

Impact of CCR5 Blockade in HIV+ Kidney Transplant Recipients

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**HIVTR-CCR5****Impact of CCR5 Blockade in HIV+ Kidney Transplant Recipients**

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Protocol Synopsis

Title	Impact of CCR5 Blockade in HIV+ Kidney Transplant Recipients
Short Title	HIVTR CCR5
Clinical Phase	Phase II
Number of Sites	10
IND Sponsor/Number	NIAID DAIDS
Study Objectives	<p>The primary clinical objectives are to evaluate the:</p> <ol style="list-style-type: none"> 1. Impact of CCR5 blockade (maraviroc, MVC) on renal function at week 52 post-transplant. 2. Overall safety and tolerability of CCR5 blockade in the HIV+ kidney transplant recipient. <p>The secondary clinical objectives are to evaluate the:</p> <ol style="list-style-type: none"> 1. Impact of CCR5 blockade (maraviroc, MVC) on inflammation in the graft at week 26 and week 52 post-transplant. 2. Impact of CCR5 blockade on long term kidney function in the HIV positive kidney transplant recipient at months 3, 6, 9 and years 1, 2, 3. 3. Impact of the addition of CCR5 blockade to cART and immunosuppression on HIV persistence following kidney transplantation 4. Impact of CCR5 blockade on the incidence and severity of rejection 5. Incidence of HIV infection/HIV nephropathy (HIVAN) in the kidney allograft 6. Safety profiles with use of CCR5 blockade 7. Pharmacokinetics and impact of CCR5 blockade exposure to CNIs <p>The primary mechanistic objectives are to:</p> <ol style="list-style-type: none"> 1. Define immunologic parameters associated with rejection in the HIV positive recipient (versus HIV+ non-rejectors) 2. Determine the impact of CCR5 blockade on the immunologic profiles in the HIV positive recipient.
Study Design	Prospective, multi-center, double-blind phase II study
Primary Clinical Endpoints	<ol style="list-style-type: none"> 1. Measured glomerular filtration rate at 52 weeks by iohexol clearance. 2. Graft loss, toxicities \geq Grade 3 and/or permanent treatment discontinuation within the first 52 weeks post-transplant.
Secondary Clinical Endpoints	<ol style="list-style-type: none"> 1. Inflammation in the graft <ol style="list-style-type: none"> a. CD45 gene expression (PTPRC) on the FFPE kidney biopsy sample at 26 weeks post-transplant. b. CD45 quantitative IHC on the FFPE sample at 26 weeks post-transplant.

	<ul style="list-style-type: none"> c. Gene expression profiling using the 11-gene tCRM module on FFPE biopsy shaves at 26 weeks and the 11-gene uCRM module on urine cell pellets at 26 weeks and 52 weeks post-transplant. <ol style="list-style-type: none"> 2. Measures of Renal Function and Injury (months 3, 6, 9, years 1, 2, 3) <ol style="list-style-type: none"> a. Proportion of participants with eGFR by CKD-EPI, cystatin C, and CKD-cystatin C, < 60 mL/min/1.73 m². b. Proportion of participants with defined CKD stage 4 or 5. c. Mean calculated eGFR by CKD-EPI, cystatin C, and CKD-cystatin C. d. The slope of eGFR by CKD-EPI, cystatin C, and CKD-cystatin C variable models over time based on serum creatinine. 3. HIV persistence <ol style="list-style-type: none"> a. HIV reactivation (frequency of CD4+ T cells producing HIV multiply spliced RNAs upon TCR stimulation) b. HIV DNA and RNA in peripheral blood CD4+ T cells c. Plasma HIV RNA levels (single copy assay) 4. Histologic and Serologic Evidence of Rejection <ol style="list-style-type: none"> a. Incidence of clinically suspected and biopsy proven acute rejection within the first 52 weeks and 156 weeks as defined by histologic evidence of rejection and graft dysfunction as identified on central read of biopsy slides. b. The incidence of acute cellular rejection grade equal to or > than IA, by the Banff 2007 criteria, within the study period (3 years) as identified on central read of biopsy slides. c. The severity of first and highest grade of acute cellular rejection within the study period. d. The incidence of antibody mediated rejection. e. The prevalence of de novo anti-donor HLA antibodies at 52 weeks. 5. Analysis of HIV infection in the renal allograft and HIVAN <ol style="list-style-type: none"> a. Histology/in situ hybridization to assess HIV infection in the renal allograft 6. Safety Profile measures <ol style="list-style-type: none"> a. Death b. Graft loss c. Incidence of all adverse events (AEs) >= Grade 3 d. Incidence of serious adverse events (SAEs) >= Grade 3 e. Incidence of opportunistic infections or neoplasms f. Incidence of non-opportunistic infections requiring hospitalization or systemic therapy 7. Pharmacokinetics <ol style="list-style-type: none"> a. Calcineurin inhibitor trough levels and AUC for participants on maraviroc versus placebo b. AUC and trough levels of CCR5 blockade (maraviroc)
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Mechanistic Endpoints	<p>1) Define immunologic parameters associated with rejection in the HIV+ participants by comparing the following parameters in those who experience acute rejection in the first 6 months and those who do not have acute rejections (samples collected prior to and at the time of rejection will be analyzed and compared):</p> <ul style="list-style-type: none"> a. Circulating leukocyte subsets and their activation phenotypes b. Anti-donor alloimmune responses measured by frequency of total donor-reactive T cells and donor-reactive effector T cells c. Frequencies of T cells with cross-reactivity between donor alloantigens and HIV, CMV, and EBV antigens d. DSA in serum e. Gene expression in for-cause biopsies by RNASeq analysis f. Histological analysis for evidence of cellular and antibody mediated rejection mechanisms. <p>2) Determine the impact of CCR5 blockade on the immunologic profiles in the HIV + recipients by comparing results from MVC-treated and those receiving standard of care (SOC). Change of the various parameters after initiation of MVC or SOC will be monitored and these changes will be compared between the two groups (Samples collected pre transplant and at various time points post-transplant will be analyzed):</p> <ul style="list-style-type: none"> a) Change in circulating leukocyte subsets and their activation phenotypes from pre-transplant baseline b) Change in CCR5 and other chemokine receptor expression on subsets of various circulating leukocytes from pre-transplant baseline c) Change on anti-donor alloimmune responses measured by frequency of total donor-reactive T cells and donor-reactive effector T cells d) Change in frequencies of T cells with cross-reactivity between donor alloantigens and HIV, CMV, and EBV antigens after transplant. e) DSA in serum f) Histological analysis of for-cause biopsies for mechanisms of rejection
Accrual Objective	130 randomized (65 maraviroc and 65 placebo)
Study Duration	5 years (all participants followed for 1 – 3 years depending on enrollment date)

<p>Treatment Description</p>	<p>Prior to transplantation, participants should be on a stable non-protease inhibitor-based regimen. At the time of transplantation, eligible participants will be randomized 1:1 to add maraviroc or placebo. Initiation of study drug will occur during the admission for transplantation, prior to transplant.</p> <p>Arm 1: Maraviroc</p> <p>Arm 2: Placebo</p> <p>Dose: Initial dose of 300 mg twice daily (150mg twice daily if co-prescribed with a potent CYP3A inhibitor or 600mg twice daily if co-prescribed with a potent CYP3A inducer). Will be modified if GFR < 30, if co-prescribed with a potent CYP3A inhibitor or inducer, or if the calcineurin inhibitor used for maintenance immunosuppression is changed to cyclosporine (which is only allowed for tacrolimus toxicity). Once GFR >= 30, the dose should be returned to the non-renal dosage. If GFR is consistently fluctuating (especially immediately post-transplant), the site investigator may <u>choose</u> when to resume normal dosing of study products based on clinical assessment of stability.</p>
<p>Inclusion Criteria</p>	<ol style="list-style-type: none"> 1) Participant is able to understand and provide informed consent 2) Documented HIV infection (by any licensed ELISA and confirmation by Western Blot, positive HIV ab IFA, or documented history of detectable HIV-1 RNA). 3) Participant is ≥ 18 years old. 4) CD4+ T-cell count >= 200/μL at any time in the 26 weeks prior to enrollment. 5) Most recent HIV-1 RNA < 50 copies RNA/mL. Eligibility at the time of enrollment will be determined based on the most recent HIV-1 RNA, not more than 26 weeks prior to enrollment. Subjects who require a switch in cART regimen to become study eligible must also have an eligible HIV-1 RNA result post change in cART. 6) Participant meets standard listing criteria for placement on transplant waiting list. 7) For participants with an HIV+ deceased donor: <ol style="list-style-type: none"> a) No active opportunistic infections. b) Concurrence by the study team that based on medical history and ART, viral suppression can be achieved in the recipient post-transplant. c) Must be enrolled in an IRB approved research protocol that fulfills the requirements of the DHHA Hope Act Policy (see section 9.1.2).

