ≤80mg/dL the donor will be excluded.
4. Meets institutional selection criteria for organ and bone marrow donation.
5. Ability to understand and provide informed consent for all study procedures including kidney transplant and bone marrow harvest.
6. Serologic evidence of prior EBV infection as documented by positive IgG and negative IgM antibodies against EBV.

4.2 EXCLUSION CRITERIA

4.2.1 Recipient

Recipient participants who meet *any* of the following criteria will *not* be eligible for this study:

1. Underlying renal disease with a high risk of disease recurrence in the transplanted kidney, including:
    a. focal segmental glomerulosclerosis (FSGS).
    b. type I or II membranoproliferative glomerulonephritis.
    c. hemolytic-uremic syndrome/thrombotic thrombocytopenic purpura.

2. Clinically important genital/urinary tract dysfunction.
3. Body mass index (BMI) > 40.
4. Women who are breastfeeding.
5. History of cancer within the last 5 years, except for nonmelanoma skin cancer, stage 1 renal cell carcinoma, stage 1 prostate cancers cured by local resection and any curatively treated carcinomas in situ.
6. History of positive HIV-1 or HIV-2 serologies or nucleic acid test.
7. Evidence of hepatitis B infection. Participants demonstrating any one of the following will be excluded:
   a. positive hepatitis B surface antigen (HBsAg) or
   b. positive anti-HBc IgM.
   c. positive anti-HBc IgG
   d. positive HBV PCR
8. Positive anti-hepatitis C (HCV) antibodies and a positive serum HCV RNA PCR. All positive HCV antibody results must be assessed by an EIA assay and confirmed by a quantitative serum HCV RNA assay. Participants with positive HCV antibodies but undetectable serum HCV RNA may be considered for eligibility. Participants with negative anti-HCV antibodies but unexplained liver enzyme abnormalities must undergo a quantitative serum RNA assay to rule out false negative HCV serologies.
9. History of active tuberculosis (TB).
10. Any active, severe local or systemic infection at the screening visit.
11. Autoimmune disease requiring immunosuppressive drugs for maintenance.
12. Use of investigational drug, other than the study medications specified by the protocol, within 30 days of transplantation.
13. Receipt of a live vaccine within 30 days of receipt of study therapy.
14. The presence of any medical condition that the Investigator deems incompatible with participation in the trial.
15. Positive cytotoxic crossmatch.
16. Calculated PRA greater than 90%.

4.2.2 Donor

Donor participants who meet *any* of the following criteria will *not* be eligible for this study:

1. History of type I or type II diabetes mellitus.
2. History of severe cardiovascular disease, defined as New York Heart Association Class III or IV.
3. History of blood product donation to recipient.
4. History of positive HIV-1 or HIV-2 serology or nucleic acid test.
5. Evidence of hepatitis B infection. Participants demonstrating any one of the following will be excluded:

a. positive hepatitis B surface antigen (HBsAg) or
b. positive anti-HBc IgM
c. positive anti-HBc IgG
d. positive HBV PCR

6. Positive anti-hepatitis C (HCV) antibodies and a positive serum HCV RNA PCR. All positive HCV antibody results must be assessed by an EIA assay and confirmed by a quantitative serum HCV RNA assay. Participants with positive HCV antibodies but undetectable serum HCV RNA may be considered for eligibility. Participants with negative anti-HCV antibodies but unexplained liver enzyme abnormalities must undergo a quantitative serum RNA assay to rule out false negative HCV serologies.
7. Autoimmune disease requiring immunosuppressive drugs for maintenance.
8. The presence of any medical condition that the Investigator deems incompatible with participation in the trial.

4.3 PREMATURE DISCONTINUATION OF STUDY THERAPY

Study therapy is defined as initiation of any study-mandated therapies and study procedures described in section 5.

Study therapy will be discontinued for the following reasons:

- Hypersensitivity. If a participant develops a persistent, life threatening hypersensitivity reaction to ATG, fludarabine or cyclophosphamide despite the measures described in section 5.2 before receiving the full dose of study drug.
- Adverse experience. If a participant suffers from an adverse experience that, in the judgment of the Principal Investigator or the Medical Monitor, may present an unacceptable consequence or risk to the participant.
- Intercurrent illness or infection. If during the course of the study a participant develops an illness or infection that is not associated with the condition under study and that requires treatment not consistent with protocol requirements; or, if a participant develops an intercurrent illness that in the judgment of the Principal Investigator justifies discontinuation.
- Protocol violation. If a participant cannot comply with the study protocol and the protocol deviations are sufficient to jeopardize his or her well-being or the integrity of the study.
- Pregnancy.
- Investigator discretion. If the Investigator determines that the study therapy is no longer in the best interests of the participant.
- If the participant is unwilling or unable to continue on study therapy.

4.3.1 Follow–up for Participants Prematurely Discontinued from Study Therapy

All participants who discontinue study therapy will be asked to complete Safety Follow-up procedures as specified in Appendix 5.

4.4 PREMATURE TERMINATION OF A PARTICIPANT FROM THE STUDY

Participants will be prematurely terminated from the study for the following reasons:

Withdrawal of consent. If for any reason a participant withdraws consent during the study they will be terminated from the study.

Failure to return. Participants who do not return for visits and who do not respond to repeated attempts by the site staff to have them return will be considered *lost to follow-up*.

4.4.1 Follow-up for Participants Prematurely Terminated from the Study

Participants who wish to withdraw consent will be asked to complete the Safety Follow-up schedule in Appendix 5. If they decline they will be asked to undergo a study termination visit containing the assessments listed in visit 401 of Appendix 5. If they decline they will be terminated from the study with no further study data.

4.5 REPLACEMENT OF STUDY PARTICIPANTS

Enrolled participants who receive any form of study therapy, including ATG, fludarabine, cyclophosphamide and TBI, but who do not fulfill the PP analysis sample definition will be replaced. Participants who meet the PP analysis sample definition (see section 9.1) but who for any reason are no longer being treated according to the study protocol will undergo safety follow-up per Appendix 5 and will not be replaced. Participants who meet the PP analysis sample definition but who prematurely terminate from the study (section 4.4) will not be replaced.

5. STUDY MEDICATIONS AND PROCEDURES

5.1 OVERVIEW OF CONDITIONING AND POST-TRANSPLANT REGIMEN

The treatment regimen is outlined in Figure 5**.**

The recipient will receive Antithymocyte globulin (ATG) from Day -9 to Day -7; fludarabine and low-dose cyclophosphamide on Days -4 and -3; fludarabine on Days -4, -3 and -2. Recipients will undergo hemodialysis 6 to 8 hours after each fludarabine dose. Recipients will undergo TBI on Day -1.

Donor nephrectomy and bone marrow harvest will take place under the same anesthetic on Day 0, with renal transplantation and bone marrow infusion into the recipient also on Day 0.

The recipient will receive high dose PT/Cy on Days 3 and 4 with hydration, MESNA and anti-emetics. Standard maintenance immunosuppression consisting of tacrolimus, with target trough level of 8 to 12 ng/ml, MMF, 15 mg/kg three times daily (maximum daily dose of 3 grams per day), and prednisone, 5 mg twice daily, will be started on Day 5.

5.2 CONDITIONING REGIMEN FOR RECIPIENTS

The following sections list study medications and procedures in chronological order of administration.

5.2.1 Indwelling central venous catheter

Placement of a double lumen central venous catheter will be required for administration of IV medications and transfusion of blood products including donor bone marrow.

5.2.2 Preparative regimen

5.2.2.1 Antithymocyte Globulin (Days -9, -8, -7)

Antithymocyte globulin (ATG) will be administered daily at a specialized outpatient BMT unit as an intravenous infusion on Days -9, -8 and -7. ATG will be infused through a 0.22 micro filter with premedications: acetaminophen 650 mg orally and diphenhydramine 25mg orally. An anaphylaxis kit must be kept at bedside during ATG administration. An initial dose of 0.5 mg/kg IV will be administered over 6 hours on Day -9. Thereafter the daily dose will be increased to 2 mg/kg IV given over 4 hours on Days -8 and -7. No more than 150 mg of ATG may be administered per day.

All participants must receive at least 2 full doses of ATG to continue on study therapy. A full dose is defined as at least 80% of the dose volume. Participants who fail to receive at least 2 full ATG doses within the first week post-transplant (Day -9 through Day -7) will undergo safety follow-up (see Appendix 5. Safety Follow-up: Recipients).

A steroid taper will be given to prevent reactions to ATG as follows: On Days -9 to -7 methylprednisolone 1mg/kg IV 1 hour prior ATG. This dose may be repeated once 3 hours after the first dose of steroids. On Day -6 and -5, methylprednisolone 0.75 mg/kg/ IV as a single dose; on Days -4 and -3, methylprednisolone 0.5 mg/kg/ IV as a single dose; on Day -2, methylprednisolone 0.25 mg/kg/ IV as a single dose.

For additional information on the preparation, storage and handling of ATG, refer to the package insert for Thymoglobulin® (Genzyme) at the following website: http://www.thymoglobulin.com/home/thymo_pdf_pi.pdf.

5.2.2.2 Fludarabine (Days -4 through -2)

Participants will be admitted to the inpatient bone marrow unit for administration of fludarabine. Fludarabine will be administered daily by intravenous infusion over 30 minutes on Day -4 to Day -2. The daily dose will be 30 mg/m^2. Hemodialysis will be performed no earlier than 6 hours after and no later than 12 hours after each dose.

For additional information on the preparation, storage and handling of fludarabine, refer to the package insert for fludarabine at the following website: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=6f1094e3-4c28-40ae-a82f-37cdfa865f49

5.2.2.3 Cyclophosphamide (Days -4, -3)

Cyclophosphamide will be administered as an intravenous infusion over 1-2 hours, (depending on volume) on Days -4 and -3. The dose of pre-transplantation cyclophosphamide is 14.5

mg/kg/day. Dose is calculated based on ideal body weight (IBW). Actual body weight should be used to dose cyclophosphamide in the case that actual body weight is less than ideal body weight. Body weight and height will be measured directly. Ideal body weight will be calculated using the following:

Males IBW = 50kg + 2.3kg/inch over 5 feet

Females IBW = 45.5kg + 2.3kg/inch over 5 feet.

Note: Hydration and MESNA will not be required for the Day -4 and -3 pre-BMT cyclophosphamide doses.

For additional information on the preparation, storage and handling of cyclophosphamide, refer to the cyclophosphamide drug label at the following website: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=591d9955-3d9c-4cdc-a308-2f1288376b9f

5.2.2.4 Total Body Irradiation (Day -1)

Total body irradiation, consisting of 200 cGy AP/PA with 4MV or 6MV photons at 8-12 cGy/min at the point of prescription (average separation of measurements at mediastinum, abdomen, and hips), will be administered in a single day on Day -1.

5.2.2.5 Bone Marrow Infusion (Day 0)

Unprocessed, unmanipulated bone marrow will be harvested from the donor and infused into the recipient on Day 0. The nucleated cell target range will be between 8 to 16 x 10^8/kg of recipient ideal body weight with the volume not to exceed 20 mL/kg of donor's weight once the minimal target of 8 x 10^8/kg has been reached. All efforts will be made to assure a cell count of at least 2-4 x 10^8/kg of recipient ideal body weight. However there is no minimum donor bone marrow cell count threshold; the bone marrow infusion will proceed regardless of donor cell counts recovered.

5.2.3 High Dose Post-Transplant Cyclophosphamide (Days 3, 4)

High dose post-transplant cyclophosphamide (PT/Cy) [50mg/kg (Ideal Body Weight)] will be administered on Day 3 post-transplant (within 60 to 72 hours of marrow infusion) and on Day 4 post-transplant. Cyclophosphamide will be given as an IV infusion over 1-2 hours depending on volume. Participants requiring hemodialysis for renal insufficiency on days 3 and 4 post-transplant will undergo hemodialysis no earlier than 12 hours after each cyclophosphamide dose.

For additional information on the preparation, storage and handling of cyclophosphamide, refer to the cyclophosphamide drug label at the following website: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=591d9955-3d9c-4cdc-a308-2f1288376b9f

It is crucial that no immunosuppressive agents are given from the time of the transplant until 24 hours after the completion of the post-transplant cyclophosphamide. This includes steroids as anti-emetics.

5.2.4 MESNA (Days 3, 4)

A series of MESNA doses will be administered for each dose of high dose, post-transplant cyclophosphamide. MESNA will be given intravenously in divided doses 30 minutes before and at 3, 6, and 8 hours after cyclophosphamide administration, or administered per institutional standards. The MESNA dose will be based on the cyclophosphamide dose being given. The total daily dose of MESNA is equal to 80% of the total daily dose of cyclophosphamide. For participants with renal insufficiency requiring hemodialysis, continuous bladder irrigation with normal saline at 200 ml/hour via a 3-way Foley catheter will be used instead of MESNA for hemorrhagic cystitis prevention. Continuous bladder irrigation will be continued for at least 12 hours after the last dose of high dose , post-transplant cyclophosphamide[66].

5.3 MAINTENANCE THERAPY (DAY 5 THROUGH ELIGIBILITY ASSESSMENT FOR IMMUNOSUPPRESSION WITHDRAWAL)

5.3.1 MMF

MMF will be administered at a dose of 15 mg/kg orally three times per day based upon actual body weight, with the maximum of 3 grams a day from Day 5 to 35. The dose will then be reduced to the standard 1 gram twice daily thereafter. Participants may not tolerate three grams a day due to GI side effects. A reduced dose of 500 or 750 mg twice daily will be allowed for those who are intolerant. Mycophenolic acid (Myfortic®) may be substituted for severe MMF intolerance.

For additional information about the risks of MMF, refer to the drug label at the following website: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=209efe01-7908-46e4-a964-0665078c0acd#druglabelcontent.

5.3.2 Prednisone

Prednisone will be administered at a dose of 10 mg orally daily from Day 5 for 12 weeks. Thereafter the dose will be reduced to 5 mg orally daily.

For further information about the risks associated with prednisone, please refer to the drug label at the following website: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=3115aef0-fd50-4ec8-a064-3effb695f3f2#druglabelcontent .

5.3.3 Tacrolimus

The first dose of tacrolimus will be administered orally on Day 5. The participant will receive tacrolimus daily to sustain target trough levels of between 8 to 12 ng/ml. Intravenous administration of tacrolimus will not be allowed due to its association with vasoconstriction and renal allograft loss.

For additional information on preparation, storage and handling of tacrolimus, refer to the drug label at the following website: http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=bd447ffa-9196-4c3c-accf-5adf29b84665#druglabelcontent

5.3.4 Filgrastim (Day 5 to ANC Reconstitution)

All recipients will receive 5 µg/kg per day of filgrastim as a single, subcutaneous injection from Day -5 until the ANC is greater than 1000/µl on three consecutive measurements over at least 2 days[31].

For additional information on preparation, storage and handling of filgrastim, refer to the drug label at the following website:
http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=3bc802bd-76b4-4f45-8571-a436ec26228e

5.4 STUDY REGIMEN FOR DONORS

5.4.1 Bone Marrow Harvest (Day 0)

Unprocessed, unmanipulated bone marrow will be harvested from the donor and infused into the recipient on Day 0. The nucleated cell target range will be between 8 to 16 x 10^8/kg of recipient ideal body weight with the volume not to exceed 20 mL/kg of donor's weight once the minimal target of 8 x 10^8/kg has been reached. All efforts will be made to assure a cell count of at least 2-4 x 10^8/kg of recipient ideal body weight. However there is no minimum donor bone marrow cell count threshold; the bone marrow infusion will proceed regardless of donor cell counts recovered.

5.5 MODIFICATION OF RECIPIENT STUDY MEDICATIONS

5.5.1 Bacterial Prophylaxis

Allograft recipients will receive ciprofloxacin 500 mg orally twice daily, starting on Day 1, until the ANC ≥1000/mm^3 for three consecutive days.

5.5.2 Tacrolimus

In the event of toxicity related to tacrolimus (nephrotoxicity, neurotoxicity hyperglycemia, etc.), the dose of tacrolimus may be adjusted at the discretion of the Investigator to maintain trough levels lower than those specified in section 5.3.3.

In the event of development of posterior reversible encephalopathy syndrome (PRES)[58] sirolimus will be substituted for tacrolimus at the discretion of the investigator. Sirolimus will be dosed to achieve a goal trough level of 8 to 10 ng/ml for the first 3 months of therapy. Thereafter the dose may be lowered to achieve a goal trough level of 6 to 8 ng/ml. All effort should be made to avoid sirolimus use in the first 10 days post-transplant to avoid its adverse effect on wound healing.

5.5.3 Mycophenolic Compounds

The dose of MMF or mycophenolic acid may be adjusted for intolerance or leukopenia. A reduced dose of MMF 500 or 750 mg twice daily will be allowed for those who are intolerant. Mycophenolic acid (Myfortic®) may be substituted for severe MMF intolerance. The maximum allowable dose of MMF is 3g daily; for mycophenolic acid, it is 1440 mg daily.

5.5.4 Corticosteroids

On Days -9 to -7 a second dose of methylprednisolone 1mg/kg IV may be given at the Investigator's discretion once 3 hours after the first dose of intravenous methylprednisolone, especially in cases of ATG infusion reaction (section 5.2.2). Otherwise the dose of study-mandated corticosteroids, including the steroid taper after ATG infusion and the course of maintenance prednisone, may not be modified.

5.6 PROPHYLACTIC MEDICATIONS AND MONITORING

Prophylactic medications associated with adverse events such as leukopenia or rash may be held or dose-adjusted per institutional standard until the episode has resolved.

5.6.1 Pneumocystis Jiroveci (PCP) Prophylaxis

Allograft recipients will receive prophylaxis for *Pneumocystis jiroveci* (PCP). Participants will receive trimethoprim sulfa (TMP-SMX) , one single- or double-strength tablet at least 3-times weekly, beginning on Day 21 or when the ANC $\geq$1000/mm^3 for three consecutive days. *Pneumocystis jiroveci* prophylaxis will continue until Day 365. For sulfa intolerant participants the following options are available:

1. Sulfa desensitization followed TMP-SMX single-strength, one tablet orally every day until Day 365.
2. Dapsone 100 mg orally every day until Day 365.
3. Pentamidine 300 mg inhaled every month until Day 365.
4. Atovaquone 1500 mg/day

For participants who are known to be allergic to or intolerant of sulfa compounds, de-sensitization will be allowed. Desensitization to TMP-SMX should be considered when there are no reasonable or available alternatives and ***the patient has not experienced severe reactions (e.g., Stevens-Johnson syndrome)*** *to sulfa drugs.* The following algorithm will be followed:
Use commercially available pediatric suspension (containing TMP 8 mg and SMX 40 mg per mL), followed by double-strength tablets, as follows:

Table 2. Rapid Oral TMP-SMX desensitization protocol

	TMP (mg)	SMX (mg) PO	Dose plus formulation
Day 1*			
9 AM	0.8	4	1 mL 1:10 dilution
11 AM	1.6	8	2 mL 1:10 dilution
1 PM	4	20	5 mL 1:10 dilution
5 PM	8	40	10 mL 1:10 dilution
Day 2			
9 AM	16	80	2 mL undiluted suspension
3 PM	32	160	4 mL undiluted suspension
9 PM	40	200	5 mL undiluted suspension
Day 3			
9 AM	80	400	1 TMP-SMX SS tab

*The solution used on Day 1 may be prepared from the oral suspension (which contains 40 mg of trimethoprim and 200 mg of sulfamethoxazole per 5 mL) by combining 2 mL of suspension and 18 mL of water[67].

Pneumocystis jiro prophylaxis will be reinstituted for 12 weeks after receiving treatment for an episode of rejection occurring after the first 52 weeks of the transplant.

5.6.2 Fungal and Yeast Prophylaxis

Allograft recipients will receive fluconazole 400 mg orally daily, starting on Day 1, for fungal and yeast prophylaxis until the ANC $\geq$1000/mm^3 for three consecutive days.

5.6.3 CMV Prophylaxis and Monitoring

Allograft recipients, regardless of CMV serological status, will receive valacyclovir 500 mg orally three times per day, starting on Day -9 until Day 365. If participants cannot tolerate oral medications, acyclovir 250mg/m^2 IV every 12 hours may be substituted for valacyclovir. The dose will be adjusted for renal dysfunction.

CMV viremia will be monitored by PCR and documented weekly beginning once the total white blood cell count is greater than 1000/mm^3. Monitoring of CMV viremia will continue weekly until Day 100; then every other week until Day 365.

5.6.4 CMV Treatment

Patients who are viremic will be treated pre-emptively with ganciclovir (5 mg/kg IV every 12 hours) for at least, 14 days, at the discretion of the physician, and then with maintenance ganciclovir (5 mg/kg IV daily) until CMV testing is negative for at least 2 weeks. Consideration should be given to administration of CMV hyperimmune globulin (Cytogam®), concomitant with ganciclovir at a dose of 150 mg/kg IV every other day times 4 doses and then weekly thereafter if the patient continues to be treated with ganciclovir for persistent

viremia. If the patient is diagnosed with CMV disease, more frequent CMV hyperimmune globulin administration may be considered. The dose will be adjusted for renal dysfunction.

Unless the patient is being treated for CMV infection or CMV disease, ganciclovir should be discontinued for development of neutropenia (ANC<500). If the participant is neutropenic, G-CSF may be used if clinically indicated. Ganciclovir should then be restarted when the ANC ≥ 500, if appropriate. Ganciclovir will be dose-adjusted appropriately for renal failure.

Initial treatment of CMV disease will be for a minimum of 2 weeks with intravenous ganciclovir at a dose of 5 mg/kg twice daily (with dosage adjustment for renal dysfunction), oral valganciclovir 900 mg orally twice daily, or other approved antiviral medication per institutional standards. CMV hyperimmune globulin (150 mg/kg every 4 weeks) will be added for seronegative participants at the discretion of the Investigator. The endpoint of intravenous therapy is the documented clearance of virus from the blood as demonstrated by CMV quantitative PCR assay.

The risk of subsequent relapse is 15-20%, so treatment may be continued for up to three months, at the discretion of the physician. With three months of oral therapy following clearance of viremia, the rate of relapse is decreased substantially.

In participants with relapsing infection, initial treatment will be repeated at the discretion of the physician. CMV hyperimmune globulin (at 150 mg/kg IV once and 100 mg/kg IV monthly for 3-6 months) is usually administered in conjunction with the intravenous ganciclovir, oral valganciclovir, or other approved therapy for seronegative recipients at the discretion of the Investigator.

5.6.5 Treatment of Herpes Infection

All participants will receive anti-viral prophylaxis for herpes infection per institutional standard for 26 weeks post-transplant.

5.7 MONITORING FOR BK VIREMIA

All participants who receive at least 1 full dose of ATG (see section 5.2.2) will be monitored for BK viremia by quantitative BK viral PCR. Participants who complete immunosuppression withdrawal will be monitored at least 1 year post-transplant. Participants who remain on immunosuppression and are moved to Safety Follow-up will be monitored at least 2 years post-transplant. For-cause biopsy visits will include a test for BK viremia. BK viremia will be monitored at the following timepoints: monthly for months 1 through 6 post-transplant; at months 9 and 12 post-transplant; at 18 and 24 months post-transplant for participants who remain on immunosuppression. Treatment of BK viremia will be left to the discretion of the site PI.

5.8 MONITORING FOR EBV REACTIVATION

All participants who receive at least 1 full dose of ATG (see section 5.2.2) will be monitored for EBV viremia by quantitative EBV PCR at the time points specified in Appendix 1 through Appendix 5. Safety Follow-up: Recipients. Treatment of EBV viremia will be left to the discretion of the site PI.

5.9 PROHIBITED MEDICATIONS AND VACCINES

- All immunosuppressive medications including oral corticosteroids are prohibited from the day of transplant to 24 hours after the last day on which high dose cyclophosphamide is administered.
- Short (≤7 days) courses of oral corticosteroids are permitted only for the treatment of unrelated illnesses such as asthma, allergy, etc. These should be avoided unless absolutely necessary.
- Otherwise, all immunosuppressive medications other than those used as specified in the protocol are prohibited during immunosuppression withdrawal unless rejection is suspected or diagnosed.
- Topical or inhaled corticosteroids or steroid mouthwashes will not be considered immunosuppressive medications.
- Live vaccines are prohibited from 30 days prior to the first dose of study therapy through the end of study participation.
- Any other investigational products are prohibited for the duration of study participation.

5.10 CONCOMITANT MEDICATIONS

All recipient concomitant medications will be documented at the screening Visit 1 (see Appendix 1A. Pre-Transplant Through Transplant: Recipient). At all subsequent visits, only immunosuppressive medications, prophylactic antibiotics, anti-viral and anti-fungal medications will be recorded.

5.11 BLOOD REPLACEMENT THERAPY

If blood component replacement therapy is required for any reason, leukocyte-filtered blood components will be administered. If clinically indicated, blood components will be irradiated.

6. STUDY PROCEDURES

6.1 VISIT WINDOWS

6.1.1 Scheduled Visits

The schedule of events for this trial can be found in Appendix 1A. Pre-Transplant Through Transplant: Recipient through Appendix 5. Safety follow-up: Recipients. For both the recipient and donor, the initial screening visit, Visit 1, must occur within 30 days of the first administration of study drug. All other scheduled study visits must occur within the time limits specified below:

Table 3. Designated study windows

Appendix	Trial Phase	Visit Window
1.A	Pre-transplant through transplant - Recipient	Visits should take place on the day indicated in the schedule of events Assessments listed in the screening visit should take place within 30 days of the first administration of study drug unless otherwise indicated Screening laboratory assessments • hematology, chemistry and pregnancy assessments must be completed within 30 days of Day -9 • viral serology assessments: results of CMV, EBV, HIV-1 and 2, Hep B and HCV testing must be reported by day -14 • previously performed high resolution molecular HLA typing, ABO typing and positive viral serologies may be used, provided the respective assays were performed using the same technology currently in use by the site laboratory • cytocytoxic crossmatch • calculated PRA If visit 2 falls on a weekend, baseline specimens may be collected up to 6 days prior to the visit
1.B	Pre-transplant through post-transplant - Donor	Visits pre-transplant through transplant should take place on the day indicated in the schedule of events If visit 2 falls on a weekend, baseline specimens may be collected up to 6 days prior to the visit Assessments listed in the screening visit should take place within 30 days of transplant (Day 0) unless otherwise indicated Screening laboratory assessments • hematology, chemistry and pregnancy assessments must be completed within 30 days of Day 0

Appendix	Trial Phase	Visit Window
		• viral serology assessments: CMV, EBV, HIV-1 and 2, Hep B and HCV must be completed on day -14, +/- 2 days • previously performed high resolution molecular HLA typing, ABO typing and positive viral serologies may be used, provided the respective assays were performed using the same technology currently in use by the site laboratory The Day 7 post-transplant visit has a +3-day window The Day 56 post-transplant visit has a +14-day window
1.C	Reinitiation of Conditioning: Pre-Transplant Through Transplant: Recipient	Visits 501 through 504 has a +7-day window Visits 505 through 514 should take place on the day indicated in the schedule of events Assessments listed in Visit 505 should take place within 30 days of the first administration of study drug during the reinitiation of conditioning, unless otherwise indicated For eligibility laboratory assessments that must be repeated, the following guidelines apply • hematology, chemistry and pregnancy assessments must be completed within 30 days of Day -9 • viral serology assessments: results of CMV, EBV, HIV-1 and 2, Hep B and HCV testing must be reported by day -14. • previously performed high resolution molecular HLA typing, ABO typing and positive viral serologies may be used, provided the respective assays were

Appendix	Trial Phase	Visit Window	
		performed using the same technology currently in use by the site laboratory • cytocytoxic crossmatch • calculated PRA If Visit 506 falls on a weekend, baseline specimens may be collected up to 6 days prior to the visit	
1.D	Reinitiation of Conditioning: Pre-Transplant Through Post-Transplant: Donor	Visits pre-transplant through transplant should take place on the day indicated in the schedule of events If visit 506 falls on a weekend, baseline specimens may be collected up to 6 days prior to the visit Assessments listed in the screening visit should take place within 30 days of transplant (Day 0) unless otherwise indicated Screening laboratory assessments • hematology, chemistry and pregnancy assessments must be completed within 30 days of Day 0 • viral serology assessments: CMV, EBV, HIV-1 and 2, Hep B and HCV must be completed on day -14, +/- 2 days • previously performed high resolution molecular HLA typing, ABO typing and positive viral serologies may be used, provided the respective assays were performed using the same technology currently in use by the site laboratory The Day 7 post-transplant visit has a +3-day window The Day 56 post-transplant visit has a +14-day window	
2	Post-transplant through evaluation for withdrawal eligibility	Visits 101-104	Day indicated
		Visits 105-106	+/- 2 days
		Visit 107	+/- 7 days
		The *Initiation of IS withdrawal eligibility biopsy* may take place as early as Week 22 or as late as Week 30	

Appendix	Trial Phase	Visit Window	
		post-transplant at Investigator discretion, based on participant medical status	
3	Immunosuppression withdrawal	Visits 201-214	+/- 7 days
		Visits 215-218 (if needed)	+/- 7 days
		The *Initiation of tacrolimus withdrawal eligibility biopsy* may take place as early as Week 34 or as late as Week 46 post-transplant based on progress of immunosuppression withdrawal and at Investigator discretion, based on participant medical status	
4	Post immunosuppression withdrawal follow-up	Visits 301-305	+/- 7 days
		Visits 306-322	+/- 2 weeks
		The *Week 52 post IS withdrawal completion biopsy* should take place 52 weeks after completion of immunosuppression withdrawal +/- 2 weeks	
		The *Week 104 post IS withdrawal completion biopsy* should take place 104 weeks after completion of immunosuppression withdrawal +/- 2 weeks	
		The *Week 208 post IS withdrawal completion biopsy* should take place 208 weeks after completion of immunosuppression withdrawal +/- 2 weeks	
5	Safety follow-up for participants who are ineligible or have failed immunosuppression withdrawal. All participants who receive the preparative regimen will be followed for at least 2 years as indicated in Appendix 5. Safety Follow-up: Recipients.	Visit 401	Participants who experience a rejection event should begin safety follow-up within 4-6 weeks of the date of diagnosis of rejection. Participants who move to safety follow-up following non-rejection events should begin this schedule within 2 weeks of the determination of their ineligibility for further progress in the protocol.
		Visits 402-410	+/- 2 weeks

6.2 GENERAL ASSESSMENTS

Recipient Assessments:

- Informed consent
- Medical, demographic, and blood products from donor history
- Complete physical examination including height
- Limited physical examination (to include: respiratory, cardiovascular, gastrointestinal, skin, neurologic and renal/urinary systems)
- Vital signs – Weight, temperature, blood pressure, respiration, and pulse
- Cardiac function – echocardiogram
- Pulmonary function – chest x-ray and spirometry
- Acute GVHD assessment – staged and graded as in section 6.7
- Chronic GVHD assessment – staged and graded as in section 6.6
- Screening for status change since prior visit including assessment of change in administration of blood products; adverse events and concomitant medications.

Donor Assessments:

- Informed consent
- Medical and demographic history including screening for any autoimmune disease requiring immunosuppressive drugs for maintenance
- Physical examination including height and weight
- Vital signs – temperature, blood pressure, respiration, and pulse
- Screening for status change since prior visit including assessment of change in administration of blood products; adverse events and concomitant medications.

6.3 CLINICAL LABORATORY ASSESSMENTS

Recipient Assessments:

- Hematology – CBC with differential and platelets
- Comprehensive Metabolic Panel (Na, K, Cl, HCO3, BUN, creatinine, glucose, albumin, total bilirubin, AST, ALT, alkaline phosphatase)
- Urine or serum pregnancy test
- CMV and EBV serologies
- BK virus (blood)

- HIV-1 and 2 serologies and nucleic acid test
- Anti-hepatitis C (HCV) antibodies, hepatitis B surface antigen (HBsAg), total hepatitis B core antibody (anti-HBc IgM and IgG), hepatitis B surface antibody (anti-HBsAb)
- ABO typing
- High resolution molecular HLA typing and DNA (A, B, C, DRB1, DRB3-5, DQA1, DQB1, DPB1)
- HLA alloantibodies includes DSA, flow PRA
- CMV and EBV viral load by PCR
- T and B cell lymphocyte crossmatch
- Cytotoxic crossmatch
- Calculated PRA
- Tacrolimus levels
- Kidney biopsy

Donor Assessments:

- High resolution molecular HLA typing and DNA (A, B, C, DRB1, DRB3-5, DQA1, DQB1, DPB1)
- CMV and EBV serology
- HIV-1 and 2 serologies and nucleic acid test
- Anti-hepatitis C (HCV) antibodies, hepatitis B surface antigen (HBsAg), total hepatitis B core antibody (anti-HBc IgM and IgG), hepatitis B surface antibody (anti-HBsAb)
- ABO typing
- Hematology – CBC with differential and platelets
- Comprehensive Metabolic Panel (Na, K, Cl, HCO3, BUN, creatinine, glucose, albumin, total bilirubin, AST, ALT, alkaline phosphatase)
- PBMC's for T and B cell lymphocyte crossmatch

6.4 MECHANISTIC ASSESSMENTS

Recipient Assessments:

- Blood chimerism (whole blood and CD3+ T cell)
- T cell assays (MLR, CML)
- Plasma cytokines
- B cell assays (HLA tetramer selection and culture)

- Whole blood – gene expression profiling
- Kidney biopsy – histology
- Kidney biopsy – gene expression profiling
- B cell repertoire sequencing
- Flow cytometry panel staining (frozen PBMC)
- PBMC collection

Donor Assessments:

- Blood chimerism (whole blood and CD3+ T cell)
- T cell assays (MLR, CML)
- B cell assays (HLA tetramer selection and culture)
- PBMC collection

See section 7 for detailed discussion of additional mechanistic assays.

6.5 ASSESSMENT AND MANAGEMENT OF ALLOGRAFT DYSFUNCTION AND REJECTION

6.5.1 Definition of Allograft Dysfunction

Allograft dysfunction is defined as a sustained increase in creatinine levels of 25% or greater over 72 hours relative to baseline. Baseline thresholds are defined as follows:

- Prior to and During Immunosuppression Withdrawal: Baseline is defined as the mean of the three lowest creatinines during weeks 2 to 4 post-transplant, excluding days on dialysis.
- After Completion of Immunosuppression Withdrawal: Baseline is defined as the mean of the three lowest creatinines in the 8 weeks prior to the final dose of immunosuppression.

6.5.2 Indications for Increased Monitoring and For-cause Biopsy

If an episode of allograft dysfunction occurs, a serum creatinine must be drawn and recorded every 2 weeks for at least 1 month after the episode begins. If allograft dysfunction is unexplained and persists for more than 72 hours, a renal biopsy must be performed to rule out acute rejection. *Unexplained* is defined as demonstration of patent vessels and no significant hydronephrosis by sonogram or other imaging method and the absence of other factors known to affect renal function (i.e., dehydration, urosepsis, drug toxicity, polyoma viral infection).

If a biopsy is performed for allograft dysfunction, all mechanistic assessments listed for Visit 308, Appendix 3. Immunosuppression Withdrawal (Initiated Between 26 and 3 Weeks Post-Transplant) should be performed. If the biopsy does not demonstrate rejection, further evaluation for other causes of renal dysfunction will be performed.

If a transient increase in creatinine occurs as defined by a level that meets the threshold for allograft dysfunction but is not sustained for 72 hours, a follow-up creatinine level must be drawn one week after the last elevated value to confirm return to baseline. If the repeat creatinine remains above the threshold, and is unexplained, a biopsy must be performed.

6.5.3 Modification of Creatinine Threshold for Performance of a For-cause Biopsy

The baseline creatinine values used to determine thresholds for performance of a for-cause biopsy may be modified if a participant's creatinine remains persistently elevated (i.e., above threshold levels greater than 72 hours) despite a biopsy demonstrating the absence of rejection. Such modification requires approval by the NIAID Medical Monitor, the ITN Clinical Trial Physician, the ITN Therapeutic Area Advisor and the Protocol Chair after review of the clinical history and biopsy findings. This biopsy will be reviewed by both local and central pathologists and the worst read will be used. The new baseline will be defined as the mean of the three consecutive values immediately prior to the date of the for cause biopsy (FCB).

NOTE: These adjusted baseline values will be used to determine thresholds for repeat biopsy (RB) only. For the purposes of calculating rates of allograft dysfunction or sustained renal impairment previously defined baseline values will be used.

6.5.4 Diagnosis of Rejection

6.5.4.1 Biopsy-Proven Rejection

Unless medically contraindicated, all episodes of acute rejection must be confirmed by renal allograft biopsy. For all biopsies performed to evaluate rejection, C4d staining should be performed using the most sensitive method. Biopsies will be read by both local and central pathology laboratories. For cases where rejection is diagnosed a Banff score should be assigned by both pathologists according to the 2007 Banff Classification of Renal Allograft Pathology[1].

See section 6.5.7 for guidelines on use and interpretation of kidney biopsies.

The types of rejections are defined as follows:

- Acute humoral (antibody-mediated) rejection is defined according to the 2007 Banff Classification of Renal Allograft Pathology[1].
- Acute cellular rejection is defined by a Banff score of Grade IA or above per the 2007 Banff Classification of Renal Allograft Pathology[1].
- Chronic rejection includes both chronic T cell -mediated and chronic antibody-mediated rejection as defined according to the 2007 Banff Classification of Renal Allograft Pathology[1]. This assessment should also include progressive interstitial fibrosis/tubular atrophy (IF/TA), transplant glomerulopathy or chronic obliterative arteriopathy without an alternative, non-rejection-related cause. See Banff 2007 Classification Renal Allograft Pathology for definition of terms[1].

Biopsy-proven indeterminate (borderline) acute cellular rejection, defined by a Banff score between normal and less than Banff Grade IA, will not be classified as rejection.

6.5.4.2 Rejection without Biopsy Confirmation

If a participant is unable to undergo a biopsy to evaluate unexplained allograft dysfunction or to confirm suspected rejection, this event will be classified as rejection without biopsy confirmation.

If there is unresolvable disagreement with regard to this classification, an adjudication committee will be convened. This committee, consisting of the NIAID Medical Monitor, the ITN Clinical Trial Physician and the Protocol Chairs, will determine whether the event should be classified as rejection.

A participant will be considered to have experienced presumed rejection regardless of biopsy findings if he or she receives more than a single dose of steroids for the purpose of treating rejection.