	<p>d) HIV+ deceased donor must have no evidence of invasive opportunistic complications of HIV infection, and must have a pre-implant biopsy.</p> <p>8) Antiretroviral (ARV) Use: Participant is on a stable cART regimen for at least 3 months prior to enrollment (unless changes are made due to toxicity, drug interactions, convenience or to an eligible non-protease inhibitor-based regimen). Switch should not be due to virologic failure. A regimen consisting of 2 NRTIs and an integrase inhibitor is preferred due to minimal drug interaction but any non-protease inhibitor regimen may be used.</p> <ul style="list-style-type: none"> • If on a protease inhibitor based regimen, participant must be switched to a non-protease inhibitor-based regimen based on lack of any prior drug resistance or antiretroviral-treatment failure, and be willing to remain on indefinitely unless a change is medically necessary. Participants who need to be switched must have been on a stable cART regimen for at least 3 months prior, and must have an eligible HIV-1 RNA result post change in cART. • If already on a stable non-protease inhibitor-based regimen, participant is willing to remain on this regimen indefinitely unless a change in regimen is medically indicated. • If untreated, must initiate and be willing to remain on indefinitely a non-protease inhibitor-based antiretroviral regimen unless a change is medically necessary. <p>9) No known allergy or intolerance to components of MVC or its formulation.</p> <p>10) No known contraindication to MVC.</p> <p>11) Female participants of child-bearing potential must have a negative serum beta-HCG pregnancy test within 30 days of randomization.</p>
<p>Exclusion Criteria</p>	<ol style="list-style-type: none"> 1. Participant is currently on maraviroc. 2. Participant needs multi-organ transplant. 3. Participant has a live donor who is HIV+. 4. Participant is unable to switch to a non-protease inhibitor-based cART regimen. 5. Participant has received immunosuppressant medication in the 6 months prior to enrollment. Note: Low dose maintenance steroids (\leq 10 mg per day of prednisone, or equivalent strength steroid) will not be considered immunosuppression. 6. Opportunistic Complication History: Any history of progressive multifocal leukoencephalopathy (PML), chronic intestinal cryptosporidiosis of > 1 month duration, or primary CNS lymphoma. Note: History of pulmonary

	<p>coccidiomycosis will be treated per local site policy regarding this infection in HIV negative transplant candidates, generally requiring a 5-year disease-free interval.</p> <ol style="list-style-type: none"> 7. Participant has a history of any neoplasm except for the following: resolved kaposi’s sarcoma, <i>in situ</i> anogenital carcinoma, adequately treated basal or squamous cell carcinoma of the skin, solid tumors (except primary CNS lymphoma) treated with curative therapy and disease free for more than 5 years. History of renal cell carcinoma requires disease free state for 2 years. History of leukemia and disease-free duration will be per site policy. 8. Substance use that in the opinion of the investigator would interfere with compliance with the study requirements. 9. Participant is pregnant or breastfeeding. Note: Participants who become pregnant post-transplant will continue to be followed in the study and will be managed per local site practice. Women that become pregnant should not breastfeed. 10. Participant has used IL-2 or GM-CSF in the prior six months. 11. Participant has received interferon-alpha therapy in the prior 12 weeks. 12. Use of investigational drugs within 4 weeks of enrollment. 13. Past or current medical problems or findings from medical history, physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant’s ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.
<p>Participant Stopping Rules</p>	<p>Treatment discontinuation</p> <ol style="list-style-type: none"> 1. Participant meets criteria to discontinue study treatment (MVC or placebo) 2. The investigator no longer believes continuing study treatment is in the best interest of the participant. 3. Participant refuses to continue study treatment. 4. Treatment is held or discontinued for clinical reasons for greater than 60 days. <p>Study discontinuation</p> <ol style="list-style-type: none"> 1. The participant elects to withdraw consent from all future study activities, including follow-up. 2. The participant is “lost to follow-up” (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed). 3. For those on the waiting list, confirmed or predicted failure to receive transplant by the end of year 4.

	<ol style="list-style-type: none"> 4. Study closed by sponsor or FDA. 5. Despite being randomized and receiving study treatment, participant did not receive a transplant as expected.
<p>Study Stopping Rules</p>	<p>The study may be discontinued at any time by the EC/IRB, NIAID, the pharmaceutical supporter(s) or designee, the FDA, or other government entities as part of their duties to ensure that research participants are protected.</p> <p>In addition, the incidence of specific safety-related events of particular concern will be continuously monitored throughout the study to determine if any of their observed subject-based incidence rates exceed a threshold incidence rate of concern pre-specified for each particular event.</p> <p>These events and their corresponding thresholds of concern are:</p> <ol style="list-style-type: none"> 1. During year 1 of follow-up, death due to any reason except accidental death above 10%. 2. During year 1 of follow-up, graft loss due to any reason except accidental death above 16%. 3. During year 1 of follow-up, site-reported treated acute rejection above 35%

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Glossary of Abbreviations

3TC	lamivudine, EPIVIR
Ab	Antibody
ABC	abacavir sulfate, ZIAGEN
AE	Adverse event
AIDS	Acquired immune deficiency syndrome
AUC	Area under the curve
BID	Twice a day
BKV	BK Polyoma Virus
cART	Combination antiretroviral therapy
CCR5-Δ 32	CCR5 delta 32
CFR	Code of Federal Regulations
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration equation
CMV	Cytomegalovirus
CNI	Calcineurin inhibitor
CNS	Central nervous system
CRF	Case Report Form
CRM	Common Response Module
DAERS	DAIDS Adverse Experience Reporting System
DAIDS	Division of AIDS
DAIT	Division of Allergy, Immunology, and Transplantation
dd-cfDNA	Donor Derived Cell Free DNA
Dolutegravir	Tivicay
DSMB	Data Safety Monitoring Board
eGFR	estimated glomerular filtration rate
EBV	Epstein Barr Virus
EMMES IDES	EMMES internet data entry system
ESRD	End-stage renal disease
FDA	Food and Drug Administration
FTC	emtricitabine, EMTRIVA
GCP	Good Clinical Practice
GFR	Glomerular filtration rate

HBV	Hepatitis B
HCV	Hepatitis C
HIV	Human immunodeficiency virus
HIV VL	Human immunodeficiency virus viral load
HIVAN	HIV Associated Nephropathy
HLA	Human leukocyte antigen
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
kSORT	Kidney Solid Organ Rejection Test
MAC	Mycobacterium avium complex
MOP	Manual of Procedures
MMF	Mycophenolate mofetil
MVC	Maraviroc
NIAID	National Institute of Allergy and Infectious Diseases
NNRTI	Non-nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside/nucleotide reverse transcriptase inhibitor
PCP	Pneumocystis carinii pneumonia
PI	[Site] Principal Investigator
PK	Pharmacokinetic
PPD	Tuberculosis skin test
PPSC	Publications and Presentations Sub-committee
Pre-tx	Pre-transplant
QD	Once daily
Raltegravir	ISENTRESS
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SOC	Standard of care
SOP	Standard Operating Procedure
SUSAR	Serious Unexpected Suspected Adverse Reaction
TDF	tenofovir, VIREAD
uPRO	Urine Proteins

Study Definitions Page

Acute Rejection	Banff grade of greater than or equal to 1A with or without clinical symptoms.
Antibody Mediated Rejection (AMR)	Diffusely positive staining for C4d, presence of circulating anti-donor antibodies, and morphologic evidence of acute tissue injury.
Banff 2007 criteria	The Banff classification for assessment of renal allograft biopsies was introduced as a standardized international classification of renal allograft pathology and acute rejection. Subsequent debate and evaluation studies have attempted to develop and refine the classification. The 2007 version is the most current.
CKD Stage 4	Decrease in the glomerular filtration rate (GFR) to 15-30 ml/min
CKD Stage 5	End stage renal disease (ESRD) with a glomerular filtration rate (GFR) of 15 ml/min or less.
Cystatin C	a protein encoded by the CST3 gene, mainly used as a biomarker of kidney function.
Graft loss	For protocol, defined as the first observation of any of the following events: death, retransplantation, or initial return to chronic dialysis. Chronic dialysis is defined as 30 days or a treatment plan of permanent ongoing dialysis support.
HIV breakthrough	2 consecutive plasma HIV viral load > 200 copies/mL or one HIV viral load > 1000 copies/mL after a period of virologic control post-transplant.
HIV persistent virologic failure	HIV Viral load > 1000 copies/mL for more than 90 days that is not the result of an investigator/physician approved interruption in antiretroviral treatment.
Iohexol	Radiographic contrast agent whose clearance from serum can be used to estimate GFR (kidney function)
MDRD4 variable model	<p>The most recently advocated formula for calculating the GFR is the one that was developed by the <i>Modification of Diet in Renal Disease Study Group</i>.¹ The most commonly used formula is the “4-variable MDRD,” which estimates GFR using four variables: serum creatinine, age, ethnicity, and gender. ²</p> <p>¹National Kidney Foundation (February 2002). “K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification”. <i>American Journal of Kidney Diseases</i> 39 (2 Suppl 1): S1–266.</p> <p>²Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D (March 1999). “A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group”. <i>Annals of Internal Medicine</i> 130 (6): 461–70.</p>
Neoplasm	An abnormal mass of tissue that results when cells divide more than they should or do not die when they should. Neoplasms may be benign (not cancer), or malignant (cancer). Also called tumor.

Opportunistic infection	An infection by a microorganism that normally does not cause disease but becomes pathogenic when the body's immune system is impaired and unable to fight off infection.
Protocol Mandated Procedures	Any procedure performed solely for the purpose of this research study, not considered site specific standard of care.
Randomized	A participant who met all eligibility criteria; met with the study investigator or designee to discuss the study purpose, requirements (i.e., time requirements, schedule of events, etc.), discussed all risks and benefits, signed the informed consent document and was randomly assigned to one of 2 treatment groups.
Study Therapy	The investigational therapeutic regimen.
Visit window	2 weeks prior to time point, or after the time point up to the mid-way point between the target date and the subsequent study visit date.

1 Study Hypotheses/Objectives

1.1 Hypotheses

It has been established that persistent HIV infection leads to immune dysregulation, which is characterized by increased basal activation of T cells, B cells, and myeloid cells. This global dysregulation can lead to augmented anti-donor alloimmune responses through specific expansion of donor HLA and pathogen cross-reactive cells (heterologous immunity) and/or non-specific expansion of effector T cells and antibody-producing B cells through homeostatic proliferation. CCR5 expression is increased on activated T cells, B cells, and myeloid cells and it is known to play an important role in orchestrating coordinated activation of immune effector functions. In addition, CCR5 also contributes to the spread and maintenance of HIV reservoir by serving as a receptor for HIV entry in CD4 T cells and myeloid cells, thus perpetuating and exacerbating the immune dysregulation. Therefore, we hypothesize that CCR5 contributes to the enhanced alloimmune responses in HIV+ participants and CCR5 blockade will be effective in reducing immunologic graft injury and improving long-term kidney function through reducing HIV reservoir and intercepting the vicious cycle of immune activation and dysregulation. Our specific hypotheses are:

- CCR5 blockade in HIV+ renal transplant participants will minimize immunologic graft injury and improve renal function. The high rate of kidney transplant rejection in HIV+ patients is due to high frequency of heterologous memory donor-reactive T cells that arise in response to chronic infections in this population which include HIV and a multitude of other co-pathogens such as CMV, EBV, HCV, and HBV.
- CCR5 blockade in combination with immunosuppressive therapy used following kidney transplantation will reduce HIV persistence in CD4+ T lymphocytes.