6.5.5 Treatment and Management of Participants with Rejection

Immunosuppression according to institutional standard will be reinstituted for all participants who experience rejection as confirmed by local pathology read. These participants will undergo safety follow-up per Appendix 5. Safety Follow-up: Recipients.

All participants who experience presumed rejection without biopsy confirmation (see section 6.5.4.2) will also resume immunosuppression according to institutional standard and will undergo safety follow-up per Appendix 5. Safety Follow-up: Recipients.

All participants with biopsy-confirmed rejection assessed by either central or local pathology reading will undergo a repeat biopsy (RB) 4 weeks after diagnosis to document that the rejection has resolved (see section 6.5.7.3) .

6.5.6 Types of Kidney Biopsies

The following types of renal biopsies are defined for this trial:

6.5.6.1 Protocol biopsies

Participants will undergo renal biopsies at times specified in the schedule of events. These biopsies will be used to monitor renal function, to screen for subclinical rejection, to determine eligibility for immunosuppression withdrawal and for mechanistic analyses.

6.5.6.2 For-cause biopsies

For-cause biopsies (FCB) will be obtained to confirm suspected rejection following unexplained allograft dysfunction and for performing mechanistic analyses.

6.5.6.3 Repeat biopsies

Participants will undergo a repeat biopsy (RB) at a specified interval after an initial protocol biopsy or for-cause biopsy (FCB) to confirm the resolution of histological abnormalities or to ensure that none have evolved.

6.5.7 Use and Interpretation of Kidney Biopsies

All renal biopsies will be interpreted and analyzed by both local and central pathology laboratories. The guidelines for the use of these pathology reports are outlined below.

6.5.7.1 Protocol Biopsies

For purposes of determining study outcomes such as eligibility for immunosuppression withdrawal or endpoint classification, the local and central pathologist will each report a Banff score according to the 2007 Banff classification[1]. If significant discrepancies between the two readings are identified, the two pathologists will be asked to conduct a collaborative consultation. This consultation may include the exchange of images or biopsy material. A 'significant discrepancy' is defined as greater than or equal to one letter grade difference in Banff score (i.e., IA versus IB) or reports that result in discrepancies in eligibility or endpoint classification. If discrepancies cannot be resolved through this collaborative consultation, an independent third party will be used for adjudication of biopsy classification. In cases where there is no local read the central read will be used.

For the purposes of clinical management the local pathology read will be used.

6.5.7.1.1 Biopsy for Determination of the Primary Endpoint

A protocol biopsy is required for determination of the primary endpoint. Participants who decline to undergo the post immunosuppression withdrawal protocol biopsy, as designated in Appendix 3. Immunosuppression Withdrawal (Initiated Between 26 and 3 Weeks Post-Transplant), will be considered to have failed the primary endpoint and will be restarted on immunosuppression at the discretion of the site Investigator. Participants will be counseled on the risks of remaining off immunosuppression without appropriate surveillance biopsies.

6.5.7.2 For-cause Biopsies

For all FCBs the local pathology read will be used for clinical management and determination of study outcomes such as continued immunosuppression withdrawal. For the purposes of analysis the central and local findings will be considered in conjunction with any clinical treatment to determine rates of rejection. Significant discrepancies between central and local readings will be adjudicated by a third party pathologist.

6.5.7.3 Repeat Biopsies

Participants will undergo a RB after an initial biopsy under the circumstances listed below.

6.5.7.3.1 Biopsy-confirmed Rejection

All participants with biopsy-confirmed rejection assessed by either central or local pathology reading will undergo a RB 4 weeks after diagnosis to document that the rejection has resolved. This RB will be interpreted and analyzed using the guidelines FCB (see section 6.5.7.2).

6.5.7.3.2 Indeterminate Rejection on Withdrawal Eligibility Biopsy

Participants with indeterminate acute cellular rejection (<IA) as the worst read on eligibility biopsy for immunosuppression withdrawal as read by either local or central pathology lab will undergo a RB within 4 weeks of their initial biopsy unless medically contraindicated. The results of this biopsy will be used to determine eligibility for immunosuppression withdrawal.

These biopsies will be considered protocol biopsies and will be interpreted and analyzed using the guidelines above for all other protocol biopsies.

6.5.7.3.3 Development of DSA off Immunosuppression

A participant who undergoes a FCB for the development of DSA off immunosuppression after successful completion of immunosuppression withdrawal will undergo a RB 3 months after the DSA biopsy is obtained (see section 6.6). This RB will be interpreted and analyzed using the guidelines for FCB (see section 6.5.7.2).

6.5.7.4 Determination of Acute Rejection Rate for Stopping Guidelines

For the purposes of determining rates of acute rejection for stopping guidelines both local and central pathology reads will be used as described above; the local read will be used for for-cause biopsies and the worst read will be used for protocol biopsies. In addition all episodes of presumed rejection without biopsy confirmation will count towards this determination.

6.5.8 Biopsy Technique and Handling

The Day 0 protocol biopsy will be performed as a core biopsy on the back table prior to transplantation of the kidney into the recipient. Otherwise, the protocol and for-cause biopsies will be performed percutaneously under ultrasound guidance with a 15- to 18-gauge needle. Use of a 16-gauge needle or larger is preferred. A minimum of two core biopsies should be obtained with a minimum of 1 cm of tissue per core biopsy. Where possible, trained personnel should confirm, by visual inspection, that biopsy tissue contains glomeruli (cortex). If insufficient tissue is obtained, cortical tissue should be allocated first to the ITN Pathology core and then to processing for gene expression studies. A consent specific for this procedure will be obtained according to the guidelines at each study site.

6.5.9 Recording of Biopsy Interpretations

The local pathology read shall be recorded on the eCRF by the site. The central pathology read will be recorded by the ITN central pathology core laboratory and the data will be transferred at regular intervals to the clinical database.

6.6 MONITORING AND MANAGEMENT OF DONOR SPECIFIC ANTIBODIES

Participants will be monitored at regular, frequent intervals for the occurrence of HLA alloantibodies including donor-specific antibodies (DSA) per SOE (see Appendix 1A. Pre-Transplant Through Transplant: Recipient through Appendix 5. Safety Follow-up: Recipients).

Participants who develop DSA prior to initiation of immunosuppression withdrawal will be deemed ineligible for withdrawal.

Participants who develop DSA during immunosuppression withdrawal or while on tacrolimus monotherapy will restart immunosuppression and will move to safety follow up per Appendix 5. Safety Follow-up: Recipients

Participants who develop DSA while off immunosuppression after successful completion of immunosuppression withdrawal will undergo a FCB. Immunosuppression will be restarted

and the participant will move to safety follow-up (Appendix 5. Safety Follow-up: Recipients) if any of the below are observed by the local pathologist:

- Diffuse c4d+ (defined as positive staining of >50% of the PTCs stained) AND either:
    a. Glomerulitis ≥ Banff g1 or
    b. Transplant glomerulopathy ≥ Banff cg1, or
- Rejection of any type (see section 6.5.4.1).

Other concerning biopsy findings, including but not limited to capillaritis or inflammation with focal or absent c4d staining, will prompt a review by a committee consisting of the protocol chairs, the NIAID Medical Monitor, the ITN Clinical Trial Physician and the central pathologist. Immunosuppression will be restarted per committee recommendation.

All participants who develop DSA off immunosuppression will undergo a RB 3 months after the initial FCB and the same criteria for restarting immunosuppression will apply.

6.7 GRAFT-VERSUS-HOST DISEASE

All participants will be monitored closely for GVHD. All episodes of GVHD will be graded. Treatment of GVHD will be initiated per standard of care at the discretion of the investigator.

6.7.1 Acute GVHD

Acute GVHD will be graded by standard criteria (see

Table 4)[68]. The occurrence of acute GVHD will be assessed at all study visits between transplant and Day 200 post-transplant using an acute GVHD assessment worksheet (Appendix 6. Acute GVHD Assessment Worksheet). Time to onset, organ involvement, maximum grade and dates of treatment will be recorded. All suspected cases of acute GVHD greater than grade 1with skin involvement must be evaluated histologically by a skin biopsy. Biopsies from other sites or organs will be obtained as clinically indicated.

Table 4. Consensus conference on clinical grading of acute GVHD

Grading of acute GVHD

Stage	Skin	Liver	Intestinal Tract
1	Maculopapular rash <25% of body surface	Bilirubin 2-3 mg/dL	> 500 ml diarrhea/day
2	Maculopapular rash 25 - 50% of body surface	Bilirubin 3-6 mg/dL 1	> 1000 ml diarrhea/day
3	Generalized erythroderma	Bilirubin 6-15 mg/dL	> 1500 ml diarrhea/day
4	Generalized erythroderma with bullous formation and desquamation	Bilirubin > 15 mg/dL	Severe abdominal pain, with or without ileus

Grade	Overall Clinical Grading of Severity of Acute GVHD
I	Stage 1 - 2 skin rash; no gut involvement; no liver involvement; no decrease in clinical performance
II	Stage 1 - 3 skin rash; stage 1 gut involvement or stage 1 liver involvement (or both); mild decrease in clinical performance
III	Stage 2 - 3 skin rash; stage 2 - 3 gut involvement or 2 - 4 liver involvement (or both); marked decrease in clinical performance
IV	Similar to Grade III with stage 2 - 4 organ involvement and extreme decrease in clinical performance

6.7.2 Chronic GVHD

Chronic GVHD will be graded by NIH consensus criteria (see Table 5)[69]. The occurrence of chronic GVHD will be assessed in all participants, regardless of donor chimerism, at every study visit between 60 days and 2 years post-transplant using the Chronic GVHD Assessment Worksheet (see Appendix 7. Chronic GVHD Assessment Worksheet) . If a participant's chronic GVHD persists beyond 2 years post-transplant or if he or she is diagnosed with chronic GVHD >2 years post transplant, the chronic GVHD Assessment Worksheet will be graded at every subsequent study visit until resolution of the episode or until the end of study participation.

Time to onset and date of biopsy proof (if any) will be recorded, as well as dates and types of treatment.

Table 5. Clinical Grading of Chronic GVHD

Grade	Overall Clinical Grading of Severity of Chronic GVHD based on Global Scoring of Individual Organs
Mild	Involves only 1 or 2 organs or sites (except the lung) with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites).
Moderate	• At least 1 organ or site with clinically significant but no major disability (maximum score of 2 in any affected organ or site) OR • Three or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites).
Severe	• Major disability caused by chronic GVHD (score of 3 in any organ or site). • A lung score of 2 or greater will also be considered severe chronic GVHD

6.8 ENGRAFTMENT SYNDROME

Engraftment syndrome will be assessed by the clinical study team based on the following criteria:

1. Symptoms consistent with engraftment syndrome (see below)
2. A renal biopsy consistent with engraftment syndrome[70]

Symptoms:

The presence of all symptoms or symptoms 1, 2 and 4 will trigger a for-cause biopsy to confirm engraftment syndrome:

1. Temperature >39 deg C
2. Erythroderma/rash > 50% body surface
3. Non-cardiogenic pulmonary edema/pulmonary infiltrates
4. Creatinine >4

occurring in the time interval between day 0 and 1 week after recovery of ANC, without apparent other cause.

Biopsy:

Engraftment syndrome will be confirmed by the presence of histological features on renal biopsy consistent with this diagnosis[70]. These may include but are not limited to:

1. Prominent interstitial haemorrhage, congestion and edema
2. Acute tubular injury
3. Minimal or no interstitial inflammation or tubulitis
4. Occasional intracapillary mononuclear or polymorphonuclear cells

7. MECHANISTIC ASSAYS

7.1 RATIONALE FOR IMMUNE STUDIES

A battery of mechanistic assays will measure the immune response in pre- and post-transplant samples from patients undergoing combined kidney and HSCT. Results from these assays will correlate the degree of hematopoietic chimerism to the rate and success in achieving clinical tolerance. Additionally, these assays will identify potential biomarkers that are predictive of patient success for immunosuppression withdrawal (ISW). Biomarkers may be most easily identified after immunosuppression withdrawal since the process of withdrawal or its outcome may well create or magnify differences between the two populations (immunosuppression withdrawal-success and immunosuppression withdrawal-failure). For the derived biomarker(s) to be clinically useful as an indicator, the differences must be appreciable in specimens collected at baseline, prior to attempted withdrawal.

The central hypothesis of the study is that stable or rising T cell chimerism greater than 50% at six months post transplantation is sufficient, but not necessary, for evidence of hypo-responsiveness to host and donor antigens *in vitro* and for kidney allograft tolerance without maintenance immunosuppression. The exact mechanism(s) responsible for cyclophosphamide induced tolerance is not clear, but may include deletion of alloreactive clones and/or up-regulation of regulatory T cells. It is additionally hypothesized that hematopoietic chimerism plus cyclophosphamide-induced tolerance affects B-cell responses producing a durable elimination of HLA antibody, thereby increasing the probability for successful kidney transplantation. It is unclear whether the B cell response is a direct response to the study regimen or is an indirect result of targeting T cells and the subsequent modulation of the adaptive immune response. The assays described below have been designed to test these hypotheses and monitor the patient's overall immune response including changes in HLA antibody, peripheral blood cell subsets, immune response in biopsy samples, cellular activation, T and B cell alloreactivity and changes in gene expression etc.

7.2 PLANNED MECHANISTIC ASSAYS

7.2.1 Histological Assessments of Tolerance Mechanisms

Biopsies are used to identify the cause or process responsible for allograft dysfunction. In the case of patients with normally functioning allografts, biopsies are used to monitor structural integrity of long surviving allograft as well as response to the treatment. Renal biopsies are used to determine the absence of acute, intermediate and chronic rejection at intervals before and after immunosuppression withdrawal. Biopsies are thus necessary to rule out misclassification errors that would compromise subsequent attempts to identify "biomarkers of tolerance".

Multiplex antigen labeling (immunohistochemistry IHC) by the ITN core pathology laboratory will be used to determine state of the allograft tissue during immunosuppression withdrawal at baseline and longitudinally in banked specimens. Suggested staining panels are described in Table 6 with their key attributes and will be finalized once longitudinal banked specimens are available. We will test the hypothesis that the renal allograft itself plays a critical role in development and maintenance of tolerance. It may achieve this via gradual erosion of donor reactivity through inefficient stimulation by donor antigen presenting cells and a microenvironment hostile to effector and memory cell development and maintenance. In a

recently completed ITN liver transplant trial (ITN029ST), patients immunosuppression withdrawal status correlated with differences in C4d expression, γδT cell ratio, and MHC class II positive cell frequency[71] (and unpublished observations). Additional ITN studies have demonstrated increased CD20, FoxP3, and CD3 gene expression in urinary cell sediment of tolerant kidney transplant recipients compared to SI or healthy controls (ITN507ST)[72].

Detailed biopsy analysis using these markers and additional IHC panels proposed in Table 6 will evaluate factors associated with donor reactive cells, absence of inflammation (effector T cells), and increased ratios of regulatory cells and effector to memory cell ratio in the transplant organ niche. The analyses will reflect systemic immune reactivity toward the allograft and the allograft's ability to suppress the immune response. These findings will be interpreted in conjunction with other cellular, molecular, and immunologic assays performed within this trial.

Table 6. Proposed Multiplex IHC Staining Panel

1.	Ki-67 (proliferation marker)
2.	CD68 (pan monocyte, macrophage, and dendritic cell marker)/ DC-SIGN (myeloid dendritic cell marker); check for infiltration of CD68+ macrophages (DC-SIGN negative) with ISW
3.	CD8 (T subset)
4.	CD20 (B cell infiltrates)
5.	NKG2D (NK, CD8+ activated, NK1.1+ T, some myeloid cells)
6.	CD34 (hematopoietic precursors, capillary endothelial cells)
7.	Myeloperoxidase (MPO, heme protein secreted by activated phagocytes)
8.	C4d (deposition suggests antibody mediated rejection; Is C4D deposition associated with failed ISW?)
9.	CD3/CD45RO/CD45RA (ratio of naïve to memory T cells, do increased memory cells associate with failed IS withdrawal?)
10.	CD3/γδ-1/ γδ-2 (Is increased ratio of γδ-1/ γδ-2 associated with ISW or tolerance)
11.	HLA-DR/CD31 (is expression of HLA-DR associated with failed ISW?)
12.	CD4/Tbet/GATA-3/IL-17/FoxP3 (regulatory T cells)
13.	IL10/TGFβ/HLA-DR (is increased IL-10 expression associated with transplant tolerance?)
14.	CD56/PD-1/CD3 (ratio of CD3+, CD56+, and PD-1+ lymphocytes and whether changes in NKT cells are associated with operational tolerance?)
15.	CD5/CD19/CD27/IgG (ratios of naïve to memory B and B1:B2; do increased numbers of memory or regulatory B contribute to allograft acceptance or rejection?)
16.	CD3/CD45RO/CD45RA (ratio of naïve to memory T cells, test if increased numbers of memory cells are associated with failed ISW?)

7.2.2 Gene Expression in peripheral blood and biopsies

Total RNA and miRNA may be extracted and batch processed from banked specimens for gene expression experiments. As patient numbers are insufficient for *de novo* gene discovery, only *a-priori* hypothesis based assays are feasible. Ongoing studies in peripheral blood of renal transplant recipients by the ITN and a European consortium identified 25 immune-function related genes that differentiated operationally tolerant patients from patients receiving

continued immunosuppression[72-74]. Table 7 lists the 3 genes published by the ITN[72] and an additional 10 genes published from RISET. Expression of these genes, and additional genes reported with a strong association to tolerance, may be assessed using samples from this trial. Follow up work by the ITN demonstrates that the published ITN renal signature is broadly indicative of tolerance irrespective of the therapeutic intervention (unpublished data). It will therefore be informative to test if these reported tolerance signatures differ based on the presence or absence of hematopoietic chimerism and/or the induction regime used in this protocol.

Biopsy tissue, if available, for gene expression studies, may be compared pre- and post-withdrawal and between immunosuppression withdrawal success and failure patients. A gene panel for biopsy and micro-RNA will be finalized before the start of the experiment based on current literature.

Drug effects may confound mechanistic observations; therefore, care needs to be taken in the interpretation of data comparing patients on immunosuppression versus those that have withdrawn from immunosuppression. This increases complexity given that greater and more frequent gene expression differences are typically seen between pre- and post-immunosuppression withdrawal samples. Comparisons at baseline will be important since patients will be on the same or similar treatment regimens and a detectable signature at the baseline would allow selection of the patients most likely to be successful in immunosuppression withdrawal. Gene expression levels will be quantified in whole blood (and biopsy tissue if available) by RT-PCR in conjunction with samples from the ITN biorepository to assess comparative levels of the known "tolerance" signatures.

Table 7. Thirteen (13) of 25 genes used to derive renal tolerance signatures in 3 clinical studies

	Genes*[1]	Description	ITN/ RISET
1.	IGKV1D -13	Part of the immunoglobulin kappa variable cluster (IGKV@), 1D-13 involved in antigen binding*[2].	ITN
2.	IGKV4-1	Part of the immunoglobulin kappa variable cluster (IGKV@), involved in antigen binding 4-1*[2].	ITN
3.	IGLL1	Immunoglobulin lambda like polypeptide-1 is found on the surface of proB and preB cells. Gene encodes one of the surrogate light chain subunits of the preB cell receptor.	ITN
4.	TLR5	Fundamental role in pathogen recognition (bacterial flagellins) and activation of innate immunity by recognition of pathogen-associated molecular patterns that are expressed on infectious agents. Acts via MYD88 and TRAF6, leading to NF-κB activation, cytokine secretion, and the inflammatory response. It is highly expressed in peripheral blood leukocytes, particularly monocytes.	RISET
5.	PNOC	Secreted protein that binds the opioid receptor–like receptor (OPRL1). Altered plasma levels have been reported in patients with various pain states, depression, and liver disease.	RISET

	Genes*[1]	Description	ITN/ RISET
6.	SH2D1B	Single SH2-domain adapter that binds to specific tyrosine residues in cytoplasmic tail of SLAM and related receptors. Signals downstream of CD84 (upregulated in memory B). Stimulated B cells undergo early apoptotic events in the presence of SH2D1B.	RISET
7.	SLC8A1	Transmembrane protein that plays a fundamental role in Ca2+ refilling in the endoplasmic reticulum. In macrophages and monocytes, it restores Ca2+ signals that induce TNF-α production.	RISET
8.	H3ST1	Member of the heparan sulfate biosynthetic enzyme family, is a rate-limiting enzyme for synthesis of anticoagulant heparin	RISET
9.	FCRL1	Membrane protein, considered as a B cell co-receptor. Specifically expressed by mature B lineage cells, with higher expression in naive versus memory B cells.	RISET
10.	FCRL2	Membrane protein, expressed preferentially by memory B cells.	RISET
11.	MS4A1	B lymphocyte–specific, cell-surface molecule involved in B cell activation and differentiation.	RISET
12.	TCL1A	Located downstream of the BCR signaling pathway. Overexpression prolongs naive B cell survival.	RISET
13.	CD79B	Part of the B cell antigen receptor (BCR) complex	RISET

*[1]: Twelve (12) additional genes from recent ITN507ST and RISET clinical study results are unpublished at this time and therefore not included in this table, but will likely be included in these studies.

*[2]: During lymphoid differentiation into Ab. producing cells, an IG kappa variable region is transcriptionally activated by rearrangement linking it to a kappa constant (C kappa) region gene that is transcribed prior to somatic mutation.

7.2.3 Multi-Parameter Flow Cytometry (MFC)

In an ITN study (FACTOR, ITN507ST) renal allograft tolerance was strongly associated with a baseline B cell signature using flow cytometry as well as gene expression assays[72]. The baseline flow cytometry B-cell signature was associated with elevated numbers of peripheral blood total B cells, and sub-populations of naive and transitional B cells in tolerant participants when compared to participants on standard immunosuppression.

In a recently completed, unpublished, frozen flow cytometry analysis of ITN507ST longitudinal samples, the tolerance associated B cell phenotype (higher frequencies of total B cell, naïve B cells and T1/ 2 transitional B cells when compared to the standard immunosuppression group), was reproducible and persisted longitudinally. The group on standard immunosuppression displayed higher frequencies of activated B memory cells. These results highlight a critical role for B cells in regulating alloimmunity.

Peripheral blood will be evaluated using flow cytometry on frozen/banked specimens to identify specific cells or cellular subsets that correlate with tolerance or other clinical

phenotype. Frozen flow, performed on banked specimens, has the advantage that it can be batch processed, allowing for the same reagents to be used for each sample. This typically generating tighter data sets for longitudinal samples. A second significant advantage of working with stored samples for clinical trials of significant duration is that the most relevant, up-to-date antibody staining panels can be used.

Longitudinal changes in T cells, B cells, NK cells, and monocytes will be evaluated using panels of antibodies directed at cell surface markers, nuclear factors, and intracellular cytokines. Cell populations of interest include lymphoid naïve, memory and transitional subsets, dendritic cell subsets (plasmacytoid, pDC and myeloid, mDC) and NK cells as well as activated lymphoid subsets. We hypothesize that cyclophosphamide will induce tolerance through the up-regulation of regulatory T cells. Antibody panels will be finalized and validated by the ITN flow core prior to starting assays. Examples of panels that may be used for analysis are listed in Table 8.

Table 8. Frozen/banked specimen flow cytometry panel design and list of suggested cellular antigens

Function	1	2	3	4	5	6	7	8	9	10	11	12
T sub-set, T regs	CCR 7	CD12 7	CD25	CD3	CD45 RA	CD45 RO	CD4	HLA-Dr	CD8	FoxP 3	Helio s	Live/ Dead
B Cell sub-set*[1]	CD3	CD19	IgD	CD27	CD24	CD38	MTG	CD21	CD23	CD95	9G4	Live/ Dead
NK cells	NKG 2D	CD12 2	NKp4 4	CD3	CD56	CD4	HLA-Dr	CD8	CD16	Live/ Dead		
B cell subsets	IgD	CD24	CD10	CD19	CD38	IgM	HLA-Dr	CD27	CD20	Live/ Dead		
B cell cytokines	IgD	CD24	CD20	IFNg	CD38	IgM	IL-10	CD27	TGFb	Live/ Dead		
TLR5, DC and monocytes	CD11 c	CD12 3	CD20	CD3	CD56	CD16	HLA-Dr	CD14	TLR5	Live/ Dead		

***[1]: This panel was used to analyze B-cell subsets in banked baseline and longitudinal samples of ITN507ST and will be included in current trial to compare CKBMT patients with ITN507ST kidney transplant recipients.**

Data analyses will identify differences in baseline immune phenotype between participants who succeed and those who failed immunosuppression withdrawal. Longitudinal changes in cell phenotype will be assessed during and after immunosuppression withdrawal and will be correlated with successful versus failed withdrawal. Frozen flow markers used previously to analyze the B cell subset of ITN507ST participant longitudinal visits (see B cell panel above) can be included in this study to allow direct comparison between these two trials. We expect that the flow panels and analyses planned in this study will expand our understanding of mechanisms of immune tolerance as well as compare results of combined kidney and bone marrow transplant (CKBMT, this trial) with kidney transplant alone (ITN507ST).

7.2.4 Alloreactive T Cell and Plasma Cytokine Assays

T cells are central mediators of anti-donor immunity that can cause direct (cell mediated) or indirect (through B cells and innate immune mechanisms) allograft damage through pre-existing recipient alloreactive antibodies. Frequencies of proliferating alloreactive T cell can be measured *in vitro* when performed in limiting dilutions of mixed lymphocyte responses (MLR, CD4) or cell mediated lympholysis (CML, CD8) assays, done at pre-transplant and longitudinal intervals. In addition, T cell alloreactivity can also be measured by monitoring specific cytokine levels directly from the patient's peripheral blood, *ex vivo*. These multiplex cytokine panels can be designed to measure the alloreactivity mediated by direct or indirect pathways and can define the phenotype of corresponding T cell (such as Th1 or Th2). Taken together, these *in vitro* and *ex vivo* assays provide a comprehensive readout of the alloreactive T cells and their state of tolerance throughout the immune system.

Whole PBMCs and isolated CD3+ T cells from real-time specimens will be compared in MLR and CML assays. These methods have been successfully developed and tested at the Human Immunology Core Lab of the JHU (Dr. Alan Hess, Director, Human Immunology Core Laboratory). Depletion of $CD25^+$ (marker of lymphocyte activation) cells was shown to enhance third party CML and MLR responses in ITN036ST, a renal transplant study, and may be used in addition to above[75]. Donor specific suppressive activity of regulatory cells may be present pre-transplant or induced post-transplant and these can be measured *in vitro*. Donor antigens and/or third party stimulators will be cultured with recipient PBMC from pre-transplant and post-transplant collections for these "stimulated suppression assays". In addition, supernatants will be collected after five days of the mixed lymphocyte culture and analyzed for the levels of multiple cytokines using multiplex assays, e.g. the Bio-Rad Pro Human Cytokine 17-plex assay: (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP1β, and TNF-α).

These experiments, in conjunction with other mechanistic assays, will test whether tolerance in CKBMT recipients is due to deletion or anergy of donor reactive T cells. Results of cytokine assays from mixed lymphocyte cultures will be related with cytokine levels in patient's serum/plasma taken at the onset of fever between days 2-5 after transplantation, effectively representing an *in vivo* MLR. The same cytokine panels, as those used for the MLR described above, will be tested in the patient's serum/plasma samples. We will compare levels of individual cytokines between patients with, versus without, sustained donor hematopoietic chimerism to determine if there is a cytokine pattern that predicts donor hematopoietic engraftment and kidney allograft tolerance.

7.2.5 Alloreactive B Cell Assays and B cell Repertoire Analysis

Activation of alloreactive B cells or pathogenic alloantibodies can contribute to graft rejection either directly or indirectly, in addition to alloreactive T cell response. Antibodies to donor HLA antigens are a major barrier to transplant acceptance and characterizing these specific anti-HLA-antibody secreting B cells provides a method to identify humorally sensitized patients. These HLA-specific B cells can be identified/ phenotyped, enumerated and isolated by staining with HLA tetramers[76,77].

B cells specific for mismatched HLA antigens (including: HLA-A1, A2, A11, A24, B7, or B15) will be quantified and isolated (by fluorescent PE labeled tetramers) from both the recipient and donor (real-time specimens) at pre-specified time points per the schedules of events (see appendices 1 through 5). Whenever possible, HLA tetramers for mismatched,

autologous, and third party antigens will be used. These methods have been successfully developed and tested at the Immunogenetics Laboratory of the JHU and will be carried out there[75-77]. Isolated HLA-specific B lymphocytes (and B lymphocytes isolated from PBMC) will be tested for their ability to respond to alloantigenic challenge by flow cytometric measurement of activation markers[78] and may also be tested for possible deletion or expansion of particular clones using high throughput DNA sequencing of the B cell receptor [79].

Experimentally, multiplex PCR may be run to amplify rearranged BCR loci from the genomic DNA or cDNA. The amplified products that uniquely identify the V and J segments of the corresponding CDR3 region may then be sequenced on the Illumina platform and compared to germline sequences catalogued on public websites (e.g. International ImmunoGenetics database). The aligned sequences or groups of sequences reflect the B cell composition in the original sample and can be analyzed statistically by their V-J recombination frequency, BCR diversity, distribution of BCR clonotype frequencies that represent clonal expansions or deletions etc.

The ITN and Dr. Maria H. Fuentes (Kings College London and ITN collaborator) have consistently and independently[72,80] seen expansion of B cell populations in renal transplant tolerant participants versus participants on SI. During these analyses, a consistent "B cell gene signature" was also observed from several immunoglobulin variable genes regions (IGKV1D-13, IGKV4-1 and 4 other unpublished genes). The expanded B cells may preferentially use these variable region immunoglobulin gene segments. We may therefore, test for hyper expansion of the Ig variable regions (heavy and light chains) as well as somatic mutations within such clonal expansions that may represent clonal cell lineage. These analyses may be done using longitudinal samples, and the results correlated with other mechanistic data as well as clinical outcomes. These BCR sequencing experiments would need to be combined with other ITN renal transplant studies such as ITN507ST or ITN036ST to generate sufficient sample size.

7.2.6 Blood Chimerism

There is increasing evidence to support that patients with hematopoietic chimerism are tolerant of solid organ allografts from the same donor and can be weaned off immunosuppression even when chimerism is transient. In a recent renal transplant study at ITN, 4 out of 5 patients with end stage renal disease (ESRD) that received CKBMT after non-myeloablative regimen had transient hematopoietic chimerism (21 days) and were successfully weaned off all immunosuppression[25]. In longitudinal analysis of these 5 patients along with 5 additional patients treated similarly (ITN036ST), 7 out of the total 10 patients with transient hematopoietic chimerism were successfully weaned off drugs although idiopathic capillary leak syndrome was observed in 9 of the 10 patients. It is unclear whether sustained hematopoietic chimerism will result in durable renal allograft tolerance.

A similar treatment regimen as that described in this protocol was shown to achieve full, or near full, donor chimerism in 5 (also completely off immunosuppression) of 19 patients with sickle cell anemia or thalassemia, receiving transplantation from HLA matched or HLA-haploidentical first degree relatives[81]. For 5 of 19 patients that experienced graft failure in that study, donor chimerism was lost early after transplantation. Furthermore, durable, but not full, hematopoietic chimerism was achieved in >50% of the patients. We expect to induce durable chimerism in most of the patients transplanted during this trial, and at least temporary chimerism.

Experimentally, a buccal swab will be collected from the donor and recipient pre-transplant to measure degree of donor chimerism at the Pathology Molecular Diagnostics Lab of the JHU labs. Following transplantation, peripheral blood from transplant recipients and CD3+ cells separated from peripheral blood using the RoboSep® automated instrument (StemCell Technologies) will be used to measure the degree of chimerism. Fifteen microsatellite markers and the amelogenin locus will be amplified by PCR using AmpFlSTR® Identifiler PCR Amplification Kit (Applied Biosystems). The resulting PCR products will be analyzed by capillary electrophoresis and peak heights of the informative alleles compared to calculate a percentage engraftment[82,83]. The true limit of detection for an individual reaction is both locus and PCR dependent. The formal limit of detection is 5%. A micro chimerism assay with improved sensitivity is also being developed by this lab and may be adopted if validated prior to the start of this study. The degree and durability of chimerism will be correlated with organ allograft tolerance. Chimerism levels may be correlated with T and B cell phenotype, alloreactivity, and possibly gene expression to identify predictors and mechanisms of tolerance.

7.2.7 Anti-donor HLA Alloantibody and Flow Cross Match

HLA Abs have been strongly implicated as a risk factor in kidney transplant recipients, their presence at baseline may preclude successful immunosuppression withdrawal. Moreover, after initiation of immunosuppression withdrawal, monitoring the increasing breadth and/or strength of alloantibodies particularly if they are directed against donor antigens, may be important as this may signal the presence of an immune response that will be deleterious to long-term allograft histology.

Serum samples collected from participants will therefore be evaluated for alloantibodies, including donor-specific alloantibodies (DSA), in real time at the Immunogenetics Laboratory of the JHU. Assessments will be performed prior to immunosuppression withdrawal and at multiple time points during and after immunosuppression withdrawal. An initial screen will determine if the participant has detectable alloantibodies. If the screening assay is positive, antibody specificities will then be determined using beads coated with single HLA-antigens.

HLA typing of donor and recipient will be necessary to assign donor specificity. Whole blood will be collected from all donors and recipients for HLA typing. Details of HLA typing assessment are provided below.

The flow cytometry crossmatch (FCXM) is a highly sensitive method for confirming the presence of DSA. FCXM testing will be performed selectively, that is, only after observing DSA in participant serum. Experimentally, three color FCXM is performed by incubating donor cells or surrogates with recipient serum followed by addition of a fluoresceinated (FITC) goat, anti-human polyclonal immunoglobulin. A phycoerythrin (PE) labeled monoclonal antibody that detects B cells along with chlorophyll protein (PerCP)-conjugated monoclonal antibody that detects T cells will be analyzed by flow cytometry and expressed as positive or negative based on a shift in median channel fluorescence intensity (MFI) of the test serum with respect to negative control. This method can also be adapted to assess anti-monocyte Abs if needed. If necessary, pronase treatments to reduce non-specific background staining or dithiothreitol (DTT) treatments to eliminate interference from IgM class Abs can be included.

Results from Alloantibody and FCXM may be used to evaluate: 1) whether *de novo* anti-HLA alloantibody correlates with successful IS withdrawal or failure, 2) whether donor-specific alloantibody (DSA) correlates with IS withdrawal success or failure, 3) compare temporal patterns of recipient anti-HLA antibodies between IS withdrawal success and failure participants; and longitudinally with chimerism durability, 4) whether baseline anti-HLA antibody and/or donor-specific anti-HLA antibody profiles classify participants with donor chimerism strength and durability as well as with other clinical outcomes 5) determine relationship between serum HLA alloantibody strength and tissue immune histopathological measurements, such as complement pathway products or activated lymphocytes.

7.2.8 HLA Typing

HLA typing information will be collected as standard of clinical care for transplantation. Information will be used to assess presence of DSA and will be done on early/ baseline specimens collected in this trial from both allograft donors and recipients. Whole blood collected from participants (recipients and donors) will be used to perform sequence-based HLA-typing (HLA-A, B, C, DRB1, DRB3-5, DQA1, DQB1, DPB1) in real time at the Immunogenetics Laboratory of the JHU.

7.3 OVERVIEW OF DATA ANALYSIS

This study is designed to assess ability of a novel conditioning regimen, bone marrow transplantation and high dose PT/Cy to enable discontinuation of immunosuppressive therapy, as well as to assess safety in haploidentical living related donor renal transplant recipients. The study size (total of 6 participants) is limited by considerations of safety of the novel testing regimen that may include risk of graft-versus-host disease (GVHD), graft rejection, graft loss, infection and malignancy etc. The accrual of six participants is planned in two stages (3:3) with a pre-specified interim safety analysis. If safety of conditioning regimen is deemed unacceptable, the experimental pre-transplant conditioning regimen may be omitted. The change, if it occurs, will effectively create two participant subgroups (with and without conditioning regimen), and may impact the scope of data analysis. Based on the nature of the study, the primary mechanistic data analysis strategy is individual participant-based longitudinal profiling which includes graphic plotting and descriptive statistics etc. Exploratory analysis is also planned to discover potential molecular and cellular patterns that might be linked to withdrawal outcome, explained below.

7.3.1 Individual based longitudinal profiling

Mechanistic samples from patients enrolled in this study will be assayed as described in the individual sections above with the aim to identify specific cell phenotypes, donor chimerism, intracellular responses and /or gene sets (tissue and/or peripheral blood) that correlate with successful immunosuppression withdrawal or predict failure thereof. These assay data may also be assessed in support of study safety evaluation of the experimental pre-transplant regimen (such as by flow cytometry or alloreactive assays). Graphic plots will facilitate longitudinal monitoring of relevant cellular populations, immune cell repertoire, donor chimerism, DSA and gene expression etc., in conjunction with the treatment process, adverse events and clinical outcomes. Descriptive statistics will be provided as appropriate.

7.3.2 Exploratory analysis

The study participants may be grouped based on primary clinical outcome (withdrawal success vs. failure) and/or degree of chimerism. However, the classification variables will be obtained at the end of the study (withdrawal outcome) or after marrow transplant (donor chimerism) rather than pre-defined before participant recruitment. Hence, the limited and uncertain sample size of these groups limits the use of formal statistical hypothesis testing approaches. The primary objective of exploratory analysis is to discover molecular, cellular, and immunogenetic patterns among the six participants at clinical milestones during the study that may provide insights into potential association with donor chimerism and/ or withdrawal outcome. Pattern recognition methods including hierarchical clustering and principal component analysis may be used for the analysis. The exploratory analysis will be conducted on individual assay datasets (flow cytometry, gene expression, histological assessment assays, etc.) as well as integrated multi-assay data.

7.4 FUTURE/UNPLANNED STUDIES

Specimens stored during the trial may be used in future assays to reevaluate biological responses as research tests are developed over time. Additionally, samples may be used for assays/ experiments outside the scope of this proposal, such as investigation of differences in the T cell receptor (TCR) repertoire as evaluated by sequencing, proteomics or other assays that may emerge and be compelling. Note that with only 6 participants in this study, it is likely that any future work would be in conjunction with other studies. Re-evaluations or new assays will only be performed on samples of participants who have consented for future research. The ITN sample sharing policy will apply for the provision of samples to study or outside investigators (www.immunetolerance.org). Any research conducted using stored samples for future use may also need appropriate regulatory approval, such as Institutional Review Board per the study consent.