1.2 Objectives

1.2.1 The primary clinical objectives are to evaluate the:

1. Impact of CCR5 blockade (maraviroc, MVC) on renal function at week 52 post-transplant.
2. Overall safety and tolerability of CCR5 blockade in the HIV+ kidney transplant recipient

1.2.2 The secondary clinical objectives are to evaluate the:

1. Impact of CCR5 blockade (maraviroc, MVC) on inflammation in the graft at week 26 and week 52 post-transplant.
2. Impact of CCR5 blockade on long term kidney function in the HIV positive kidney transplant recipient at months 3, 6, 9 and years 1, 2, 3.
3. Impact of the addition of CCR5 blockade to cART and immunosuppression on HIV persistence following kidney transplantation
4. Impact of CCR5 blockade on the incidence and severity of rejection
5. Incidence of HIV infection/HIV nephropathy (HIVAN) in the kidney allograft
6. Safety profiles with use of CCR5 blockade
7. Pharmacokinetics and impact of CCR5 blockade exposure to CNIs

1.2.3 The primary mechanistic objectives are to:

1. Define immunologic parameters associated with rejection in the HIV positive recipient (versus HIV+ non-rejectors)
2. Determine the impact of CCR5 blockade on the immunologic profiles in the HIV positive recipient.

2 Background and Rationale

2.1 Background and Scientific Rationale

Despite considerable refinement in immunosuppressive regimens over the last 25 years and decreased rates of acute rejection following kidney transplantation, long-term transplant outcomes have remained stagnant. Furthermore, there has been a relative paucity in the development of new immunosuppressive strategies to minimize acute and chronic injury. Maraviroc is a CCR5 inhibitor that may have a novel role in modulating the immune response following transplantation. An ideal setting to test the impact on the alloimmune response is in HIV infected patients undergoing kidney transplantation since CCR5 inhibition with maraviroc has been effective as an antiretroviral agent that has been safely used as a component of cART. Furthermore, HIV infected adults have unexpectedly high rejection rates (2-3 fold higher than HIV uninfected recipients) [1, 2], facilitating studies exploring the immunosuppressive efficacy of CCR5 blockade.

HIV persists indefinitely during cART. Many mechanisms likely contribute to this persistence, including persistent immune activation and dysfunction, as well as persistent replenishment of the reservoir due to low-level HIV spread/replication. There are theoretical suggestions that CCR5 blockade may affect these pathways, leading to reduction in HIV reservoir. Careful measurements of HIV persistence/viral load will be performed in our study. These measurements are important as changes in HIV load can directly impact immune function and graft survival. Of equal significance, any agent that affects HIV persistence will contribute to the emerging global cure efforts.

2.2 Rationale for Selection of Investigational Product or Intervention

CCR5 is a chemokine receptor widely expressed on immune cells, including dendritic cells, activated macrophages [3], resting and activated NK cells [4], and activated Th1 and CD8 cells [5, 6]. CCR5 plays a major role in regulating immunity by facilitating CD4-CD8 T cell collaborations [7], serving as a co-stimulatory molecule for Th1 cell activation [8], activating pro-inflammatory M1 macrophages [9], and recruiting monocytes, macrophages, NK cells, and T cells to sites of inflammation [10]. CCR5 blockade promotes graft survival in rodent models of heart [11, 12], kidney [13], islet [14] and aorta transplantation [15]. Kidney transplant recipients who do not express CCR5 as a consequence of a homozygous CCR5 delta 32 deletion ("CCR5-Δ 32") have significantly prolonged long term allograft survival compared to recipients lacking this genetic deletion [16]. Similarly, liver transplant recipients homozygous for the CCR5-Δ 32 mutation have reduced acute rejection rates [17]. The findings collectively support the idea that CCR5 blockade using maraviroc may be beneficial in kidney transplantation. Consistent with this notion, a recently concluded clinical trial in bone marrow transplant recipients demonstrated that maraviroc blocked lymphocyte chemotaxis and visceral graft-versus host disease [18]. *We hypothesize that the addition of CCR5 blockade to cART in HIV+ renal transplant recipients will minimize immunologic graft injury and improve renal function.*

Several lines of evidence have suggested that inhibition of CCR5 could reduce the size of the reservoir during long-term antiretroviral therapy. Chronic inflammation and T cell activation are now well-accepted parts of HIV pathogenesis. The frequency of activated T cells is high during untreated disease and declines during treatment, but it rarely fully normalizes. Among long-term effectively treated individuals, the frequency of activated T cells is higher than normal [19], which is associated with risk of disease progression [20], and correlated with the size of the HIV reservoir. Chronic inflammation may contribute to HIV viral persistence through many mechanisms, including: 1) increased HIV production from CD4+ cells; 2) increased number of activated CD4+ T cells, which are the preferred target for viral replication; 3) homeostatic proliferation of latently infected cells; and 4) changes in immunoregulatory environment, leading to reduced clearance mechanisms. As binding of CCR5 on T cells to its ligands regulates chemotaxis and migration to sites of inflammation, inhibiting CCR5 might prevent CD4+ T cells from accumulating in areas where ongoing cell-to-cell

transmission of HIV occurs and alters several inflammatory pathways that lead to HIV persistence [10, 21]. Any of these mechanisms may have contributed to the absence of HIV viral persistence in the “Berlin patient”, the HIV+ recipient of a bone marrow transplant from a homozygous CCR5-Δ 32 donor [22]. *We hypothesize that CCR5 blockade in combination with immunosuppressive therapy used following kidney transplantation will reduce HIV persistence in CD4+ T lymphocytes*

The finding that HIV+ patients experience three-fold more rejection than HIV- recipients not only presents an important clinical challenge, but also an intriguing mechanistic question which might ultimately lead to identification of novel interventions for the broader transplantation field. The question which we will address in this protocol is why do HIV-infected adults - who despite cART often have readily measured immunodeficiency - mount a stronger response to transplanted grafts than HIV-uninfected adults? The answer may lie in heterologous immunity, which is emerging as a central focus in transplantation biology [23]. Experiments in animal models of transplantation demonstrate that multiple viral infections can induce the generation of memory allo-reactive T cells; this occurs as a result of cross-reactivity between alloantigen- and pathogen-reactive T cells [24-30]. These cross-reactive memory T cells have a lower threshold for activation, can directly traffic to grafts, and are capable of expressing effector functions upon first encounter with donor antigens, all of which contribute to accelerated graft rejections and resistance to tolerance induction. Cross-reactivity between human alloantigen and viral antigen-reactive T cell lines and clones has also been demonstrated [31]. However, association between increased heterologous immunity and enhanced graft rejection in transplant patients has not been documented. *We hypothesize that the high rate of kidney transplant rejection in HIV+ patients is due to high frequency of heterologous memory donor-reactive T cells that arise in response to chronic infections in this population which include HIV and a multitude of other co-pathogens such as CMV, EBV, HCV, and HBV.* Thus, transplantation in HIV+ patients may provide a unique opportunity to determine the importance of heterologous immunity to transplant outcomes in humans.

2.3 Preclinical Experience

Maraviroc is a CCR5 inhibitor that has been approved for use as a component of combined antiretroviral therapy (see Clinical Studies). Since CCR5 is a chemokine receptor widely expressed on immune cells, blocking this receptor has theoretic benefits in terms of modulating the alloimmune response by blocking the migration of pro-inflammatory cells to the allograft. Several pre-clinical studies have demonstrated the efficacy of CCR5 blockade in promoting allograft survival in rodent models of heart [11, 12], kidney [13], islet [14] and aorta transplantation [15].

Although maraviroc has not been approved for use as an immunosuppressive agent, a recently concluded trial in bone marrow transplant recipients demonstrated that maraviroc blocked lymphocyte chemotaxis and visceral graft-versus-host disease (20). Thirty-eight subjects who had undergone reduced-intensity allogeneic hematopoietic stem cell transplantation were treated with standard graft-versus-host disease (GVHD) prophylaxis and maraviroc (ClinicalTrials.gov number, NCT00948753). Among 35 evaluable subjects, the observed incidence rate of grade III or IV acute GVHD was far less common than that observed in historical controls. Mechanistic studies suggested that inhibition of lymphocyte trafficking lead to prevention of GVHD.

The potential efficacy of CCR5 blockade in terms of blocking the alloimmune response in solid organ transplantation is supported by the consistent observation that transplant recipients with homozygous CCR5-Δ 32 allele (no cell surface expression of CCR5) have significantly prolonged long term allograft survival compared to recipients with no mutations in CCR5; this has been shown for both kidney [16] and liver [17] transplants. The findings collectively support the idea that CCR5 blockade using maraviroc may be beneficial in kidney transplantation.

2.4 Clinical Studies

Maraviroc is a member of a class of antiretroviral compounds known as small molecule CCR5 antagonists that block R5 HIV entry into CD4 cells. Maraviroc has demonstrated selective and reversible binding to CCR5, as well as potent antiviral activity *in vitro* against a wide range of laboratory adapted strains of R5 HIV from Clades A, B, C, D, E, F, G, J and O. Maraviroc also retains *in vitro* antiviral activity against clinical isolates resistant to the existing drug classes, but has no activity against viruses that enter CD4+ cells using CXCR4. When used in combination with other antiretroviral drugs in subjects known to lack CXCR4-utilizing viruses, maraviroc is safe, well-tolerated and results in sustained suppression of HIV replication. Maraviroc has been approved for the management of HIV disease since 20XX. A recent FDA-mandated review of long-term (5 years) exposure to this drug in controlled studies (n=938 treated with maraviroc) found low rates of death, hepatic failure, malignancy, myocardial infarction and other adverse events, leading to the conclusion that the drug has safety profile consistent with other antiretroviral drugs [32].

Maraviroc is an oral medication that is rapidly absorbed when taken without food. The standard dose is 300 mg bid (without concomitant potent CYP3A4 inhibitors or inducers. Approximately 25% is excreted unchanged in the feces and 33% is excreted unchanged in the urine, the rest being metabolized by the liver. The drug accumulates with advanced renal or liver failure [33]. Maraviroc is taken up by an OATP1 uptake transporter and the homozygous 521T>C mutation decreases uptake by 75%.[34]. Maraviroc is metabolized by cytochrome P4503A4, and any concomitant medications that inhibit CYP3A4, such as tacrolimus, will increase maraviroc concentrations. In one case report in a liver transplant subjects, tacrolimus AUC increased ~15%, while maraviroc AUC was approximately twice normal[35].

With regard to our proposed study, maraviroc has no known nephrotoxicity and appears to be safe and well-tolerated in subjects with impaired renal function. A recent small open-label study of maraviroc in subjects with mild to severe renal impairment (including end-stage renal disease) found that renal function had no readily apparent effect on plasma concentration-time curve and no dosing adjustments among those with impaired renal function[36].