7.5 SPECIMEN LOGISTICS

The clinical site will be trained in collection, processing, shipment, and tracking of mechanistic research specimens. The ITN will monitor specimen quality, shipping compliance etc. and retrain the clinical site if not producing optimum quality mechanistic samples. Johns Hopkins University clinical site will process all mechanistic samples according to the ITN standard procedures and use the ITN Specimen Tracking System (STS) software to identify and track all mechanistic specimens. The site is required to have its own calibrated laboratory equipment for use in standard ITN procedures, such as a centrifuge for spinning primary blood tubes, a micropipettor to aliquot specimens, and -70 or 80° C freezer to store frozen specimens until they can be shipped. JHU will use an appropriate courier service for shipping specimens to ITN repositories/ core labs, per ITN standard procedures. All shipping will conform to Department of Transportation regulations (49 CFR 173.199) for Diagnostic Specimens.

7.6 SPECIMEN TRACKING PROCEDURES

The ITN will track all mechanistic specimens until the final disposition of all material is known. Samples will remain in either JHU or ITN repositories until used for assays or destroyed.

7.7 SPECIMEN STORAGE

Samples will be stored under specific conditions to maintain long-term sample integrity, as well as specimen tracking from receipt to shipment to alternate locations. The ITN specimen tracking system will be used to track date of shipment, location shipped to, carrier, items shipped, amount shipped, barcode numbers, protocol number and associated comments about each individual specimen. Storage temperature, location, processing, aliquoting, and freeze/thaw events may also be recorded.

If the study subject allows storage for future studies, the subject's specimens will be stored indefinitely. The subject can change their mind at any time and have their stored specimens destroyed by notifying the study physician in writing. In such cases, the site coordinator would send all requests for sample destruction to the ITN. The site will receive confirmation that the specimen was destroyed as requested. If the subject's samples have already been analyzed, then the data will be used as part of the overall analysis. The subject can only request to have samples destroyed if they still exist, i.e. have not already been used in an experiment.

Specimens can only be transferred from JHU or the ITN repository to another destination with appropriate authorization per ITN standard procedures. Some items checked prior to authorization include: purpose for accessing/transferring the specimen (within study assay as defined by the protocol or future studies), evaluation of subject consent for the purpose provided, verification of specimen identifiers, and quality and quantity of the specimen.

8. ADVERSE EVENTS

8.1 OVERVIEW

The Principal Investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE (adverse event) or SAE (serious adverse event) as described in sections 8.2.1 and 8.2.3 in this protocol. All AEs and SAEs will be recorded in the source documents and on the appropriate electronic CRF(s). All data will be reviewed periodically by the DSMB, which may provide recommendations to NIAID about withdrawing any participant and/or terminating the study because of safety concerns.

Adverse events that are classified as serious according to the definition of health authorities must be reported promptly and appropriately to the NIAID, ITN, Principal Investigators in the trial, IRBs and health authorities. This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording and reporting them. Information in this section complies with 21CFR 312; ICH Guideline E2A*: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*; and ICH Guideline E-6: *Guidelines for Good Clinical Practice*; and applies the standards set forth in the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events, Version 4.0* (May 28, 2009).

8.2 DEFINITIONS

8.2.1 Adverse Event

An AE is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that occurs during participation in the trial. An AE for the recipient will be followed until it resolves or until 30 days after the recipient terminates from the study, whichever comes first. An AE for the donor will be followed until it resolves or until 48 hours after the final donor study visit, whichever comes first. All AEs will be reported as specified in section 8.3.2.1 whether they are or are not related to disease progression or study participation.

8.2.2 Adverse Reaction and Suspected Adverse Reaction

An adverse reaction means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

8.2.3 Serious Adverse Event or Serious Suspected Adverse Reaction

An AE or SAR is considered "serious" if, in the view of either the Investigator or NIAID Medical Monitor, it results in any of the following outcomes (21 CFR 312.32(a)):

- Death: A death that occurs during the study or that comes to the attention of the Principal Investigator during the protocol-defined follow-up period as defined in section 8.3.2.2 must be reported whether it is considered treatment-related or not.
- A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the Investigator or NIAID Medical Monitor, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- An event that requires 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 serious 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.

- Congenital anomaly or birth defect.

8.2.4 Unexpected Adverse Events

A SAR is considered "unexpected" if it is not identified in the package insert and/or drug label, investigator brochure, or protocol, or is not listed at the specificity, or severity that has been observed (21 CFR 312.32(a)).

8.3 COLLECTING AND RECORDING ADVERSE EVENTS

8.3.1 Methods of Collection

Adverse events for recipients will be collected from the time the participant receives the first dose of study medication as specified in section 8.3.2. Adverse events will be followed until the time an event is resolved or until 30 days after the recipient completes or terminates from the study, whichever comes first. The methods for collecting AEs will include:

- Observing the participant.
- Questioning the participant in an objective manner.
- Receiving an unsolicited complaint from the participant.

An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an AE if it is determined by the Investigator to be clinically significant. If this is the case, it must be recorded in the source document and as an AE on the appropriate AE form(s). The evaluation that produced the value or result should be repeated until that value or result returns to normal or can be explained and the participant's safety is not at risk.

8.3.2 Severity of Adverse Events to be Collected

8.3.2.1 Adverse Events

All AE grades will be defined per NCI-CTCAE version 4.0 criteria unless otherwise specified.

Recipient

With the following exceptions, all AEs of grade 3 or higher will be collected from the time at which the participant receives the first dose of ATG until the time the participant completes or prematurely withdraws from the study:

- During the first 30 days post-bone marrow transplant, expected hematological abnormalities associated with the non-myeloablative conditioning regimen, such as cytopenias, will not be collected as AEs. Rather, participants will undergo frequent complete blood counts as specified in the SOE (see Appendix 2. Post-Transplant Through

Evaluation for Withdrawal Eligibility). Delayed hematological recovery will be captured as an AE as follows:

- Delayed ANC recovery is defined as failure to achieve neutrophil recovery by day 30 following the post-transplant neutrophil nadir. The neutrophil nadir is defined as the first day post-transplant on which the ANC is below 500 per µL.
- Neutrophil recovery is defined as greater than or equal to 500/uL, the first of three consecutive measurements over at least two days.
- Delayed platelet recovery is defined as failure of recovery by post-transplant day 30.
- Platelet recovery is defined as greater than or equal to 20,000/uL with no history of platelet transfusions over the prior seven days.

- Hematological abnormalities will be collected as AEs per NCI-CTCAE v. 4.0 criteria once participants have achieved platelet and neutrophil recovery.

- All episodes of renal allograft rejection as defined in section 3.6.2 and GVHD as defined in section 6.7, regardless of grade, will be collected as AEs.

Donor

Only AEs related to bone marrow harvest and study-mandated blood draws will be collected for the donor.

8.3.2.2 Serious Adverse Events

Serious AEs, regardless of grade, will be collected from the time at which the recipient receives the first dose of ATG until 30 days after the recipient completes, or prematurely terminates, from the study. Serious AEs, regardless of grade, will be collected from the time the donor undergoes the first study-mandated blood draw until 48 hours after the final donor study visit.

8.3.3 Recording Method

8.3.3.1 Adverse Events

Throughout the study, the Investigator will record AEs on the appropriate eCRF regardless of their relation to study participation. The Investigator will treat participants experiencing AEs appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

8.3.3.2 Serious Adverse Events

Serious AEs will be recorded on the AE eCRF and on the SAE eCRF, and health authorities will be notified as outlined in section 8.5.

8.4 GRADING AND ATTRIBUTION OF ADVERSE EVENTS

8.4.1 Grading Criteria

The study site will grade the severity of AEs experienced by study participants according to the criteria set forth in the National Cancer Institute's *Common Terminology Criteria for Adverse Events Version 4.0* (published May 28, 2009). This document (referred to herein as the "NCI-CTCAE v. 4.0 manual") provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs.

Severity of adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE v. 4.0 manual:

- 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.

For additional information and a printable version of the NCI-CTCAE v. 4.0 manual, go to http://ctep.cancer.gov/reporting/ctc.html.

8.4.2 Attribution Definitions

Adverse events will be categorized for their relation to one or more of the following:

- bone marrow transplantation
- study medications
- immunosuppression withdrawal including withdrawal from mycophenolate compounds, prednisone and tacrolimus
- other protocol-directed study procedures

The Principal Investigator will do the initial determination of the relation, or attribution, of an AE to study participation and will record the initial determination on the appropriate eCRF and/or SAE reporting form. The relation of an AE to study participation will be determined using definitions in Table 9. Final determination of attribution for safety reporting will be decided by NIAID Medical Monitor.

Table 9. Attribution of adverse events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy)
Unrelated Categories		
1	Unrelated	The adverse event is clearly not related.
2	Unlikely	The adverse event is unlikely related.
Related Categories		
3	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
4	Probable	The adverse event is likely related.
5	Definite	The adverse event is clearly related.

8.5 REPORTING SERIOUS ADVERSE EVENTS

8.5.1 Reporting SAEs to the IND Sponsor

The following process for reporting a SAE ensures compliance with 21CFR 312 and ICH guidelines. After learning that a participant has experienced an SAE, the Investigator or designee will report the SAE via the electronic SAE report form (SAE eCRF) within 24 hours of becoming aware of the event to the sponsor. Initial SAE eCRF should include as much information as possible, but at a minimum must include the following:

- AE term.
- Relationship to study-mandated therapy and procedures.
- Reason why the event is serious.
- Supplementary CRF pages that are current at the time of SAE reporting: medical history, concomitant medications, demographics, study drug administration, death.

As additional details become available, the SAE eCRF should be updated and submitted to the sponsor. Every time the SAE eCRF is submitted, it should be electronically signed by the Investigator or sub-Investigator.

For additional information regarding SAE reporting, contact Rho Product Safety:

[redacted]

8.5.2 Reporting SAEs to Health Authorities

After the SAE has been reported by the site Investigator and assessed by the IND sponsor, the IND sponsor must report an event to the appropriate health authorities based on the following criteria:

- **Standard reporting.** This option applies if the AE is classified as one of the following and will be reported in the IND annual report:
  - Serious, expected, suspected adverse reactions (see sections 8.2.3 and 8.2.4).
  - Serious and not a suspected adverse reaction (see section 8.2.3).
- **Expedited reporting.** This option applies if the AE is considered serious, related to study medications described in section 5, and unexpected per section 8.2.4.

  These events must be reported by the sponsor to the appropriate health authorities within 15 calendar days; fatal or life-threatening events must be reported within 7 calendar days.

8.5.3 Reporting SAEs to IRBs and Ethics Committees

All Investigators must report IND Safety Reports and related safety information to their respective IRBs or Ethics Committees as mandated by them.

8.5.4 Reporting SAEs to the DSMB

The NIAID and ITN will provide the DSMB with data of all SAEs on an ongoing basis.

8.5.5 Reporting Pregnancy

The Investigator should be informed immediately of any pregnancy in a study participant or a partner of a study participant and all available pregnancy information should be entered into the electronic data capture (EDC) system within 24 hours of becoming aware of the event. The Investigator should counsel the participant and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the participant should continue until the conclusion of the pregnancy. Follow-up information detailing the outcome of the pregnancy should be entered into the EDC system as it becomes available. Any premature termination of the pregnancy will be reported.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or SAE as described in sections 8.2.1 and 8.2.3.

9. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

9.1 ANALYSIS SAMPLES

Safety sample: All participants who provide informed consent for study participation and receive any form of study therapy, including ATG, cyclophosphamide, fludarabine, tacrolimus, prednisone, or mycophenolate compounds.

Per protocol (PP) sample: All participants who receive a combined bone marrow and renal allograft transplant and at least one dose of high dose PT/Cy.

9.2 ANALYSIS PLAN

The principal features of the plan for statistical data analysis are outlined in this protocol and will be described in greater detail in the statistical analysis plan (SAP).

Analysis of study data will be conducted to address all objectives of the trial and other interrelationships among all data elements of interest to the Investigators and of relevance to the objectives of the study. Analyses by gender, age, and race/ethnicity, as appropriate, are also planned.

Primary analysis of treatment effect will be conducted on the PP sample.

9.2.1 Primary Endpoint

The primary endpoint is defined in section 3.5.1.

The primary endpoint will be analyzed using the PP sample and descriptively summarized with a point estimate and two-sided, 95% exact binomial confidence interval. All participants who fail to complete immunosuppression withdrawal, regardless of reason, will be considered to have failed the primary endpoint. Participants who restart immunosuppression or experience acute rejection after 52 weeks off immunosuppression will be considered successfully withdrawn for the purpose of the primary endpoint.

A sensitivity analysis of the primary endpoint will be performed comparing sensitized versus unsensitized participants.

9.2.2 Secondary Endpoints

- The secondary endpoints are defined in section 3.5.2. These will be analyzed using the PP sample.
- Proportion endpoints will be descriptively summarized using frequency tables with frequencies and percentages and 95% exact binomial confidence intervals.
- Time-to-event endpoints will be assessed using Kaplan-Meier survival estimates and associated two-sided 95% confidence intervals. Subjects lost due to competing risks or being lost to follow-up will be censored at the time of occurrence of these events.
- Continuous endpoints will be summarized using descriptive statistics (n, mean, standard deviation, median, minimum, maximum).
- Categorical variables will be summarized using counts (n) and percentages (%).

9.2.3 Safety Analysis

The safety analyses will be performed using the safety sample. All adverse events will be classified by body system and preferred term according to the MedDRA dictionary. Frequency

tables by category of event (e.g. serious, related to study therapy) and by NCI-CTCAE v. 4.0 grade will be presented. Selected laboratory values will be summarized and displayed graphically.

9.2.3.1 Donor Safety Analysis: Selected Adverse Events

The incidence of the following selected donor AEs related to study procedures will be summarized:

- Pain, bleeding and infection associated with the bone marrow harvest.
- The requirement for blood product transfusion.

9.2.4 Baseline Characteristics and Demographics

Baseline and demographic characteristics will be collected for all transplanted participants. Demographic data will include age, race, sex, ethnicity, body weight and height; these data will be presented in the following manner:

- Continuous data (i.e., age, body weight and height) will be summarized descriptively by mean, standard deviation, median, and range.
- Categorical data (i.e., sex and race) will be presented as frequencies and percentages.

9.2.5 Medical History

Medical history within the past 12 months, including the existence of current signs and symptoms, will be collected for each body system and summarized in tabular form.

9.2.6 Use of Medications

All prophylactic antibiotics, immunosuppressive medications, and steroids taken by or administered to study participants throughout the study will be collected.

9.2.7 Study Completion

The percentage of participants who complete the study, losses to follow-up, times to lost to follow-up and reasons for termination (e.g., adverse events) will be presented.

9.3 SAMPLE SIZE

Given a sample size of 6 for this pilot study, there is insufficient power for hypothesis testing. Thus the analyses will be primarily descriptive. For example, if 5 participants successfully withdraw from immunosuppression, the point estimate and 95% exact binomial confidence interval for the proportion of participants successfully withdrawn will be 0.83 (0.36, 0.996). If 3 subjects are successfully withdrawn, the resulting estimate and 95% confidence interval will be 0.50 (0.12, 0.88).

9.4 INTERIM ANALYSIS

No formal interim efficacy analysis is planned for this study. The DSMB will receive periodic safety reports on participants who have received a transplant along with all study therapy discontinuation cases. The DSMB may request modifications to the protocol based on its review of the findings.

Additionally, a safety review will be performed after the third transplanted participant has been followed for 3 months post-transplant, as described in section 3.1.4.

9.5 REPORTING DEVIATIONS FROM THE ORIGINAL STATISTICAL PLAN

The principal features of both the study design and the plan for statistical data analysis are outlined in this protocol and in the statistical analysis plan (SAP). Any change in these features requires either a protocol or an SAP amendment, which is subject to review by study sponsor(s), and the health authorities. These changes will be described in the final study report as appropriate.

10. ACCESS TO SOURCE DATA/DOCUMENTS

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants 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 sites must permit authorized representatives of the ITN, sponsor, and health authorities to examine (and to copy when required by applicable law) 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 (and any personally identifying information must be obscured). 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 sites will normally be notified in advance of auditing visits.

11. QUALITY CONTROL AND QUALITY ASSURANCE

The Investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The Investigator is required to ensure that all CRFs are 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.

The CRFs will be completed online via a web-based electronic data capture (EDC) system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations.

Study staff at the site will enter information into the electronic CRFs, and the data will be stored remotely at a central database. Data quality will be ensured through the EDC system's continuous monitoring of data and real-time detection and correction of errors. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations.

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, current Good Clinical Practice (GCP) guidelines—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 the sponsor and an appropriate ethics review committee or institutional review board (IRB). Any amendments to the protocol or consent materials must also be approved by the Sponsor, the IRB and submitted to FDA before they are implemented.

12.2 INFORMED CONSENT

The informed consent form is a means of providing information about 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 before participating in the study, taking the 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 the appropriate language.

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 participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The attending physician, in the presence of a witness, will review the consent and answer questions. The participant will be informed that participation is voluntary and that he/she may withdraw from the study at any time, for any reason.

12.3 PRIVACY AND CONFIDENTIALITY

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number. This number, rather than the participant's name, will be used to collect, store, and report participant information.

13. PUBLICATION POLICY

The ITN policy on publication of study results will apply to this study. Authorized participants may find details regarding the policy statement on the ITN internet website at http://www.immunetolerance.org.

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APPENDIX 1A. PRE-TRANSPLANT THROUGH TRANSPLANT: RECIPIENT

Study Day	Screen[1,2]	-14 to -9	-8	-7	Days of rest		-4	-3	-2	-1	0
Visit (Windows: Visits should take place on the day indicated in this schedule.)	1	2	3	4	5	6	7	8	9	10	11
1A - GENERAL ASSESSMENTS											
Informed consent	X										
Medical and demographic history including history of blood products donation and concomitant medications	X										
Complete physical examination including height	X										
Limited physical exam[3]		X	X	X			X	X	X	X	X
Vital signs including w eight	X	X	X	X			X	X	X	X	X
Cardiac function – echocardiogram	X										
Pulmonary function – chest radiography and spirometry	X										

[1] Recipient screening must occur within 30 days of first administration of study medication. Study medication dosing is initiated on day -9. The screening assessments in Visit 1 or Visit 505 may be performed over multiple days within the 30 day window.

[2] All standard-of-care laboratory assessments (hematology, chemistry, pregnancy and viral serologies) must be completed within 30 days of day -9. High resolution molecular HLA typing and ABO typing, and any positive viral serology obtained per site standard of care, may be completed greater than 30 days prior to Visit -9,with no upper window, provided the respective assays were performed using the same technology currently in use by the site laboratory.

[3] To include assessment of at least the following body systems: respiratory, cardiovascular, gastrointestinal, skin, neurologic and renal/urinary.

Study Day	Screen[1,2]	-14 to -9	-8	-7	Days of rest		-4	-3	-2	-1	0
Visit (Windows: Visits should take place on the day indicated in this schedule.)	1	2	3	4	5	6	7	8	9	10	11
Status change since prior visit[4]		X	X	X			X	X	X	X	X
1A - LOCAL CLINICAL LABORATORY ASSESSMENTS[5]											
Hematology – CBC with differential and platelets	X	X	X	X			X	X	X	X	X
Comprehensive Metabolic Panel (Na, K, Cl, HCO3, BUN, creatinine, glucose, albumin, total bilirubin, AST, ALT, alkaline phosphatase)	X	X	X	X			X	X	X	X	X
Urine or serum pregnancy test	X	X[6]									
CMV and EBV serologies	X[7]										
HIV-1 and 2 serologies and nucleic acid test	X[7]										
Anti-hepatitis C (HCV) antibodies [8]	X[7]										

[4] Includes assessment of change in administration of blood products; adverse events and concomitant medications. (See section 5.10 for specific concomitant medications to be assessed.) Changes should be recorded in eCRFs as appropriate.

[5] All blood collections should be performed prior to any study drug administration, if blood collection and drug administration occur on the same day.

[6] Pregnancy test must be repeated within 72 hours of first study drug administration.

[7] Collect screening blood samples for serologies such that results are available by day -14. Previously performed viral serologies will be used for eligibility criteria.

[8] All positive HCV antibody results must be assessed by an EIA assay and confirmed by a quantitative serum HCV RNA assay. Participants with positive HCV antibodies but undetectable serum HCV RNA may be considered for eligibility. Participants with negative anti-HCV antibodies but unexplained liver enzyme abnormalities must undergo a quantitative serum RNA assay to rule out false negative HCV serologies.

Study Day	Screen[1,2]	-14 to -9	-8	-7	Days of rest		-4	-3	-2	-1	0
Visit (Windows: Visits should take place on the day indicated in this schedule.)	1	2	3	4	5	6	7	8	9	10	11
Hepatitis B surface antigen (HBsAg), total hepatitis B core antibody (anti-HBc IgM and IgG), hepatitis B surface antibody (anti-HBsAb)[9]	X[7]										
Quantiferon test	X										
ABO typing[10]	X										
High resolution molecular HLA typing and DNA (A, B, C, DRB1, DRB3-5, DQA1, DQB1, DPB1) [11]	X										
HLA alloantibodies includes DSA, flow PRA	X										
T and B cell lymphocyte crossmatch	X[12]	X[12]									
Cytotoxic crossmatch (CDC)	X										
Calculated PRA	X										
Kidney biopsy											X
1A - LOCAL MECHANISTIC ASSESSMENTS[5,13]											
Chimerism (buccal swab)		X									
T cell assays (MLR, CML)		X									
B cell assays (HLA tetramer selection and culture)		X									
B cell sequencing		X									

[9] Participants will undergo a quantitative HBV DNA PCR test per institutional standard and will be excluded if HBV DNA is detectable.

[10] ABO typing to be taken from medical records.

[11] High resolution HLA typing to be performed on DNA sample banked from routine initial evalutation.

[12] Screening T and B cell lymphocyte crossmatch to be performed as close to day -30 as possible. Final T and B cell lymphocyte crossmatch to be performed on day -14, before recipient receives study drug.

[13] To be collected no later than study day -9 once candidate has been successfully screened for eligibility criteria.

Study Day	Screen[1,2]	-14 to -9	-8	-7	Days of rest		-4	-3	-2	-1	0
Visit (Windows: Visits should take place on the day indicated in this schedule.)	1	2	3	4	5	6	7	8	9	10	11
1A - CENTRAL MECHANISTIC ASSESSMENTS[5]											
Plasma cytokines		X									
Flow cytometry panel staining (frozen PBMC)		X									
PBMC collection		X									
Whole blood – gene expression profiling		X									
Kidney biopsy – histology											X
Kidney biopsy – gene expression profiling											X
1A - STUDY TREATMENT											
Hemodialysis (6-12 hours after fludarabine dosing)							X	X	X		
Total body irradiation										X	
Renal transplantation											X
Bone marrow infusion											X
1A - STUDY MEDICATION											
ATG[14]		X	X	X							
Methylprednisolone (ATG premedication)		X	X	X			X	X	X		
Fludarabine							X	X	X		
Low dose cyclophosphamide							X	X			

[14] ATG to be administered along with premedication as specified in section 5.2.2.

APPENDIX 1B. PRE-TRANSPLANT THROUGH POST-TRANSPLANT: DONOR

Study Day	Screen[15,2]	-14 to -9	-1	0	7	56
Visit (Visits pre-transplant through transplant should take place on the day indicated in the schedule of events; Day 7 post-transplant visit has a +3-day window; Day 56 post-transplant visit has a +14-day window.)	1	2	10	11	104	106
1B - GENERAL ASSESSMENTS						
Informed consent	X					
Medical and demographic history	X					
Screening for any autoimmune disease requiring immunosuppressive drugs for maintenance	X					
Complete physical examination including height	X					
Vital signs including weight	X					
Status change since prior visit[16,17]		X	X	X	X	X
1B - LOCAL CLINICAL LABORATORY ASSESSMENTS[5]						
Hematology – CBC with differential and platelets	X	X	X	X	X	X
Comprehensive Metabolic Panel (Na, K, Cl, HCO3, BUN, creatinine, glucose, albumin, total bilirubin, AST, ALT, alkaline phosphatase)	X	X	X	X	X	X
CMV and EBV serology	X[7]					
HIV-1 and 2 serologies and nucleic acid test	X[7]					
Anti-hepatitis C (HCV) antibodies [8]	X[7]					
Hepatitis B surface antigen (HBsAg), total hepatitis B core antibody (anti-HBc IgM and IgG), hepatitis B surface antibody (anti-HBsAb)[9]	X[7]					
ABO typing[10]	X					
High resolution molecular HLA typing and DNA (A, B, C, DRB1, DRB3-5, DQA1, DQB1, DPB1) [11]	X					

[15] Donor screening must occur within 30 days of Day 0. The screening assessments in Visit 1 or Visit 505 may be performed over multiple days within the visit study window.

[16] Includes assessment of change in adverse events, concomitant medications and administration of blood products. (See section 5.10 for specified concomitant medications.) Changes should be recorded in eCRFs as appropriate.

[17] Only AEs related to bone marrow harvest and study-mandated blood draw will be collected for the donor.

Study Day	Screen[15,2]	-14 to -9	-1	0	7	56
Visit (Visits pre-transplant through transplant should take place on the day indicated in the schedule of events; Day 7 post-transplant visit has a +3-day window; Day 56 post-transplant visit has a +14-day window.)	1	2	10	11	104	106
T and B cell lymphocyte crossmatch	X[12]	X[12]				
PBMC's for future T and B cell lymphocyte crossmatches	X[18]					X
1B - LOCAL MECHANISTIC ASSESSMENTS[5]						
Chimerism (buccal swab)		X[18]				
T cell assays (MLR, CML)		X[18]				
B cell assays (HLA tetramer selection and culture)						X
1B - CENTRAL MECHANISTIC ASSESSMENTS						
PBMC collection						X
1B - STUDY TREATMENT						
Bone marrow harvest and nephrectomy				X		

[18] Collect donor baseline blood samples on day -14 (+/-2 days).

APPENDIX 1C. REINITIATION OF CONDITIONING: PRE-TRANSPLANT THROUGH TRANSPLANT: RECIPIENT

	Post 1st Attempt Conditioning				2nd Attempt Conditioning[19]										Transplant
Study Day	+2-6 weeks	*+12 weeks*	*+18 weeks*	*+24-26 weeks*	Eligibility[1,2,20]	-14 to -9	-8	-7	Days of rest (-6 and -5)		-4	-3	-2	-1	0
Visit	501	*502*[20]	*503*[20]	*504*[20]	505	506	507	508	509	510	511	512	513	514	11
1C - GENERAL ASSESSMENTS															
Informed consent					X										
Medical and demographic history including history of blood products donation and concomitant medications					X										
Complete physical examination including height					X										
Limited physical exam[3]	X	*X*	*X*	*X*		X	X	X			X	X	X	X	X
Vital signs including weight	X	*X*	*X*	*X*	X	X	X	X			X	X	X	X	X
Cardiac function – echocardiogram[21]					X										
Pulmonary function – chest radiography and spirometry[21]					X										
Status change since prior visit[4]	X	*X*	*X*	*X*	X	X	X	X			X	X	X	X	X
1C - LOCAL CLINICAL LABORATORY ASSESSMENTS[5]															
Hematology – CBC with differential and platelets	X	X	X	X	X	X	X	X			X	X	X	X	X

[19] Visits 506-513 are to be completed per investigators discretion. The determination of when to administer reconditioning and how much reconditioning, if any, is necessary to proceed with the transplantation will be left to the discretion of the investigator and may be adjusted based on the amount of conditioning initially received and the time elapsed since the last dose.

[20] Visits 502, 503, 504 are to be completed as needed between conditioning attempts. Eligibility assessment can occur any time after complete ANC recovery.

[21] Pulmonary function and cardiac tests may be repeated if deemed necessary by the investigator.

	Post 1st Attempt Conditioning				2nd Attempt Conditioning[19]										Transplant
Study Day	+2-6 weeks	*+12 weeks*	*+18 weeks*	*+24-26 weeks*	Eligibility[1,2,20]	-14 to -9	-8	-7	Days of rest (-6 and -5)		-4	-3	-2	-1	0
Visit	501	*502*[20]	*503*[20]	*504*[20]	505	506	507	508	509	510	511	512	513	514	11
Comprehensive Metabolic Panel (Na, K, Cl, HCO3, BUN, creatinine, glucose, albumin, total bilirubin, AST, ALT, alkaline phosphatase)	X	X	X	X	X	X	X	X			X	X	X	X	X
Urine or serum pregnancy test					X	X[6]									
CMV and EBV serologies					X[7]										
HIV-1 and 2 serologies and nucleic acid test					X[7]										
Anti-hepatitis C (HCV) antibodies[8]					X[7]										
Hepatitis B surface antigen (HBsAg), total hepatitis B core antibody (anti-HBc IgM and IgG), hepatitis B surface antibody (anti-HBsAb)[9]					X[7]										
Chest x-ray[22]					X										
ABO typing[10]															
High resolution molecular HLA typing and DNA (A, B, C, DRB1, DRB3-5, DQA1, DQB1, DPB1) [11]															
HLA alloantibodies includes DSA, flow PRA					X										
T and B cell lymphocyte crossmatch					X[12]	X[12]									
Cytotoxic crossmatch (CDC)					X										
Calculated PRA					X										

[22] Chest x-ray will only occur for those recipients who were previously Quantiferon positive.

	Post 1st Attempt Conditioning				2nd Attempt Conditioning[19]										Transplant
Study Day	+2-6 weeks	*+12 weeks*	*+18 weeks*	*+24-26 weeks*	Eligibility[1,2,20]	-14 to -9	-8	-7	Days of rest (-6 and -5)		-4	-3	-2	-1	0
Visit	501	*502*[20]	*503*[20]	*504*[20]	505	506	507	508	509	510	511	512	513	514	11
Kidney biopsy															X
1C - LOCAL MECHANISTIC ASSESSMENTS[5,13]															
Chimerism (buccal swab)					X[23]										
T cell assays (MLR, CML)					X[23]										
B cell assays (HLA tetramer selection and culture)					X[23]										
B cell sequencing					X[23]										
1C - CENTRAL MECHANISTIC ASSESSMENTS[5]															
Plasma cytokines					X[23]										
Flow cytometry panel staining (frozen PBMC)					X[23]										
PBMC collection					X[23]										
Whole blood – gene expression profiling					X[23]										
Kidney biopsy – histology															X
Kidney biopsy – gene expression profiling															X
1C - STUDY TREATMENT															
Hemodialysis (6-12 hours after fludarabine dosing)											X	X	X		
Total body irradiation														X	
Renal transplantation															X
Bone marrow infusion															X
1C - STUDY MEDICATION															
ATG[14]						X	X	X							
Methylprednisolone (ATG premedication)						X	X	X			X	X	X		
Fludarabine											X	X	X		
Low dose cyclophosphamide											X	X			

[23] To be collected only if first attempted collection is inadequate.

APPENDIX 1D. REINITIATION OF CONDITIONING: PRE-TRANSPLANT THROUGH POST-TRANSPLANT: DONOR

	(Recipient's) 2nd Attempt Conditioning[24]			Transplant	Post-Transplant	
Study Day	Screen[15,2]	-14 to -9	-1	0	7	56
Visit	505	506	514	11	104	106
1D - GENERAL ASSESSMENTS						
Informed consent	X					
Medical and demographic history	X					
Screening for any autoimmune disease requiring immunosuppressive drugs for maintenance	X					
Complete physical examination including height	X					
Vital signs including w eight	X					
Status change since prior visit[16,17]		X	X	X	X	X
1D - LOCAL CLINICAL ASSESSMENTS[5]						
Hematology – CBC w ith differential and platelets	X	X	X	X	X	X
Comprehensive Metabolic Panel (Na, K, Cl, HCO3, BUN, creatinine, glucose, albumin, total bilirubin, AST, ALT, alkaline phosphatase)	X	X	X	X	X	X
CMV and EBV serology	X[7]					
HIV-1 and 2 serologies and nucleic acid test	X[7]					
Anti-hepatitis C (HCV) antibodies[8]	X[7]					
Hepatitis B surface antigen (HBsAg), total hepatitis B core antibody (anti-HBc IgM and IgG), hepatitis B surface antibody (anti-HBsAb)[9]	X[7]					
ABO typing[10]	X					
High resolution molecular HLA typing and DNA (A, B, C, DRB1, DRB3-5, DQA1, DQB1, DPB1)[11]	X					
T and B cell lymphocyte crossmatch	X[12]	X[12]				
PBMC's for future T and B cell lymphocyte crossmatches	X[18]					X
1D - LOCAL MECHANISTIC ASSESSMENTS[5]						
Chimerism (buccal swab)		X[18]				
T cell assays (MLR, CML)		X[18]				
B cell assays (HLA tetramer selection and culture)						X
1D - CENTRAL MECHANISTIC ASSESSMENTS						

[24] Visit 506 to be completed per investigators discretion.

	(Recipient's) 2nd Attempt Conditioning[24]			Transplant	Post-Transplant	
Study Day	Screen[15,2]	-14 to -9	-1	0	7	56
Visit	505	506	514	11	104	106
PBMC collection						X
1D - STUDY TREATMENT						
Bone marrow harvest and nephrectomy				X		

APPENDIX 2. POST-TRANSPLANT THROUGH EVALUATION FOR WITHDRAWAL ELIGIBILITY

Post-transplant Week	0	0	0	0	4	8	22	*30*
Post-transplant Day	3	4	5	7	28	56	154	*210*
Post-transplant Visit (Windows: Visits 101-104 to be conducted on day indicated; Visits 105-106 +/- 2 days; Visit 107 +/- 7 days)	101	102	103	104	105	106	107 [1]	*108A,B*
2 - GENERAL ASSESSMENTS								
Vital signs including weight	X	X	X	X	X	X	X	*X*
Limited physical exam [2]	X	X	X	X	X	X	X	*X*
Status change since prior visit [3]	X	X	X	X	X	X	X	*X*
Acute GVHD assessment [4]	X	X	X	X	X	X	X	*X*
Chronic GVHD assessment [5]						X	X	*X*
Absolute neutrophil count recovery [6]	X							

[1] Participants may be evaluated for prednisone and MMF IS withdrawal as early as Week 22 (Day 154) post-transplant. Immunosuppression withdrawal may begin as early as Week 26 (Day 182) as per Appendix 3. Participants who are not ready to undergo eligibility biopsy at Week 22, should undergo routine study visit at this time point and may return for eligibility evaluation no later than Week 30 (Day 210, Visit 108) post-transplant. If the eligibility biopsy must be repeated, perform all assessments indicated for Visit 108.

[2] To include assessment of at least the following body systems: respiratory, cardiovascular, gastrointestinal, skin, neurologic and renal/urinary.

[3] Includes assessment of change in administration of blood products, adverse events and concomitant medications. (See section 5.10 for specific concomitant medications to be assessed.) Changes should be recorded in eCRFs as appropriate.

[4] Acute GVHD will be staged and graded as in section 6.7, post-transplant Day 0 through approximately Day 200, at the visits indicated.

[5] Chronic GVHD will be staged and graded as in section 6.7.2, approximately post-transplant Day 60 through approximately 2 years post-transplant, at the visits indicated, using the Chronic GVHD Assessment Worksheet (Appendix 7. Chronic GVHD Assessment Worksheet). If a participant's chronic GVHD persists beyond 2 years post-transplant or if he/she is diagnosed with chronic GVHD >2 years post-transplant, the chronic GVHD Assessment Worksheet will be graded at every subsequent study visit until resolution of the episode, or until the end of study participation.

[6] Time of absolute neutrophil count (ANC) recovery to be captured on the Absolute Neutrophil Count recovery eCRF.

Post-transplant Week	0	0	0	0	4	8	22	*30*
Post-transplant Day	3	4	5	7	28	56	154	*210*
Post-transplant Visit (Windows: Visits 101-104 to be conducted on day indicated; Visits 105-106 +/- 2 days; Visit 107 +/- 7 days)	101	102	103	104	105	106	107[1]	*108A,B*
Platelet count recovery[7]	X							
2 - LOCAL CLINICAL LABORATORY ASSESSMENTS[8]								
Hematology – CBC with differential and platelets[9]					X	X	X	*X*
Comprehensive Metabolic Panel (Na, K, Cl, HCO3, BUN, creatinine, glucose, albumin, total bilirubin, AST, ALT, alkaline phosphatase)[10]					X	X	X	*X*
BK virus (blood)					X	X	X	*X*
Tacrolimus levels					X	X	X	*X*
HLA alloantibodies includes DSA, flow PRA				X	X	X	X	*X*
CMV and EBV viral load[11]					X	X	X	
Kidney biopsy[12]							*X*[13]	
2 - LOCAL MECHANISTIC ASSESSMENTS[8,14]								

[7] Time to platelet count recovery to be captured on the Platelet Count Recovery eCRF.

[8] All blood collections should be performed prior to any study drug administration, if blood collection and drug administration occur on the same day.

[9] Values from more frequent, standard of care testing on non-study visit days may also be recorded in the participant's eCRF study record.

[10] Creatinine values used to establish baseline value during weeks 2-4 post-transplant will be recorded on the Baseline Creatinine eCRF.

[11] CMV viremia (by PCR) will be monitored and documented weekly post-transplant beginning once the total white blood cell count is greater than 1000/mm3. Monitoring of CMV viremia will continue weekly post-transplant until Day 100; then every other week until Day 365.

[12] In the event of a for cause biopsy or repeat biopsy, follow assessments listed in Appendix 4 visit 308.

[13] The immunosuppression withdrawal eligibility biopsy may take place as early as week 22 or as late as week 30 post-transplant.

[14] Mechanistic draws must be completed prior to biopsy procedures on visits when both biopsies and mechanistic draws are being performed.