Studies in Healthy Volunteers

In Phase 1 and 2a clinical studies conducted in healthy volunteers and HIV+ individuals, maraviroc has been administered as single oral doses up to 1200 mg and multiple oral doses up to 300 mg BID for 28 days, and up to 900 mg BID and 1200 mg QD for 7 days. Single doses up to 900mg and multiple doses up to 300mg BID were well tolerated, with the adverse event profile of 300mg BID being similar to placebo. Doses above 300mg (the approved dose) were associated with an increased incidence of adverse events, particularly postural hypotension, compared to placebo. The most common Adverse Events (AEs), regardless of causality, reported following single and multiple dosing have been asthenia, headache, dizziness, nausea, rhinitis and postural hypotension. Postural hypotension is potentially the dose limiting adverse event [37].

No treatment related Serious Adverse Events (SAEs) were reported in the healthy volunteer (Phase 1) studies, and most AEs were judged to be mild to moderate in severity. Mean QTcI differences from placebo for maraviroc were not clinically significant for single doses up to and including 900 mg.

Studies in treatment-experienced subjects with R5 HIV

Two identical phase 2B/3 registration studies of maraviroc have been completed (MOTIVATE 1 and 2). MOTIVATE 1 enrolled 585 subjects from North America whereas MOTIVATE 2 enrolled 464 subjects from Europe, Australia and North America. Subjects in both studies were infected with R5 HIV-1, and have experienced more than six months treatment with three of four approved antiretroviral drug classes, (PIs, NRTIs, NNRTIs, and FIs) AND/OR resistance to at least one

member of three of the four approved classes of antiretroviral medications. Subjects were randomized (2:2:1) to receive optimal background therapy (OBT) plus maraviroc 300 mg QD or BID (reduced to 150 mg QD/BID in presence of a PI). Both studies showed the reduction in HIV RNA from baseline to week 24 was statistically significantly greater in the OBT + maraviroc QD or BID groups compared to the OBT + placebo group. These studies also demonstrated that maraviroc (QD or BID) had equivalent acceptable safety and tolerability profiles, without indications of increased risk of hepatotoxicity, secondary infection, malignancy or QTc interval prolongation, relative to placebo at an interim analysis conducted at 24 weeks [38, 39].

Studies in treatment-experienced subjects with non-R5 HIV

In cohort studies the detection of X4 virus has been associated with more rapid CD4 decline and HIV-1 disease progression. A safety study in treatment-experienced subjects infected with non-CCR5 tropic HIV-1 (Study A4001029) was conducted to address the theoretical concern that giving maraviroc to a subject with dual or mixed tropic virus (R5/X4) may cause a more rapid disease progression or CD4 cell loss due to the selection of X4 virus at time of virologic failure. Study A4001029 is an ongoing, prospective, randomized (1:1:1), double-blind, placebo controlled, multi-country, phase 2b clinical trial of placebo vs. a nominal dose of maraviroc 300 mg QD or BID (reduced to 150 mg QD/BID in presence of a PI or delavirdine) added to OBT in HIV-1-infected treatment-experienced subjects with non-R5 virus. This study found no apparent differences between either of the maraviroc containing groups and the placebo group in the reported frequency of AEs, SAEs, deaths, drug interruption or discontinuations due to AEs in A4001029. There were no differences in Grade 3 or 4 liver function test abnormalities (AST, ALT, total bilirubin) across the three treatment groups. No cases of lymphoma or other HIV associated malignancies were reported in the first 24 weeks of the A4001029 trial. Overall, Study A4001029 demonstrated no harm and no benefit in efficacy, safety or tolerability when maraviroc in subjects infected with non-R5- tropic HIV-1 (compared to OBT + placebo).

Studies in treatment-naive subjects with non-R5 HIV

A registrational phase 3 study of maraviroc was performed in antiretroviral-naive subjects with R5 HIV infection (maraviroc versus efavirenz in Treatment-Naive Patients, or MERIT study; NCT00098293)[40]. Treatment-naïve adults lacking evidence of X4 infection were randomized zidovudine/lamivudine with maraviroc (300 mg once or twice daily) or efavirenz. The once-daily maraviroc arm was stopped early due to lack of efficacy. At week 48, maraviroc 300 mg bid (n = 721) was non-inferior to maraviroc in terms of proportion with an HIV RNA level below 400 copies/mL (70.6% for maraviroc vs 73.1% for efavirenz) but not for <50 copies/mL (65.3% vs 69.3%). Maraviroc was associated with high rates of virologic failure but lower rates of premature discontinuation for adverse events. In a post-hoc reanalysis that used a very sensitive co-receptor tropism assay to exclude those individuals with low-level X4 viruses, the efficacy of maraviroc was comparable to that of efavirenz.

Studies of maraviroc intensification

Given the potential immunodulatory activity of maraviroc, a series of studies have been performed in which maraviroc was added to a stable regimen ("intensification") to improve immune function in antiretroviral-treated adults with undetectable HIV RNA levels [41-49]. These observations provide the strongest evidence that maraviroc affects immune function independent of its effect on HIV replication. Several studies found that maraviroc intensification lead to reductions in CD8+ T cell activation [46, 48, 49], but a randomized placebo-controlled study of 45 immunologic non-responders (defined as a persistent CD4+ T cell count below < 350 cells per mm³) found that compared to placebo, maraviroc-treated subjects experienced a greater median increase in % CD38+HLA-DR+ peripheral blood CD8+ T cells at week 24 (+2.2% vs -0.7%, P = .014), and less of a decline in activated CD4+ T cells (P < .001). Plasma CC chemokine

receptor type 5 (CCR5) ligand (macrophage-inflammatory protein 1beta) levels increased 2.4-fold during maraviroc intensification ($P < .001$) and plasma lipopolysaccharide declined[44]. In most studies, circulating CD8+ T cell counts increased rapidly, providing strong evidence that the drug affects trafficking of these cells[47, 50, 51]. These intensification studies also provided preliminary data that maraviroc intensification reduced the size of the reservoir during effective cART[42, 43, 46]; this effect may be due to increased NF κ B activity, which might reverse latency, leading to HIV production and clearance of infected cells[52]. Although the immunologic and virologic effect of maraviroc intensification remains controversial, these studies collectively indicate that the approach is safe and well-tolerated.

Studies involving the use of HIV+ donors

The data in HIV positive donors in South Africa[53] is comparable to our data using HIV negative donors in HIV positive recipients[1]. Since we do not have the appropriate data in the US comparing HIV positive to HIV negative donors, we compiled the South African data. Despite some differences between the South African study (with HIV positive deceased donors, N=27) and HIVTR (with HIV negative donors, N=150) in eligibility criteria, characteristics and ARV/IS medications used, we compared the two studies in order to get an assessment of expected impact on the addition of HIV positive donors in our HIVTR-CCR5 study. The primary efficacy endpoint in HIVTR-CCR5 is the mean GFR at week 52 post-transplant, and in the sample size considerations section, we indicate that the expected mean GFR level at week 52 for the control group (Arm 2), based on the data observed in HIVTR and other post-transplant studies, is 60 ml/min per 1.73 m². Based on Dr. Muller et al.'s NEJM 2015 publication data, the mean GFR level at week 52 was 59 ml/min per 1.73 m², which demonstrates that use of HIV positive donors did not impact expected graft function at week 52 compared to the use of HIV negative donors. We have also compared some of the safety endpoints between the two studies. In HIVTR, the 1-year patient survival was 95% (CI 90,98) while in the South African study, the 1-year patient survival was 84% (CI, 62, 94). The 1-year rates of acute rejection in the HIVTR and South African studies were 29% and 8%, respectively.

Graft function and safety profiles with use of HIV positive deceased donors in the South African experience is very promising[53], and with the implementation of the HOPE Act, many centers are expected to utilize these valuable organs in their HIV+ kidney transplant candidates who would otherwise have long waiting times on the list.

2.5 Rationale for studying the impact of maraviroc on the reservoir

HIV persists indefinitely in essentially all antiretroviral-treated individuals. Several mechanisms contribute to persistence, including cell-to-cell transfer of virus ("replication") and the establishment of latent (or quiescent) infection of long-lived memory CD4+ T cells. The latter cell population is maintained in part by homeostatic proliferation.

The eradication of the HIV-1 reservoir resulting in the cure of the Berlin Patient in 2009 and the sustained reduction of the HIV-1 reservoir to undetectable levels in two infected individuals in Boston in 2012 by allogeneic hematopoietic stem cell transplant (HSCT) has renewed interest in novel HIV eradication strategies. [54-57] Although many factors contributed to these remarkable outcomes, it is noteworthy that all three individuals were heterozygous for the CCR5 Δ 32 mutation at baseline. This common genetic feature of the three individuals provides evidence that predisposition to HIV eradication may be linked to lower CCR5 cell surface expression. Given the central role of CCR5 in HIV-replication and the intriguing observations in the Berlin and Boston patients, we measured cellular HIV-1 *pol* and 2-LTR circle DNA, cell-associated HIV-1 RNA, and immunologic cell surface markers in 18 CCR5 delta-32 heterozygotes and 54 CCR5 wild type individuals on suppressive antiretroviral therapy. While no difference was observed in HIV-1 *pol* DNA levels, cell-associated HIV-1 RNA ($p=0.035$), RNA:DNA transcriptional ratios ($p=0.013$), and frequency of detectable 2-LTR circle DNA ($p=0.013$) were significantly lower in CD4+ T cells from CCR5 delta-32 heterozygotes. Cell-associated HIV-1 RNA was significantly correlated with CCR5 cell surface expression on CD4+ T cells ($r^2=0.136$, $p=0.002$). Despite

previously reported data describing effects of CCR5-mediated signaling on HIV-1 transcription via NF- κ B, NF- κ B pathway and target genes were expressed at similar levels between genotype groups. Taken together, these data suggest that limited CCR5 cell surface expression impacts the stability of the HIV-1 reservoir. The mechanism for this effect is unknown. We will explore in this study the possibility that therapeutic manipulation of CCR5 will have a similar effect.

Although a number of studies have investigated the impact of maraviric intensification on immune function, few have done detailed virologic studies. Most smaller studies that compared maraviroc-based regimens with non-maraviroc-based regimens have failed to detect an effect [58, 59], one provocative study based in Madrid found that maraviroc intensification resulted in decline in level of replication-competent HIV [42] and another in Barcelona suggested the drug had unique virologic effects[46]. More data on this question could be informative to emerging efforts to cure HIV infection [60, 61] and will allow us to interpret the mechanisms whereby maraviroc might influence clinical outcomes in a transplant setting.