Post-transplant Week	0	0	0	0	4	8	22	*30*
Post-transplant Day	3	4	5	7	28	56	154	*210*
Post-transplant Visit (Windows: Visits 101-104 to be conducted on day indicated; Visits 105-106 +/- 2 days; Visit 107 +/- 7 days)	101	102	103	104	105	106	107[1]	*108A,B*
Blood chimerism (w hole blood and CD3+ T cell)					X	X	*X*[15]	
T cell assays (MLR, CML)							*X*[15]	
B cell assays (HLA tetramer selection and culture)							*X*[15]	
B cell repertoire sequencing							*X*[15]	
2 - CENTRAL MECHANISTIC ASSESSMENTS[8,14]								
Plasma cytokines	X				X		*X*[15]	
Flow cytometry panel staining (frozen PBMC)					X	X	*X*[15]	
PBMC collection					X	X	*X*[15]	
Whole blood – gene expression profiling					X	X	*X*[15]	
Kidney biopsy – histology							*X*[15]	
Kidney biopsy – gene expression profiling							*X*[15]	
2 - STUDY MEDICATION								
Tacrolimus, prednisone and mycophenolate mofetil[16]			X	X	X	X	X	*X*
High dose cyclophosphamide[17]	X	X						
Filgrastim[18]			X	X	X			

[15] To be collected at the visit in which the immunosuppression withdrawal eligibility biopsy is performed.

[16] Maintenance immunosuppressive therapy to be initiated on Day 5 post-transplant per section 5.3. It is crucial that no immunosuppressive agents are given from the time of the transplant until 24 hours after the completion of the post-transplant cyclophosphamide. This includes steroids as anti-emetics. Intravenous administration of tacrolimus will not be allowed.

[17] To be administered with MESNA and anti-emetics as outlined in section 5.2.3 on post-transplant Days 3 and 4 (within 48-72 hours of marrow infusion).

[18] Filgrastim 5 µg/kg/day sc will be administered from day 5 until the absolute neutrophil count is greater than 1000/µl on three consecutive measurements over at least 2 days.

APPENDIX 3. IMMUNOSUPPRESSION WITHDRAWAL (INITIATED BETWEEN 26 AND 34 WEEKS POST-TRANSPLANT)

Immunosuppression Withdrawal Week	0	2	4	6	8	10	12	14	16	18	20	22	*24*	*26*	*28*	*30*	*32*	*34*
Immunosuppression Withdrawal Visit (Windows: +/- 7 days)	201	202	203	204	205	206	207	208	209	210	211	212	*213*	*214*	*215*	*216*	*217*	*218*
3 - GENERAL ASSESSMENTS																		
Informed consent					X[43]													
Vital signs including weight		X		X		X		X		X		X		X		X		X
Limited physical exam[44]		X		X		X		X		X		X		X		X		X
Status change since prior visit[45]	X[46]	X	X[46]	X	X[46]	X	X[46]	X	X[46]	X	X[46]	X	X[46]	X	X[46]	X	X[46]	X
Acute GVHD assessment[47]	X[48]	X	X[48]	X														
Chronic GVHD assessment[49]	X[48]	X	X[48]	X	X[48]	X	X[48]	X	X[48]	X	X[48]	X	X[48]	X	X[48]	X	X[48]	X

[43] To be obtained prior to assessment for eligibility for tacrolimus withdrawal as early as Week 8 or as late as Week 16 of the Immunosuppression Withdrawal schedule.

[44] To include assessment of at least the following body systems: respiratory, cardiovascular, gastrointestinal, skin, neurologic and renal/urinary.

[45] Includes assessment of change in administration of blood products, adverse events and concomitant medications. (See section 5.10 for specific concomitant medications to be assessed.) Changes should be recorded in eCRFs as appropriate.

[46] Visit may be conducted by telephone, unless eligibility for withdrawal is being assessed. Participants eligible for immunosuppression withdrawal should be instructed on dose change for prednisone and MMF.

[47] Acute GVHD will be staged and graded as in section 6.7 from Day 0 to approximately 200 post-transplant, at the visits indicated.

[48] Not performed if visit is conducted by telephone.

[49] Chronic GVHD will be staged and graded as in section 6.7.2, approximately post-transplant Day 60 through approximately 2 years post-transplant, at the visits indicated, using the Chronic GVHD Assessment Worksheet (Appendix 7. Chronic GVHD Assessment Worksheet). If a participant's chronic GVHD persists beyond 2 years post-transplant or if he/she is diagnosed with chronic GVHD >2 years post-transplant, the chronic GVHD Assessment Worksheet will be graded at every subsequent study visit until resolution of the episode, or until the end of study participation.

Immunosuppression Withdrawal Week	0	2	4	6	8	10	12	14	16	18	20	22	*24*	*26*	*28*	*30*	*32*	*34*
Immunosuppression Withdrawal Visit (Windows: +/- 7 days)	201	202	203	204	205	206	207	208	209	210	211	212	*213*	*214*	*215*	*216*	*217*	*218*
Absolute neutrophil count recovery[50]	X																	
Platelet count recovery[51]	X																	
3 - LOCAL CLINICAL LABORATORY ASSESSMENTS[52]																		
Hematology – CBC with differential and platelets[53]		X	X	X	X	X	X	X	X	X	X	X	*X*	*X*	*X*	*X*	*X*	*X*
Comprehensive Metabolic Panel (Na, K, Cl, HCO3, BUN, creatinine, glucose, albumin, total bilirubin, AST, ALT, alkaline phosphatase)[53]		X	X	X	X	X	X	X	X	X	X	X	*X*	*X*	*X*	*X*	*X*	*X*
BK virus (blood)[54]	X																	
Tacrolimus levels[53,55]		X	X	X	X	X	X	X	X	X	X	X	*X*	*X*	*X*	*X*	*X*	*X*
HLA alloantibodies includes DSA, flow PRA[56]		X		X		X		X		X		X		*X*		*X*		*X*
T and B cell lymphocyte crossmatch													*X*[61]					

[50] Time of absolute neutrophil count recovery to be captured on the Absolute Neutrophil Count eCRF (applicable if ANC recovery is still being monitored when participant moves to Immunosuppression Withdrawal visits).

[51] Time to platelet count recovery to be captured on the Platelet Count Recovery eCRF (applicable if platelet count recovery is still being monitored when participant moves to Immunosuppression Withdrawal visits).

[52] All blood collections should be performed prior to any study drug administration.

[53] For visits conducted by phone, results from the participant's home laboratory may be used.

[54] Monitoring for BK viremia should continue for up to 1 year post-transplant in participants who complete immunosuppression withdrawal; see section 5.7 for specific time points.

[55] Monitoring of tacrolimus levels may be discontinued once levels become undetectable.

[56] Performed only at study site laboratory.

Immunosuppression Withdrawal Week	0	2	4	6	8	10	12	14	16	18	20	22	*24*	*26*	*28*	*30*	*32*	*34*
Immunosuppression Withdrawal Visit (Windows: +/- 7 days)	201	202	203	204	205	206	207	208	209	210	211	212	*213*	*214*	*215*	*216*	*217*	*218*
CMV and EBV viral load[57]	X	X	X	X	X	X	X	X	X	X	X	X						
Kidney biopsy[58]					X[59, 60]								X[61]					
3 - LOCAL MECHANISTIC ASSESSMENTS[52,59]																		
Blood chimerism (whole blood and CD3+ T-Cell)					X[60]								X[61]					
T cell assays (MLR, CML)					X[60]								X[61]					
B cell assays (HLA tetramer selection and culture)					X[60]								X[61]					
B cell repertoire sequencing					X[60]													
3 - CENTRAL MECHANISTIC ASSESSMENTS[52,59]																		
Plasma cytokines					X[60]								X[61]					
Flow cytometry panel staining (frozen PBMC)					X[60]								X[61]					
PBMC collection					X[60]								X[61]					
Whole blood – gene expression profiling					X[60]								X[61]					
Kidney biopsy – histology					X[60]								X[61]					
Kidney biopsy – gene expression profiling					X[60]								X[61]					

[57] CMV viremia will be monitored by PCR and documented weekly post-transplant beginning once the total white blood cell count is greater than 1000/mm3. Monitoring of CMV will continue weekly post-transplant until Day 100; then every other week until Day 365.

[58] In the event of a for cause biopsy or repeat biopsy, follow assements listed in Appendix 4 visit 308.

[59] Mechanistic draws must be completed prior to biopsy procedures on visits when both biopsies and mechanistic draws are being performed.

[60] Tacrolimus withdrawal eligibility biopsy and associated mechanistic assessments may take place as early as Week 8 or as late as Week 16 of the Immunosuppression Withdrawal Schedule.

[61] To be performed on the first day following the completion of immunosuppression withdrawal; the first day the participant takes no immunosuppressive medication. This assessment may take place as early as Week 24 or as late as Week 34 of the Immunosuppression Withdrawal Schedule.

Immunosuppression Withdrawal Week	0	2	4	6	8	10	12	14	16	18	20	22	*24*	*26*	*28*	*30*	*32*	*34*
Immunosuppression Withdrawal Visit (Windows: +/- 7 days)	201	202	203	204	205	206	207	208	209	210	211	212	*213*	*214*	*215*	*216*	*217*	*218*
3 - STUDY MEDICATION																		
Tacrolimus[62]	X	X	X	X	X	X	X	X	X	X	X	X	X[21]	X[21]	X[21]	X[21]	X[21]	X[21]
Prednisone[63]	X	X	X	X[64]	X[21]													
Mycophenolate mofetil[64]	X	X	X	X[21]	X[21]													

[62] Please see section 3.3.2.1 & 3.3.2.2 for tacrolimus withdrawal eligibility criteria and immunosuppression withdrawal algorithm.

[63] Please see section 3.3.1.2 for prednisone and MMF withdrawal algorithm.

[64] Needed only if withdrawal of this medication is not complete at this time point.

[A] In the event of a for cause biopsy or repeat biopsy, follow assessments listed in Appendix 4 visit 308.

APPENDIX 4. POST IMMUNOSUPPRESSION WITHDRAWAL FOLLOW-UP (INITIATED BETWEEN 52 AND 62 WEEKS POST-TRANSPLANT)

Post Immunosuppression Withdrawal Week	3	6	9	12	26	36	48	52	60	72	84	96	108	120	132	144	156	168	180	192	204	216
Post Immunosuppression Withdrawal Visit (Windows: Visits 301-305 +/- 7 days; Visits 306-322 +/- 2 weeks)	301	302	303	304	305	306	307	308[1]	309	310	311	312	313	314	315	316	317	318	319	320	321	322
4 - GENERAL ASSESSMENTS																						
Vital signs including weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Limited physical exam[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Status change since prior visit[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[4]
Chronic GVHD[5] assessment	X	X	X	X	X	X	X	X	X	X												
4 - LOCAL CLINICAL LABORATORY ASSESSMENTS																						
Hematology – CBC with differential and platelets	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Comprehensive Metabolic Panel (Na, K,	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

[1] For For Cause Biopsy (FCB) and Repeat Biopsy (RB) visits, follow the clinical and mechanistic assessments specified in Visit 308, with the exception of B cell repertoire sequencing which does not need to be performed for FCB or RB visits. If a FCB or RB is performed within 60 days of transplant the following local mechanistic assays will not be performed; T cell assays and B cell assays. This visit should also include testing for BK virus regardless of length of time since transplant.

[2] To include assessment of at least the following body systems: respiratory, cardiovascular, gastrointestinal, skin, neurologic and renal/urinary.

[3] Includes assessment of change in administration of blood products, adverse events and concomitant medications. (See section 5.10 for specific concomitant medications to be assessed.) Changes should be recorded in eCRFs as appropriate.

[4] Any unresolved AE will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first, per section 8.2.1.

[5] Chronic GVHD will be staged and graded as in section 6.7.2, approximately post-transplant Day 60 through approximately 2 years post-transplant, at the visits indicated using the Chronic GVHD Assessment Worksheet (Appendix 7. Chronic GVHD Assessment Worksheet). If a participant's chronic GVHD persists beyond 2 years post-transplant or if he/she is diagnosed with chronic GVHD >2 years post-transplant, the chronic GVHD Assessment Worksheet will be graded at every subsequent study visit until resolution of the episode, or until the end of study participation.

Post Immunosuppression Withdrawal Week	3	6	9	12	26	36	48	52	60	72	84	96	108	120	132	144	156	168	180	192	204	216
Post Immunosuppression Withdrawal Visit (Windows: Visits 301-305 +/- 7 days; Visits 306-322 +/- 2 weeks)	301	302	303	304	305	306	307	308[1]	309	310	311	312	313	314	315	316	317	318	319	320	321	322
Cl, HCO3, BUN, creatinine, glucose, albumin, total bilirubin, AST, ALT, alkaline phosphatase)																						
HLA alloantibodies – includes DSA, flow PRA		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Kidney biopsy								X					X								X	
4 - LOCAL MECHANISTIC ASSESSMENTS[6]																						
Blood chimerism (whole blood and CD3+ T cell)					X			X					X								X	
T cell assays (MLR, CML)					X			X					X								X	
B Cell Assays (HLA tetramer selection and culture)					X			X					X								X	
B cell repertoire sequencing					X			X														
4 - CENTRAL MECHANISTIC ASSESSMENTS[6]																						
Plasma cytokines					X			X					X								X	
Flow cytometry panel staining (frozen PBMC)					X			X					X								X	
PBMC collection					X			X					X								X	
Whole blood – gene expression profiling					X			X					X								X	
Kidney biopsy – histology								X					X								X	
Kidney biopsy – gene expression profiling								X					X								X	

[6] Mechanistic draws must be completed prior to biopsy procedures on visits when both biopsies and mechanistic draws are being performed.

APPENDIX 5. SAFETY FOLLOW-UP: RECIPIENTS

Safety Follow-up Week[1]	2-6	12	24	36	48	60	72	84	96	104	~~156~~	~~208~~	~~260~~
Safety Follow-up Visit (Windows: visits 402-410 +/- 2 weeks)	401[2]	402	403	404	405	406	407	408	409	410	~~411~~[3]	~~412~~	~~413~~
5 - GENERAL ASSESSMENTS													
Vital signs including weight	X	X	X	X	X	X	X	X	X	X	~~X~~	~~X~~	~~X~~
Limited physical exam[4]	X	X	X	X	X	X	X	X	X	X	~~X~~	~~X~~	~~X~~
Status change since prior visit[5]	X	X	X	X	X	X	X	X	X	X[6]	~~X~~	~~X~~	~~X~~[6]
Acute GVHD assessment[7] (if applicable)	X	X	X										
Chronic GVHD assessment[8] (if applicable)	X	X	X	X	X	X	X	X	X	X	~~X~~	~~X~~	~~X~~

[1] The assessments outlined in Appendix 5 should be initiated for all unsuccessful participants who have completed at least one dose of ATG. Participants who experience a rejection event should begin safety follow-up within 4-6 weeks of the date of diagnosis of rejection. Participants who move to safety follow-up following non-rejection events should begin this schedule within 2 weeks of the determination of their ineligibility for further progress in the protocol.

[2] Follow the clinical assessments specified here, Visit 401, for any participant requesting early termination from study.

~~[3] Visits 411-413 to be used to ensure that all participants who receive the preparative regimen are followed for at least 5 years. The 3 additional visits should be completed for study participants who are moved to safety follow-up pre-transplant or prior to evaluation for withdrawal eligibility; who fail IS withdrawal; or who are moved to safety follow-up post-IS withdrawal.~~

[4] To include assessment of at least the following body systems: respiratory, cardiovascular, gastrointestinal, skin, neurologic and renal/urinary.

[5] Includes assessment of change in administration of blood products, adverse events and concomitant medications. (See section 5.10 for specific concomitant medications to be assessed.) Changes should be recorded in eCRFs as appropriate.

[6] Any unresolved AE will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first, per section 8.2.1.

[7] Acute GVHD will be staged and graded as in section 6.7, on each visit in the safety follow-up schedule through post-transplant Day 200 or the first visit thereafter.

[8] Chronic GVHD will be staged and graded as in section 6.7.2, at the first safety follow-up visit on or after post-transplant Day 60 and at each subsequent visit of the safety follow-up visit schedule up to the point 2 years post-transplant, at the visits indicated, using the Chronic GVHD Assessment Worksheet (Appendix 7. Chronic GVHD Assessment Worksheet). If a participant's chronic GVHD persists beyond 2 years post-transplant or if he/she is diagnosed with chronic GVHD >2 years post-transplant,

Safety Follow-up Week[1]	2-6	12	24	36	48	60	72	84	96	104	~~156~~	~~208~~	~~260~~
Safety Follow-up Visit (Windows: visits 402-410 +/- 2 weeks)	401[2]	402	403	404	405	406	407	408	409	410	~~411~~[3]	~~412~~	~~413~~
Absolute neutrophil count recovery[9]	X												
Platelet count recovery[10]	X												
5 - LOCAL CLINICAL LABORATORY ASSESSMENTS[11]													
Hematology – CBC with differential and platelets	X	X	X	X	X	X	X	X	X	X	~~X~~	~~X~~	~~X~~
Comprehensive Metabolic Panel (Na, K, Cl, HCO3, BUN, creatinine, glucose, albumin, total bilirubin, AST, ALT, alkaline phosphatase)	X	X	X	X	X	X	X	X	X	X	~~X~~	~~X~~	~~X~~
CMV and EBV viral load[12] (if applicable)	X	X	X	X	X								
BK virus (blood)	X[13]												
HLA alloantibodies includes DSA, flow PRA	X		X		X		X		X	X			
Kidney biopsy[14]					X					X			
5 - LOCAL MECHANISTIC ASSESSMENTS[11,14,15]													
Blood chimerism (whole blood and CD3+ T cell)					X					X			

the chronic GVHD Assessment Worksheet will be graded at every subsequent study visit until resolution of the episode, or until the end of study participation.

[9] Time of absolute neutrophil count (ANC) recovery to be captured on the Absolute Neutrophil Count recovery eCRF (applicable if ANC recovery is still being monitored when participant moves to safety follow-up).

[10] Time to platelet count recovery to be captured on the Platelet Count Recovery eCRF (applicable if platelet count recovery is still being monitored when participant moves to safety follow-up).

[11] All blood collections should be performed prior to any study drug administration.

[12] CMV viremia (by PCR) will be monitored and documented weekly post-transplant beginning once the total white blood cell count is greater than 1000/mm3. Monitoring of CMV viremia will continue weekly post-transplant until Day 100; then every other week until Day 365.

[13] Monitoring for BK viremia should continue for up to 2 years post-transplant in participants who remain on immunosuppression and move to safety follow-up; see section 5.7 for specific time points.

[14] Performance of per protocol kidney biopsies and mechanistic assessments is not required for participants who have not undergone per protocol renal transplantation.

[15] Mechanistic draws must be completed prior to biopsy procedures on visits when both biopsies and mechanistic draws are being performed.