3 Study Design

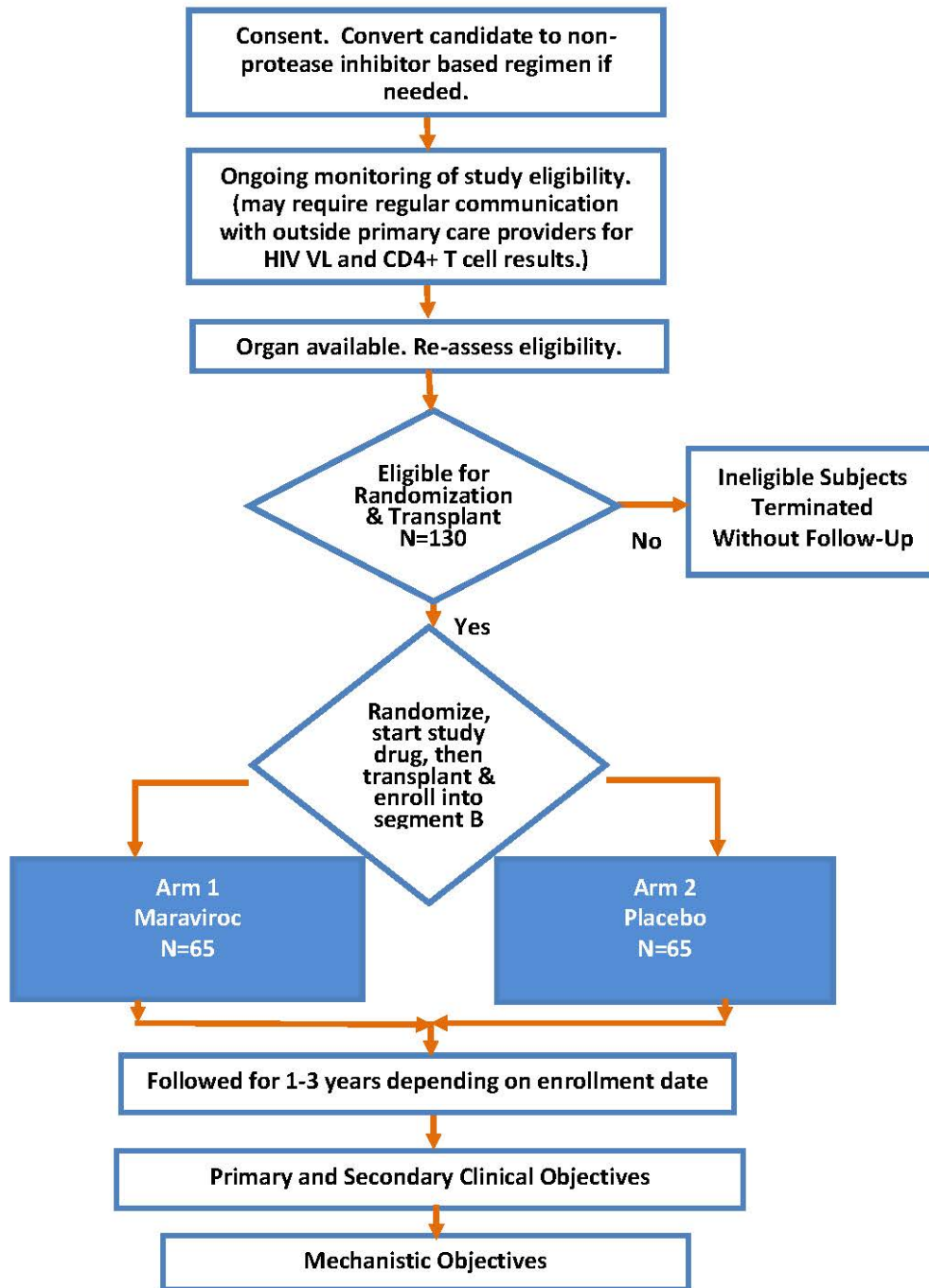


Figure 1: Study Design

3.1 Description of Study Design

This trial is a prospective, multi-center, double-blind phase II study of kidney transplantation in HIV+ individuals assessing the safety and efficacy of maraviroc or placebo given post-transplant. Participants must be stable HIV-infected adults in need of a kidney transplant who are on a non-protease inhibitor-based antiretroviral drug regimen. If not, participant must be willing and able to modify their antiretroviral regimen. This change will be initiated by the participant’s primary care provider at the request of the study team, and will occur prior to enrollment into the pre-transplant phase of the study (segment A). Randomization to maraviroc or placebo and enrollment into the post-

transplant phase of the study (segment B) will occur on the day of transplantation. Participants will be consented and enrolled until 130 eligible participants are randomized.

Major study endpoints will be determined for each participant 26 and 52 weeks after transplantation. Participants will be followed for a minimum of 1 year and a maximum of 3 (52 to 156 weeks).

3.1.1 Treatment Arms 1 and 2: HIV+

At the time of transplantation, eligible participants will be randomized 1:1 to add maraviroc or placebo. Initiation of study drug (maraviroc or placebo) will occur during the admission for transplantation, prior to transplant. If study drug is not initiated prior to transplant, it must be initiated no greater than 72 hours post randomization.

Arm 1: maraviroc

Arm 2: placebo

3.2 Primary Clinical Endpoint

1. Measured glomerular filtration rate at 52 weeks by iohexol clearance.
2. Graft loss, toxicities \geq Grade 3 and/or permanent treatment discontinuation within the first 52 weeks post-transplant.

3.3 Secondary Clinical Endpoints

1. Inflammation in the graft
 - a. CD45 gene expression (PTPRC) on the FFPE kidney biopsy sample at 26 weeks post-transplant.
 - b. CD45 quantitative IHC on the FFPE sample at 26 weeks post-transplant.
 - c. Gene expression profiling using the 11-gene tCRM module on FFPE biopsy shaves at 26 weeks and the 11-gene uCRM module on urine cell pellets at 26 weeks and 52 weeks post-transplant.
2. Measures of Renal Function and Injury (months 3, 6, 9, years 1, 2, 3)
 - a. Proportion of participants with eGFR by CKD-EPI, cystatin C, and CKD-cystatin C, < 60 mL/min/1.73 m².
 - b. Proportion of participants with defined CKD stage 4 or 5.
 - c. Mean calculated eGFR by CKD-EPI, cystatin C, and CKD-cystatin C.
 - d. The slope of eGFR by CKD-EPI, cystatin C, and CKD-cystatin C variable models over time based on serum creatinine.
3. HIV persistence
 - a. HIV reactivation (frequency of CD4+ T cells producing HIV multiply spliced RNAs upon TCR stimulation)
 - b. HIV DNA and RNA in peripheral blood CD4+ T cells
 - c. Plasma HIV RNA levels (single copy assay)
4. Histologic and Serologic Evidence of Rejection
 - a. Incidence of clinically suspected and biopsy proven acute rejection within the first 52 weeks and 156 weeks as defined by histologic evidence of rejection and graft dysfunction as identified on central read of biopsy slides.
 - b. The incidence of acute cellular rejection grade equal to or $>$ than IA, by the Banff 2007 criteria, within the study period (3 years) as identified on central read of biopsy slides.
 - c. The severity of first and highest grade of acute cellular rejection within the study period.
 - d. The incidence of antibody mediated rejection.
 - e. The prevalence of de novo anti-donor HLA antibodies at 52 weeks.

5. Analysis of HIV infection in the renal allograft and HIVAN
 - a. Histology/in situ hybridization to assess HIV infection in the renal allograft
6. Safety Profile measures
 - a. Death
 - b. Graft loss
 - c. Incidence of all adverse events (AEs) \geq Grade 3
 - d. Incidence of serious adverse events (SAEs) \geq Grade 3
 - e. Incidence of opportunistic infections or neoplasms
 - f. Incidence of non-opportunistic infections requiring hospitalization or systemic therapy
7. Pharmacokinetics
 - a. Calcineurin inhibitor trough levels and AUC for participants on maraviroc versus placebo
 - b. AUC and trough levels of CCR5 blockade (maraviroc)

3.4 Primary Mechanistic Endpoints

1. Define immunologic parameters associated with rejection in the HIV+ participants by comparing the following parameters in those who experience acute rejection in the first 6 months and those who do not have acute rejections (samples collected prior to and at the time of rejection will be analyzed and compared):
 - a. Circulating leukocyte subsets and their activation phenotypes
 - b. Anti-donor alloimmune responses measured by frequency of total donor-reactive T cells and donor-reactive effector T cells
 - c. Frequencies of T cells with cross-reactivity between donor alloantigens and HIV, CMV, and EBV antigens
 - d. DSA in serum
 - e. Gene expression in for-cause biopsies by RNASeq analysis
 - f. Histological analysis for evidence of cellular and antibody mediated rejection mechanisms.
2. Determine the impact of CCR5 blockade on the immunologic profiles in the HIV + recipients by comparing results from MVC-treated and those receiving standard of care (SOC). Change of the various parameters after initiation of MVC or SOC will be monitored and these changes will be compared between the two groups (Samples collected pre transplant and at various time points post-transplant will be analyzed):
 - a. Change in circulating leukocyte subsets and their activation phenotypes from pre-transplant baseline
 - b. Change in CCR5 and other chemokine receptor expression on subsets of various circulating leukocytes from pre-transplant baseline
 - c. Change on anti-donor alloimmune responses measured by frequency of total donor-reactive T cells and donor-reactive effector T cells
 - d. Change in frequencies of T cells with cross-reactivity between donor alloantigens and HIV, CMV, and EBV antigens after transplant.
 - e. DSA in serum
 - f. Histological analysis of for-cause biopsies for mechanisms of rejection

3.5 Stratification, Randomization, and Blinding/Masking

Participants will be randomized in a blinded fashion to one of 2 groups (maraviroc or placebo), stratified by center and by donor type (HIV negative deceased, HIV negative live, HIV positive deceased), using a web-based randomization system.