Safety Follow-up Week[1]	2-6	12	24	36	48	60	72	84	96	104	~~156~~	~~208~~	~~260~~
Safety Follow-up Visit (Windows: visits 402-410 +/- 2 weeks)	401[2]	402	403	404	405	406	407	408	409	410	~~411~~[3]	~~412~~	~~413~~
T cell assays (MLR, CML)					X					X			
B cell assays (HLA tetramer selection and culture)					X					X			
5 - CENTRAL MECHANISTIC ASSESSMENTS [11,14, 15]													
Plasma cytokines					X					X			
Flow cytometry panel staining (frozen PBMC)					X					X			
PBMC collection					X					X			
Whole blood – gene expression profiling					X					X			
Kidney biopsy – histology[14]					X					X			
Kidney biopsy – gene expression profiling[14]					X					X			

APPENDIX 6. ACUTE GVHD ASSESSMENT WORKSHEET

Participant ID	**Visit #**	**Date**

1. Record the highest level of organ abnormalities during the assessment period: Use the "rule of 9s" to compute percentage of body surface area for the skin assessment.
   a. Skin
      - ☐ None
      - ☐ Maculopapular rash < 25% of body surface
      - ☐ Maculopapular rash 25-50% of body surface
      - ☐ Generalized erythroderma
      - ☐ Generalized erythroderma with bullous formations and desquamation

   b. Intestinal Tract
      - ☐ None
      - ☐ > 500 ml/day
      - ☐ > 1000 ml/day
      - ☐ > 1500 ml/day
      - ☐ > Severe abdominal pain, with or without ileus

   c. Liver
      - ☐ None
      - ☐ Bilirubin 2-3 mg/dL
      - ☐ Bilirubin 3-6 mg/dL
      - ☐ Bilirubin 6-15 mg/dL
      - ☐ Bilirubin > 15 mg/dL
2. Overall Clinical Grading of Severity of Acute GVHD:
   - ☐ No Acute GVHD

☐ I-Stage 1-2 skin rash; no gut involvement; no liver involvement; no decrease in clinical performance

☐ II-Stage 1-3 rash; stage 1 gut involvement, or stage 1 liver involvement (or both); mild decrease in clinical performance

☐ III-Stage 2-3 skin rash; 2-3 gut involvement or stage 2-4 liver involvement (or both); marked decrease in clinical performance

☐ IV-Similar to Grade III with stage 2-4 organ involvement and extreme decrease in clinical performance

3. Record biopsy results pertaining to GVHD for this assessment period:

		Negative	Positive	Equivocal	Not Done
a.	Skin	☐	☐	☐	☐
b.	Gut	☐	☐	☐	☐
c.	Liver	☐	☐	☐	☐

4. Treatment for GVHD

☐ No treatment necessary

☐ Treatment initiated since last visit

☐ Treatment continued from last visit

☐ Treatment modified since last visit

If participant is receiving treatment, specify:

a. Treatment regimen and any modifications

b. Describe participant's response to treatment

Comments:

APPENDIX 7. CHRONIC GVHD ASSESSMENT WORKSHEET

CHRONIC GVHD SCORING Worksheet

IMPORTANT:
Include ALL GVHD related features that are present when scoring (diagnostic, distinctive, common, other)

Actual date of THIS assessment: |__|__| |__|__|__|__| |__|__|__|__|
DD MMM YYYY

Date of onset (diagnosis) of chronic GVHD: |__|__| |__|__|__|__| |__|__|__|__|
DD MMM YYYY

INSTRUCTIONS:

- When completing this form -- Do not include symptoms if there is a known cause other than GVHD
- NIH consensus GLOBAL SCORING instructions are listed on the last page

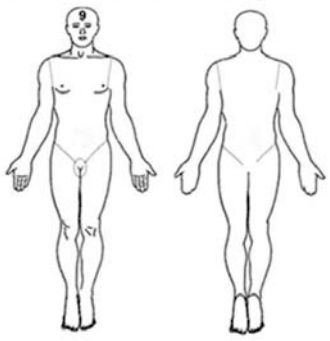
MOUTH

- ☐ **0** No symptoms related to GVHD
- ☐ **1** Mild symptoms with disease signs but not limiting oral intake significantly
- ☐ **2** Moderate symptoms with disease signs **with** partial limitation of oral intake
- ☐ **3** Severe symptoms with disease signs on examination **with** major limitation of oral intake

SKIN (including nails and scalp)

- ☐ **0** No symptoms related to GVHD
- ☐ **1** < 18% BSA with disease signs but NO sclerotic features
- ☐ **2** 19-50% BSA OR involvement with superficial sclerotic features "not hidebound" (able to pinch)
- ☐ **3** >50% BSA OR deep sclerotic features "hidebound" (unable to pinch) OR impaired mobility, ulceration or severe pruritus

If applicable, enter total % BSA affected by GVHD: _____



Calculating BSA and Rule of 9's

Head / Neck	9 %
Left upper extremity	4.5 % front; 4.5 % back
Right upper extremity	4.5 % front; 4.5 % back
Anterior torso	18 %
Posterior torso	18 %
Left lower extremity	9 % front; 9 % back
Right lower extremity	9 % front; 9 % back
Genitalia	1 %
	100 %

CHRONIC GVHD SCORING Worksheet

MUSCLE, FASCIA, JOINTS

☐ **0** No symptoms related to GVHD

☐ **1** Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL

☐ **2** Tightness of arms or legs OR joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL

☐ **3** Contractures **WITH** significant decrease of ROM **AND** significant limitation of ADL (unable to tie shoes, button shirts, dress self, etc.)

EYES

☐ **0** No symptoms related to GVHD

☐ **1** Mild dry eye symptoms not affecting ADL (requiring ≤ 3 x per day) OR asymptomatic signs of keratoconjunctivitis sicca

☐ **2** Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctual plugs), **WITHOUT** vision impairment

☐ **3** Severe dry eye symptoms significantly affecting ADL (special eye ware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca

GENITALIA

☐ **0** No symptoms related to GVHD

☐ **1** Symptomatic with mild signs on exam AND no effect on coitus and minimal discomfort with gynecologic exam

☐ **2** Symptomatic with moderate signs on exam AND with mild dyspareunia or discomfort with gynecologic exam

☐ **3** Contractures **WITH** advanced signs (stricture, labial agglutination or severe ulceration) AND severe pain with coitus or inability to insert vaginal speculum

***If no exam done and chronic GVHD has been diagnosed in one or more other organs, then use the following scoring scheme:**

☐ **0** No patient reported symptoms related to GVHD

☐ **1** Symptomatic mild patient reported symptoms

☐ **2** Symptomatic moderate patient reported symptoms including mild dyspareunia

☐ **3** Severe pain with coitus

This second scoring option is a study specific modification to the NIH consensus guidelines. It is recommended that every patient reporting symptoms should have a physical exam.

CHRONIC GVHD SCORING Worksheet

LUNGS

- ☐ **0** No symptoms related to GVHD and/or FEV1> 80% (related to GVHD)
- ☐ **1** Mild symptoms (SOB after climbing one flight of stairs)
- ☐ **2** Moderate symptoms (shortness of breath after walking on flat ground) and/or FEV1 40-59%
- ☐ **3** Severe symptoms (shortness of breath at rest; requiring O_2)

GI TRACT

- ☐ **0** No symptoms related to GVHD
- ☐ **1** Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (< 5%)
- ☐ **2** Symptoms associated with mild to moderate weight loss (5-15%)
- ☐ **3** Symptoms associated with significant weight loss > 15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation

LIVER

- ☐ **0** Normal LFTs
- ☐ **1** One or more of the following elevated < 2 X ULN due to GVHD: Total Bilirubin, Alk Phos, AST, ALT
- ☐ **2** Total Bilirubin > 3 mg/dl (i.e. > 51 µmol/L) due to GVHD
  OR
  One or more of the following elevated 2-5 x ULN due to GVHD: Total Bilirubin, Alk Phos, AST, ALT
- ☐ **3** One or more of the following elevated > 5 x ULN due to GVHD: Total Bilirubin, Alk Phos, AST, ALT

APPENDIX 8. STUDY REGIMEN WITH DE-SENSITIZATION SCHEDULE FOR SENSITIZED RECIPIENTS

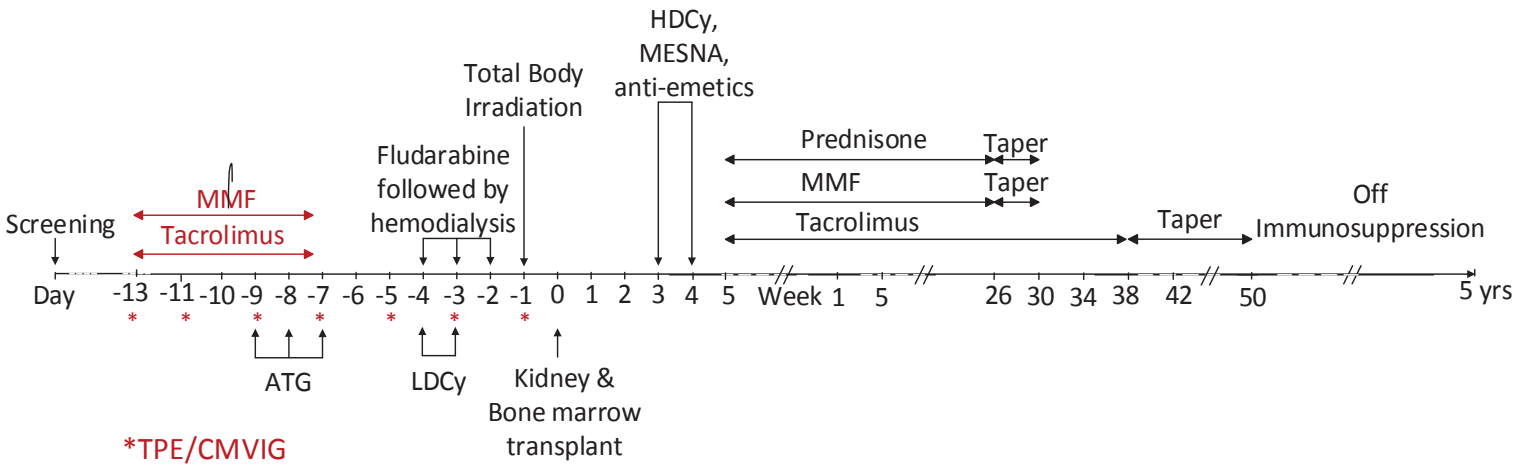


Figure 6: Study regimen for sensitized recipients

Sensitized participants with detectable DSA will undergo de-sensitization procedures per JHH standard of care as described below. Participants must demonstrate a negative flow cytometric crossmatch by day –9 to receive the first dose of study medication (ATG).

Sensitized participants with detectable DSA will receive therapeutic plasma exchange (TPE) treatments on alternating days on day -13 through day -1 prior to kidney transplantation. One and one-half plasma volume exchanges will be performed with each TPE treatment. Plasma volume will be reconstituted with human albumin, in most cases, although fresh frozen plasma may be used under certain circumstances. Pooled hyperimmune anti-CMV intravenous immune globulin (CMVIG) (Cytogam, MedImmune, Gaithersburg, MD) will be administered at 100mg/kg after each TPE.

On day -13, participants will begin treatment with tacrolimus and mycophenolate mofetil (MMF). Tacrolimus will be dosed orally to a target trough level of 8-10 ng/dL. MMF will be dosed at 1-2 grams per 24 hours. Participants will stop tacrolimus and MMF therapy on day-7.

DSA will be monitored frequently in the immediate post-operative period. Participants who demonstrate an increase in DSA will receive additional TPE and CMVIG treatments per standard of care.

APPENDIX 9. DE-SENSITIZATION SCHEDULE OF EVENTS PER JHH STANDARD OF CARE[16]

Appendix 1A Study Day	*-14 to -9*					*-8*	*-7*	*-6*	*-5*	*-4*	*-3*	*-2*	*-1*	*0*	*Post-transplant*
Sensitized Recipient Treatment Day	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	PT[2] Twice in first week, then weekly up to 2 months, months 3, 6, 9 & 12
HLA alloantibodies includes DSA, flow PRA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Therapeutic Plasma Exchange (TPE)	X		X		X		X		X		X		X		
IVIG	X		X		X		X		X		X		X		
Tacrolimus	X	X	X	X	X	X	X								
MMF	X	X	X	X	X	X	X								
Flow cytometric cross-match[3],[4]	X		X	X			X		X		X		X		*X*

[16] Appendix 9 will not be captured in electronic data capture forms

[2] DSA will be monitored frequently in the immediate post operative period. Participants who demonstrate an increase in DSA will receive additional TPE and CMVIG treatments per standard of care at JHH.

[3] If the sensitized participant has not demonstrated a negative flow cytometric crossmatch by day -9 they will be considered a screen failure and terminated from the study. A negative flow cytometric crossmatch on either day -10 or day -9 is acceptable.

[4] Flow cytometric crossmatch may be virtual or actual as needed.

APPENDIX 10. REINITIATION OF CONDITIONING REGIMEN

Recipients who receive any part of the conditioning regimen but are unable to undergo combined bone marrow and kidney transplant due to unanticipated issues with the donor harvest may undergo a second course of conditioning in order to proceed with the combined bone marrow and kidney transplant (CKBMT) per protocol.

The recipient may undergo CKBMT using the same donor or a different donor.

The determination of when to administer reconditioning and how much reconditioning, if any, is necessary to proceed with the transplantation, will be left to the discretion of the investigator and may be adjusted based on the amount of conditioning initially received and the time elapsed since the last dose received by the recipient. The general intent is for reconditioning with the full conditioning regimen.

Recipients may reinitiate the conditioning regimen no more than once. Recipients who are unable to undergo CKBMT after receiving a second course of conditioning will move to Safety Follow up (Appendix 5).Eligibility Criteria for Reinitiation of Conditioning.

INCLUSION CRITERIA

Recipient

If reinitiation of conditioning occurs more than 26 weeks after the first attempt, the recipient must fulfill *all* inclusion criteria in section 4.1.1, with the exception of donor and recipient HLA typing. In the event a new donor is used, the donor should undergo HLA typing.

If reinitiation of conditioning occurs within 26 weeks of the first conditioning attempt, recipient participants must meet *all* of the following eligibility criteria to reinitiate conditioning.

1. Complete ANC recovery defined as ANC $\geq$1000/mm^3 for three consecutive measurements over three to five days.

2. Recipient of a first renal allograft from an HLA-haploidentical, living related donor. The donor and recipient must be HLA identical for at least one allele (using high resolution DNA based typing) at the following genetic loci: HLA-A, HLA-B, HLA-C, and HLA-DRB1. Fulfillment of this criterion shall be considered sufficient evidence that the donor and recipient share one HLA haplotype. HLA typing reconfirmation is required only if a new donor is used.
3. ABO compatibility with donor.
4. DSA will be assessed by the local laboratory 30 days or less prior to transplant using solid phase micro particle technology (by Luminex® phenotype panel or Luminex single antigen bead test.) The following criteria apply:
   a. Participants without detectable DSA will be deemed eligible if they meet other entry criteria.
   b. Participants with detectable DSA and a positive flow cytometric crossmatch may undergo de-sensitization per standard of care *if they are cytotoxic crossmatch negative*. Such participants must demonstrate a negative flow cytometric

crossmatch by day -9 in order to receive the first dose of study therapy (ATG). Participants who do not demonstrate an acceptable response to de-sensitization by day -9 will be considered screen failures and will be terminated from the study.

c. Participants with a positive cytotoxicity crossmatch will be excluded.

5. If recipient had a positive QuantiFERON® (QFT) assay upon study entry, recipient must have a negative chest x-ray prior to proceeding with reinitiation of the conditioning regimen.
6. For women of childbearing potential, a negative serum or urine pregnancy test with sensitivity less than 50 mIU/m within 72 hours before the start of study medication.

Pulmonary function and cardiac tests may be repeated if deemed necessary by the investigator.

Donor

Donor participants must meet *all* of the following criteria to be eligible, regardless of whether they are the initial donor or a new donor:

1. HLA-haploidentical, first-degree relatives or half-siblings of the recipient participant at the allele or allele group. The donor and recipient must be HLA identical for at least one allele (using high resolution DNA based typing) at the following genetic loci: HLA-A, HLA-B, HLA-C and HLA-DRB1. Fulfillment of this criterion shall be considered sufficient evidence that the donor and recipient share one HLA haplotype. Reconfirmation is required only if a new donor is used.
2. Age 18 to 65 years.
3. Creatinine clearance >80 ml/minute as measured from a 24 hour urine collection within 26 weeks of the screening visit. If a serum creatinine drawn at the screening visit is > 20% higher than the serum creatinine drawn at the time of the 24 hour urine collection, the creatinine clearance must be re-evaluated by a repeat 24 hour urine test. If the new value is ≤80mg/dL the donor will be excluded. A repeat 24 hour urine collection is not required if the same donor is used.
4. Meets institutional selection criteria for organ and bone marrow donation.
5. Ability to understand and provide informed consent for all study procedures including kidney transplant and bone marrow harvest.
6. Serologic evidence of prior EBV infection as documented by positive IgG and negative IgM antibodies against EBV. EBV status does not need to be reconfirmed if the initial donor is used.

EXCLUSION CRITERIA

Recipient

Recipient participants who meet *any* of the following criteria will *not* be eligible to re-initiate conditioning:

1. Positive HIV-1 or HIV-2 nucleic acid test.

2. Evidence of hepatitis B infection. Participants demonstrating any one of the following will be excluded:
    a. positive hepatitis B surface antigen (HBsAg) or
    b. positive anti-HBc IgM
    c. positive anti-HBc IgG
    d. positive HBV PCR
3. Positive anti-hepatitis C (HCV) antibodies and a positive serum HCV RNA PCR. All positive HCV antibody results must be assessed by an EIA assay and confirmed by a quantitative serum HCV RNA assay. Participants with positive HCV antibodies but undetectable serum HCV RNA may be considered for eligibility. Participants with negative anti-HCV antibodies but unexplained liver enzyme abnormalities must undergo a quantitative serum RNA assay to rule out false negative HCV serologies.
4. Positive cytotoxic crossmatch.
5. Calculated PRA greater than 90%.
6. Any active, severe local or systemic infection at the screening visit.
7. Autoimmune disease requiring immunosuppressive drugs for maintenance.
8. Use of investigational drug, other than the study medications specified by the protocol, within 30 days of transplantation.
9. Receipt of a live vaccine within 30 days of receipt of study therapy.
10. Women who are breastfeeding.
11. The presence of any medical condition that the Investigator deems incompatible with participation in the trial.

Donor

Donor participants who meet *any* of the following criteria will *not* be eligible for this study, regardless of whether they are the initial donor or a new donor:

1. History of type I or type II diabetes mellitus.
2. History of severe cardiovascular disease, defined as New York Heart Association Class III or IV[2].
3. History of blood product donation to recipient.
4. History of positive HIV-1 or HIV-2 serology or nucleic acid test.
5. Evidence of hepatitis B infection. Participants demonstrating any one of the following will be excluded:
    a. positive hepatitis B surface antigen (HBsAg) or
    b. positive anti-HBc IgM
    c. positive anti-HBc IgG
    d. positive HBV PCR
6. Positive anti-hepatitis C (HCV) antibodies and a positive serum HCV RNA PCR. All positive HCV antibody results must be assessed by an EIA assay and confirmed by a quantitative serum HCV RNA assay. Participants with positive HCV antibodies but undetectable serum HCV RNA may be considered for eligibility. Participants with

negative anti-HCV antibodies but unexplained liver enzyme abnormalities must undergo a quantitative serum RNA assay to rule out false negative HCV serologies.

7. Autoimmune disease requiring immunosuppressive drugs for maintenance.
8. The presence of any medical condition that the Investigator deems incompatible with participation in the trial.

Monitoring of Recipient Between Conditioning Regimens

The recipient's blood counts will be monitored closely until complete ANC recovery, defined as ANC $\geq$1000/mm^3 for three consecutive measurements over three to five days. The recipient will receive antimicrobial prophylaxis per JHU standard of care.