3.5.1 Procedure for Unblinding/Unmasking

Requests can be made to unblind subjects, on a case-by-case basis immediately for emergencies or urgent situations. Requests should be communicated to the Project Manager or the EMMES data center, who will then contact the Operations Committee (See Manual of Procedures for contact information). Requests will be reviewed and approved by the Operations Committee (including the Medical Officer), and the IND holder as appropriate, unless an immediate life threatening condition has developed and the Operations Committee are not accessible. In that case, the site investigator will notify the Project Manager or EMMES data center of the unblinding event on the next business day.

All unblinding will be reported to the Data and Safety Monitoring Board (DSMB).

Unblinding information should be shared with as few individuals as possible. The unblinding information is shared only on a need-to-know basis.

A full account of the event will be recorded, including the date and time of the unblinding, the reason for the decision to unblind, and the name of the individual who made the decision and the names of the Medical Officer and others who were notified. The reasons for unblinding of a participant's treatment will be included in the final study report.

4 Selection of Participants

4.1 Rationale for Study Population

Maraviroc is a CCR5 inhibitor that may have a novel role in modulating the immune response following transplantation. An ideal setting to test its impact on the alloimmune response is in HIV-infected patients undergoing kidney transplantation as maraviroc is known to be well-tolerated in this population. This is particularly relevant since unexpectedly high rejection rates (2-3 fold higher than HIV uninfected recipients) were reported following kidney transplantation in HIV positive recipients. If the unexpectedly high rejection rates seen in HIV-infected patients undergoing kidney transplantation can be reduced, the approach could lead to benefits for all kidney transplant recipients. In addition, CCR5 inhibition may play a role in reducing HIV persistence for participants on long-term antiretroviral therapy for HIV infection.

4.2 Inclusion Criteria

All individuals with end-stage kidney disease and HIV infection who meet standard clinical criteria for transplantation and the study inclusion and exclusion criteria will be eligible for participation in the study. All participants must meet all inclusion and exclusion criteria before being enrolled into segment A (pre-randomization) and at the time of enrollment into segment B (randomization and study drug initiation).

1. Participant is able to understand and provide informed consent
2. Documented HIV infection (by any licensed ELISA and confirmation by Western Blot, positive HIV ab IFA, or documented history of detectable HIV-1 RNA).
3. Participant is ≥ 18 years old.
4. CD4+ T-cell count $\geq 200/\mu\text{L}$ at any time in the 26 weeks prior to enrollment.
5. Most recent HIV-1 RNA < 50 copies RNA/mL. Eligibility at the time of enrollment will be determined based on the most recent HIV-1 RNA, not more than 26 weeks prior to enrollment. Subjects who require a switch in cART regimen to become study eligible must also have an eligible HIV-1 RNA result post change in cART.
6. Participant meets standard listing criteria for placement on transplant waiting list.
7. For participants with an HIV+ deceased donor:
 - a) No active opportunistic infections.
 - b) Concurrence by the study team that based on medical history and ART, viral suppression can be achieved in the recipient post-transplant.
 - c) Must be enrolled in an IRB approved research protocol that fulfills the requirements of the DHHA Hope Act Policy (see section 9.1.2).
 - d) HIV+ deceased donor must have no evidence of invasive opportunistic complications of HIV infection, and must have a pre-implant biopsy.
8. Antiretroviral (ARV) Use: Participant is on a stable cART regimen for at least 3 months prior to enrollment (unless changes are made due to toxicity, drug interactions, convenience or to an eligible non-protease inhibitor-based regimen). Switch should not be due to virologic failure. A regimen consisting of 2 NRTIs and an integrase inhibitor is preferred due to minimal drug interaction but any non-protease inhibitor regimen may be used.
 - If on a non-protease inhibitor based regimen, participant must be switched to a non-protease inhibitor-based regimen based on lack of any prior drug resistance or antiretroviral-treatment failure, and be willing to remain on indefinitely unless a change is medically necessary. . Participants who need to be switched must have been on a stable cART regimen for at least 3 months prior, and must have an eligible HIV-1 RNA

result post change in cART.

- If already on a stable non-protease inhibitor-based regimen, participant is willing to remain on this regimen indefinitely unless a change in regimen is medically indicated.
 - If untreated, must initiate and be willing to remain on indefinitely a non-protease inhibitor-based antiretroviral regimen unless a change is medically necessary.
9. No known allergy or intolerance to components of MVC or its formulation.
 10. No known contraindication to MVC.
 11. Female participants of child-bearing potential must have a negative serum beta-HCG pregnancy test within 30 days of randomization.

4.3 Exclusion Criteria

Individuals who meet any of these criteria are not eligible for enrollment as study participants:

1. Participant is currently on maraviroc.
2. Participant needs multi-organ transplant.
3. Participant has a live donor who is HIV+.
4. Participant is unable to switch to non-protease inhibitor-based cART regimen.
5. Participant has received immunosuppressant medication in the 6 months prior to enrollment. Note: Low dose maintenance steroids (\leq 10 mg per day of prednisone, or equivalent strength steroid) will not be considered immunosuppression.
6. Opportunistic Complication History: Any history of progressive multifocal leukoencephalopathy (PML), chronic intestinal cryptosporidiosis of $>$ 1 month duration, or primary CNS lymphoma. Note: History of pulmonary coccidioidomycosis will be treated per local site policy regarding this infection in HIV negative transplant candidates, generally requiring a 5-year disease-free interval.
7. Participant has a history of any neoplasm except for the following: resolved kaposi's sarcoma, *in situ* anogenital carcinoma, adequately treated basal or squamous cell carcinoma of the skin, solid tumors (except primary CNS lymphoma) treated with curative therapy and disease free for more than 5 years. History of renal cell carcinoma requires disease free state for 2 years. History of leukemia and disease-free duration will be per site policy.
8. Substance use that in the opinion of the investigator would interfere with compliance with the study requirements.
9. Participant is pregnant or breastfeeding. Note: Participants who become pregnant post-transplant will continue to be followed in the study and will be managed per local site practice. Women that become pregnant should not breastfeed.
10. Participant has used IL-2 or GM-CSF in the prior six months.
11. Participant has received interferon-alpha therapy in the prior 12 weeks.
12. Use of investigational drugs within 4 weeks of enrollment
13. Past or current medical problems or findings from medical history, physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.

5 Known and Potential Risks and Benefits to Participants

5.1 Risks of Investigational Product or Intervention as cited in Investigator Brochure or Package Insert

Hepatotoxicity

Hepatotoxicity accompanied by severe rash or systemic allergic reaction including potentially life-threatening events has been reported in clinical trials and post marketing. These events occurred approximately one month after starting treatment. Among reported cases of hepatitis, some were observed in the absence of allergic features or with no pre-existing hepatic disease.

Hepatic laboratory parameters including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin should be obtained prior to starting maraviroc and at other time points during treatment as clinically indicated. If rash or symptoms or signs of hepatitis or allergic reaction develop, hepatic laboratory parameters should be monitored and discontinuation of treatment should be considered.

Caution should be used when administering maraviroc to patients with pre-existing liver dysfunction or who are coinfecting with viral hepatitis B or C. The safety and efficacy of maraviroc have not been specifically studied in patients with significant underlying liver disorders.

Severe skin and hypersensitivity reactions

Severe, potentially life-threatening skin and hypersensitivity reactions have been reported in patients taking maraviroc, in most cases concomitantly with other drugs associated with these reactions. These include cases of Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and drug rash with eosinophilia and systemic symptoms (DRESS). The cases were characterized by features including rash, constitutional findings, and sometimes organ dysfunction, including hepatic failure. Discontinue maraviroc and other suspected agents immediately if signs or symptoms of severe skin or hypersensitivity reactions develop. Delay in stopping treatment with maraviroc or other suspect drugs after the onset of rash may result in a life-threatening reaction. Clinical status, including liver aminotransferases, should be monitored and appropriate therapy initiated.

Cardiovascular events

Use with caution in patients at increased risk of cardiovascular events, because cardiovascular events, including myocardial ischemia and/or infarction, were observed in treatment-experienced and treatment-naïve patients who received maraviroc. Caution should be used when administering maraviroc in patients with a history of postural hypotension or who receive concomitant medication known to lower blood pressure. Patients should be advised that if they experience dizziness while receiving maraviroc, they should avoid driving or operating machinery.

Postural hypotension in patients with renal impairment

Maraviroc should not be used in patients with severe renal impairment or end-stage renal disease (ESRD) (CrCl <30 mL/min) who are taking potent CYP3A inhibitors or inducers due to an increased risk of postural hypotension as a result of increased maraviroc exposure in some patients.

Maraviroc should be used in patients with severe renal impairment or ESRD only if they are not receiving a concomitant potent CYP3A inhibitor or inducer and no alternative treatment options are available. If patients with severe renal impairment or ESRD not receiving a concomitant potent CYP3A inhibitor or inducer experience any symptoms of postural hypotension while taking maraviroc 300 mg twice daily, the dose should be reduced to 150 mg twice daily.

Immune reconstitution syndrome

Immune reconstitution syndrome has been reported in patients treated with combination antiretroviral therapy, including maraviroc. Autoimmune disorders (such as Graves' disease, polymyositis, and Guillain-Barré syndrome) have also been reported to occur in the setting of immune reconstitution; however, the time to onset is more variable and can occur many months after initiation of treatment.

Potential risk of infection

Maraviroc antagonizes the CCR5 coreceptor located on some immune cells, and therefore, could potentially increase the risk of developing infections. Patients should be monitored closely for evidence of infection while receiving maraviroc.

Potential risk of malignancy

While no increase in malignancy has been observed with maraviroc, due to this drug's mechanism of action, it could affect immune surveillance and lead to an increased risk of malignancy. Long-term follow-up is needed to more fully assess this risk.

5.2 Risks of Study Procedures**5.2.1 Collection of Blood:**

Collection of blood may cause slight discomfort, pain, bleeding or bruising at the injection site. Rarely, fainting or infection may occur.

5.2.2 Kidney Biopsy:

The most frequent complications of a biopsy include pain and hematuria (blood in the urine).

5.2.3 Internet-Based Data Collection

Data from this study will be entered into a computerized database through a secured web site. All information will be saved and transmitted in a coded form. Only authorized personnel requiring a password will be permitted to enter data. There is risk, although minimal, of unauthorized persons obtaining confidential information.

5.3 Participant risks associated with kidney transplant from HIV+ donors**5.3.1 Acute Rejection (AR).**

HIV+ recipients have been shown to have 2-4 fold higher rates of AR, in both the NIH HIV Multisite Study [1] and in the national experience [62]. This may be due to drug-interactions between immunosuppression and antiretroviral therapy or due to a dysregulated immune system from HIV infection. Proposed interventions to address this include avoiding ritonavir-containing ART and induction immunosuppression. It is unknown whether AR rates will be increased in HIV-to-HIV transplantation.

5.3.2 Recurrent HIV-Associated Nephropathy (HIV-AN).

There has been concern about HIV-AN recurrence after transplant; this occurred in 38% of HIV+ recipients using HIV-donors in a French cohort [63] but was not observed in the NIH Multisite study [1]. In South Africa, histologic changes consistent with HIV-AN without graft dysfunction were observed in 3 of 27 patients [53]. This risk needs to be better understood.

5.3.3 Infections and Hospitalizations.

Infections requiring hospitalization have been observed in nearly 40% of HIV+ recipients using HIV- donors although opportunistic infections (OI) were very rare (<10%) [1]. Whether the use of HIVDD will be associated with an increased risk of standard OI is unknown.

5.3.4 HIV superinfection (HIV-SI).

HIV+ recipients of HIVDD may become superinfected, i.e. acquire a second, new HIV strain from the donor.

Superinfection occurs at varying rates in populations with differing modes of transmission, including intravenous drug use (IVDU) and sexual transmission [64-66]. Although HIV+ transplant candidates are on ART [67], and HIV-SI is thought to occur rarely on fully suppressive ART, there is concern of increased risk of HIV-SI with an HIVDD because (i) the viral inoculum that occurs with transplant is likely higher than with IVDU or sexual transmission, (ii) if an HIVDD is infected with an ART resistant or X4 tropic virus, this might not be suppressed by the HIV+ recipient's ART regimen [68, 69], and (iii) stably integrated HIV-infected resting CD4+ T-cells may be transplanted. Additionally, ART regimens in HIV+ recipients ideally will avoid ritonavir-boosted protease inhibitors (PIs) [70]. However, PIs are potent and often required to treat drug resistant virus [71].

5.4 Potential Benefits

Maraviroc is a CCR5 inhibitor that may have a positive role in modulating the immune response following transplantation, and an ideal setting to test its effect on the transplant alloimmune response is in HIV infected patients undergoing kidney transplantation and who are already on cART (combination antiretroviral therapy). Potential benefits include:

- Reduction in the incidence of alloimmune rejection
- Improvement in long term allograft function by blocking migration of pro-inflammatory cells to the allograft
- Reduction in the size of the HIV reservoir by CCR5 blockade combined with immunosuppression.

If CCR5 blockade decreases immunologic graft injury and improves long-term kidney allograft function in HIV infected recipients, this strategy could be applied to HIV uninfected recipients. Any impact found on HIV persistence could lead to further research of CCR5 blockade and immunosuppression on the eradication of HIV.

6 Study Drug /Device/Intervention

Participants will be randomized to receive maraviroc or placebo in addition to their non-protease inhibitor-based cART regimen. In general, the first dose of study drug will be administered upon admission for transplantation, prior to transplant. Study drug and placebo will be provided by ViiV. Since the study drug is being administered to assess reduction in immune-mediated graft injury and is not intended as a component of antiretroviral regimen, viral tropism testing to confirm CCR5 status is not required. However, CCR5 tropism analysis will be done at the end of the study as part of the mechanistic analysis.

6.1 Study Treatment

Study Treatment for Arm 1 participants is maraviroc plus a non-protease inhibitor-based combination antiretroviral therapy (cART). Study Treatment for Arm 2 participants is placebo for maraviroc plus non-protease inhibitor-based combination antiretroviral therapy (cART). The cART is required but it will not be provided through the study.

The study-provided drugs are maraviroc and placebo for maraviroc.

All participants will be followed for at least 1 year after enrollment of the last participant.

6.2 Regimens, Administration, and Duration

6.2.1 Initial Dosage Regimen

At time of transplant, participants will be randomized (1:1) to Arm 1 or Arm 2 to receive one of the two blinded study treatment regimens while remaining on a non-protease inhibitor-based cART regimen. The study drug will be initiated during admission for transplantation and prior to transplant. If study drug is not initiated prior to transplant, it must be initiated no greater than 72 hours post randomization. In the event a participant receives the study product upon admission for transplant but the transplant is cancelled, the DCC should be notified as soon as possible, and the participant will need to be re-randomized at the next admission for transplant.

Treatment Arm 1: Maraviroc

If co-prescribed medications that do not significantly interact with maraviroc, including tacrolimus, sirolimus, tipranavir/ritonavir, rilpivirine, nevirapine, raltegravir, dolutegravir, all NRTIs, and enfuvirtide	300mg twice daily orally
If co-prescribed potent CYP3A inhibitors* (with or without a potent CYP3A inducer) including: cyclosporine, delavirdine, ketoconazole, itraconazole, clarithromycin and other potent CYP3A inhibitors (e.g., nefazodone, telithromycin)	150mg twice daily orally
If co-prescribed potent CYP3A inducer (with or without a CYP3A inhibitor) including: efavirenz, rifampin, etravirine, carbamazepine, phenobarbital, and phenytoin	600mg twice daily orally

Treatment Arm 2: Placebo for Maraviroc

If co-prescribed medications that do not significantly interact with maraviroc, including tacrolimus, sirolimus, tipranavir/ritonavir, rilpivirine, nevirapine, raltegravir, dolutegravir, all NRTIs, and enfuvirtide	300mg twice daily orally
If co-prescribed potent CYP3A inhibitors* (with or without a potent CYP3A inducer) including: cyclosporine, delavirdine, ketoconazole, itraconazole, clarithromycin and other potent CYP3A inhibitors (e.g., nefazodone, telithromycin)	150mg twice daily orally
If co-prescribed potent CYP3A inducer (with or without a CYP3A inhibitor) including: efavirenz, rifampin, etravirine, carbamazepine, phenobarbital, and phenytoin	600mg twice daily orally

Decision to use maraviroc 300 mg bid

Although the general recommendations for maraviroc dosing when given with cytochrome P450 inhibitors such as CNIs, or with participants with moderate reduction in renal function is 150 mg bid

(<https://rsc.niaid.nih.gov/sites/default/files/Maraviroc%20%28Selzentry%29%20PI%20dated%20July%202018.pdf>), in the study of CCR5 blockade by Reshef et al, the dose needed to reach the 100 ng/mL average concentration level for the study was 300 mg bid (see figure 2 below).[18] For this reason, we have decided to use 300 mg bid.

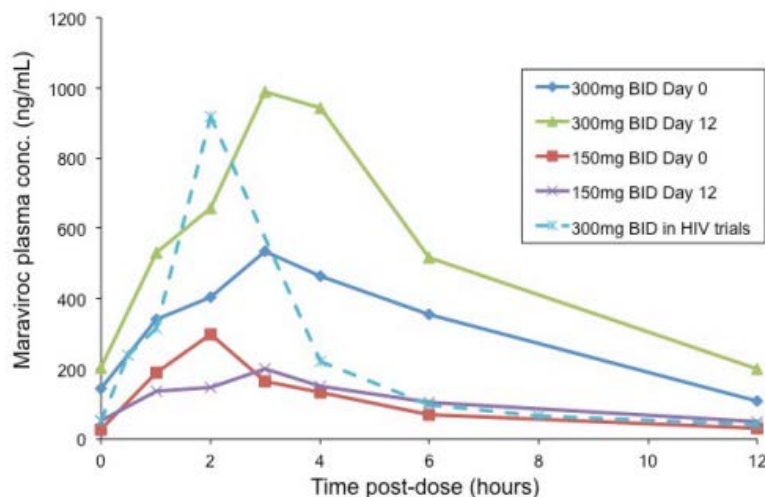


Figure 2: Maraviroc plasma concentrations as determined in 13 patients (300 mg twice daily [bid], n=6; 150 mg twice daily, n=7) on day 0 and day 12 at 0 (pre-dose), 1, 2, 3, 4, 6 and 12 hours post-dose for Blockade of Lymphocyte Chemotaxis in Visceral Graft-versus-Host Disease. Plasma levels were determined by high-performance liquid chromatography – tandem mass spectrometry.

In the study above, all of these participants were on voriconazole, which inhibits the enzyme that metabolizes maraviroc, thereby raising maraviroc levels. In our previous studies, when giving two drugs that potentially interact with each other, we either made sure that the participants always took their medications together, or we asked them to take the medications at least 4 hours apart, since that seems to decrease the degree of intestinal enzymatic inhibition. [72]

6.2.2 Modified Dosage Regimen

The study drug dose will be modified when participant's GFR < 30, if co-prescribed with a potent CYP3A inhibitor or inducer, or if the calcineurin inhibitor used for maintenance immunosuppression is changed to cyclosporine (which is only allowed for tacrolimus toxicity). Once GFR ≥ 30, the dose should be returned to the non-renal dosage. If GFR is consistently fluctuating (especially immediately post-transplant), the site investigator may choose when to resume normal dosing of study products based on clinical assessment of stability.

	Maraviroc Based on Renal Function	
	CrCl ≥30	CrCl <30 mL/min or on haemodialysis
If co-prescribed medications that do not significantly interact with maraviroc, including tacrolimus, sirolimus, tipranavir/ritonavir, rilpivirine, nevirapine, raltegravir, dolutegravir, all NRTIs, and enfuvirtide	300 mg twice daily	150 mg twice daily
If co-prescribed potent CYP3A inhibitors* (with or without a potent CYP3A inducer) including: protease inhibitors (except tipranavir/ritonavir), cyclosporine, delavirdine, ketoconazole, itraconazole, clarithromycin and other potent CYP3A inhibitors (e.g., nefazodone, telithromycin)	150 mg twice daily	150 mg once daily
If co-prescribed potent CYP3A inducer (with or without a CYP3A inhibitor) including: efavirenz, rifampin, etravirine, carbamazepine, phenobarbital, and phenytoin	600 mg twice daily	300 mg twice daily
	Placebo for Maraviroc Dose Based on Renal Function	
	CrCl ≥30	CrCl <30 mL/min or on haemodialysis
If co-prescribed medications that do not significantly interact with maraviroc, including tacrolimus, sirolimus, tipranavir/ritonavir, rilpivirine, nevirapine, raltegravir, dolutegravir, all NRTIs, and enfuvirtide	1 dose 300 mg twice daily	1 dose 150 mg twice daily
If co-prescribed potent CYP3A inhibitors* (with or without a potent CYP3A inducer) including: protease inhibitors (except tipranavir/ritonavir), cyclosporine, delavirdine, ketoconazole, itraconazole, clarithromycin and other potent CYP3A inhibitors (e.g., nefazodone, telithromycin)	1 dose 150 mg twice daily	1 dose 150 mg once daily
If co-prescribed potent CYP3A inducer (with or without a CYP3A inhibitor) including: efavirenz, rifampin, etravirine, carbamazepine, phenobarbital, and phenytoin	1 dose 600 mg twice daily	1 dose 300 mg twice daily

*For up to date lists of CYP3A4 inhibitors and inducers, we recommend checking <https://drug-interactions.medicines.uoehp.edu/Main-Table.aspx>.

All participants in both Treatment Arms must receive cART based on the current local and national dosing guidelines. cART will not be provided by this study. Study clinicians, in conjunction with participants, should determine the optimal cART regimen for each participant. Participants in both Treatment Arms will be on study drug therapy for one to three years.

6.2.3 Administration

Maraviroc 300 mg dose will be administered as one 300 mg tablet (OR two maraviroc 150 mg tablets) orally twice daily with or without food.

Placebo for Maraviroc 300 mg dose will be administered as one placebo for maraviroc 300 mg tablet (OR two placebo for maraviroc 150 mg tablets) orally twice daily with or without food.

Maraviroc 150 mg dose will be administered one 150 mg tablet orally twice daily with or without food.

Placebo for Maraviroc 150 mg dose will be administered one placebo for maraviroc 150 mg tablet orally twice daily with or without food.

6.2.4 Duration

Participants will be on the study drug for 1-3 years depending on enrollment date.

6.3 Study Drug Formulation and Preparation

Maraviroc 150 mg, Maraviroc 300 mg, Placebo for maraviroc 150 mg, and Placebo for maraviroc 300 mg will be supplied as tablets for oral administration.

Store at 15° C – 30° C (59-86°F)

6.4 Pharmacy: Product Supply, Distribution, and Accountability

6.4.1 Study Drug Acquisition/Distribution

Maraviroc 150mg, maraviroc 300 mg, Placebo for maraviroc 150 mg, and Placebo for maraviroc 300 mg will be supplied by ViiV Healthcare Ltd.

The study drugs will be available through the National Institute of Allergy and Infectious Diseases (NIAID) Clinical Research Products Management Center (CRPMC). The site pharmacist can obtain the study drug for this protocol by following the instructions in the manual *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks* in the Section Study Product Management Responsibilities.

cART will not be provided through the study.

6.4.2 Study Drug Accountability

The site pharmacist is required to maintain complete records of all study drugs received from the NIAID CRPMC and subsequently dispensed. All unused study drugs must be returned to the NIAID CRPMC after the study is completed, terminated or otherwise instructed by the study sponsor. The procedures to be followed are provided in the manual *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks*.

6.5 Premature Discontinuation of Study Drug

Study therapy may be prematurely discontinued for any participant for any of the following reasons:

1. Participant meets criteria to discontinue study treatment (MVC or placebo).
2. The investigator no longer believes continuing study treatment is in the best interest of the participant.
3. Participant refuses to continue study treatment.
4. Treatment is held or discontinued for clinical reasons for greater than 60 days.

Participants who prematurely discontinue study treatment will be treated according to the site specific standard of care, and will be followed under a modified schedule post-transplant.

7 Clinical Management Issues

7.1 Toxicity Management

Only toxicities related to study medications provided through this study (MVC, placebo) will be considered in the toxicity management section. The grading system is located in the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Corrected Version 2.1, July 2017, located at the DAIDS RSC Web Site: <https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables>

For toxicities potentially attributed to the study medication, the study medication (or the matching placebo) may be held.

7.1.1 Grade 1 or 2

Subjects who develop a Grade 1 or 2 AE or toxicity may continue study medication without alteration of the dosage at the discretion of the investigators. Subjects experiencing Grade 1 or 2 AEs who choose to discontinue all study medication should complete the premature treatment discontinuation evaluations. The site investigator should contact the Operations Committee, and the subject should be encouraged to complete follow-up protocol study evaluations.

NOTE: If subjects discontinue study medications due to Grade 1 or 2 AEs, this should be noted on the premature treatment discontinuation CRF as the reason for discontinuation.

7.1.2 Grade 3

If the investigator has evidence that the AE was NOT caused by study medication, dosing may continue. Grade 3 AE or toxicity must be evaluated and managed by the site investigator according to the standard of care. Discontinuation of study medication may be necessary. Consultation with the Operations Committee is encouraged. If study medication(s) are discontinued the subject should be reevaluated weekly until the AE stabilizes, returns to \leq Grade 2 or baseline. The study medications may be reintroduced, at the discretion of the site investigator or according to standard practice.

If the same Grade 3 AE, excluding those AEs noted in the following sections, recurs within 4 weeks of restarting treatment, the study medication must be permanently discontinued. However, if the same Grade 3 AE recurs after 4 weeks, the management scheme outlined above may be repeated.

Participants experiencing Grade 3 AEs requiring permanent discontinuation of the study medication should be followed weekly until resolution or stabilization of the AE and should be encouraged to complete the premature treatment discontinuation evaluations and continue other study evaluations according to the protocol. The Operations Committee must be notified.

7.1.3 Grade 4

Participants who develop a Grade 4 symptomatic AE or toxicity will have all study medication permanently discontinued except as indicated below. Participants experiencing Grade 4 AEs requiring permanent discontinuation of study medication should be followed weekly until resolution or stabilization of the AE and encouraged to continue other study evaluations according to the protocol. Premature treatment discontinuation evaluations should be completed and recorded on the CRF and the core team must be notified.

Asymptomatic Grade 4 abnormalities must be evaluated and managed by the site investigator according to the standard of care. Discontinuation of study medication may be necessary. Consultation with the Operations Committee is encouraged. The Operations Committee must be notified by e-mail regarding toxicities that result in a change in regimen. Participants who prematurely discontinue study treatment should continue to be followed on study.

7.1.4 AST or ALT Elevations

All study medication may be continued for asymptomatic \leq Grade 3 AST/ALT elevations, at the discretion of the site investigator. Careful assessments should be done to rule out the use of alcohol, non-study medication-related toxicity, or viral hepatitis (including viral hepatitis complicated by immune reconstitution inflammatory syndrome) as the cause of Grade 3 elevations.

For symptomatic Grade 3 (fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, or eosinophilia) or any Grade 4 elevations in AST or ALT, all study medication should be held and the Operations Committee should be consulted. In addition, for ALT ≥ 3 x ULN and bilirubin ≥ 1.5 x ULN (attempts should be made to fractionate the bilirubin), the study medications should be held and the Operations Committee consulted as this will likely result in permanent study medication discontinuation.

Participants who develop symptomatic Grade 3 or any Grade 4 AST or ALT elevation should be followed weekly until stabilization or resolution to \leq Grade 2. Subjects will be followed off study treatment, on study after study medication discontinuation.

Subjects with Grade 3 or 4 AST or ALT elevation with fever, rash or eosinophilia should stop all study medications and not be re-challenged. These individuals should be followed weekly until stabilization or resolution to \leq Grade 2 elevation and should be followed off study treatment, on study.

In consultation with the Operations Committee, careful assessments should be done to rule out the use of alcohol, non-study medication-related toxicity, or viral hepatitis (including viral hepatitis complicated by immune reconstitution inflammatory syndrome) as the cause of Grade 3 or 4 AST/ALT elevations. Evaluations to be considered (but are not required) include:

- Viral hepatitis serology including: Hepatitis A IgM antibody; Hepatitis B Surface Antigen (HBsAg) and Hepatitis B Core Antibody (IgM); Hepatitis C RNA; Hepatitis E IgM antibody;
- Cytomegalovirus IgM antibody;
- Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing);
- Syphilis screening;
- Drugs of abuse screen including alcohol;
- Serum acetaminophen test (APAP adduct test);
- Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH);
- Anti-nuclear antibody, anti-smooth muscle antibody, and Type 1 anti-liver kidney microsomal antibodies;
- Liver imaging to evaluate liver disease.

7.1.5 Calculated Creatinine Clearance (CrCl) Decline

Non-study medications should be adjusted for decline in CrCl at the discretion of the primary provider per the standard of care.

MVC dose will be modified for CrCl < 30 mL/min. See section 6.2.2 Modified Dosage Regimen.

7.1.6 Postural Hypotension

If a subject develops postural hypotension of any grade, the investigator should contact the Operations Committee. All cases of postural hypotension should also be reported as an adverse event in the EMMES IDES, regardless of grade.

7.1.7 Allergic Reaction

Subjects may continue study medication for Grade 1 or 2 allergic reactions at the discretion of the site investigator. The subject should be advised to contact the site investigator immediately if there is any worsening of symptoms or if further systemic signs or symptoms develop. Antihistamines, topical corticosteroids, or antipruritic agents may be prescribed.

Subjects with \geq Grade 3 allergic reactions that are considered to be possibly or probably related to the study medications should permanently discontinue the putative study medication and continue to be followed off study treatment, on study. The subject should not be re-challenged. The subject should be treated as clinically appropriate and followed until resolution of the AE.

Allergic reactions that include but are not limited to severe rash or any rash with fever, general malaise, fatigue, muscle or joint aches, oral lesions, conjunctivitis, facial edema or eosinophilia should result in all study medications being stopped and the subject should not be re-challenged. The subject should be followed off study treatment, on study.

All cases of allergic reaction should also be reported as an adverse event in the EMMES IDES, regardless of grade.

7.1.8 Rash

Hepatotoxicity accompanied by severe rash or systemic allergic reaction, including potentially life-threatening events, have been reported in subjects taking MVC. Severe skin and hypersensitivity reactions including SJS and TEN have also been reported in subjects taking MVC.

Subjects with an isolated Grade 1 rash may continue study medication at the site investigator's discretion. The subject should be advised to contact the site investigator immediately if there is any worsening of the rash, if any systemic signs or symptoms worsen, or if mucosal involvement develops.

Subjects may continue study medications for an isolated Grade 2 rash. However, study medications should be permanently discontinued for any \geq Grade 2 rash that is associated with an increase in ALT. The subject should be advised to contact the site investigator immediately if rash fails to resolve (after more than two weeks), if there is any worsening of the rash, if any systemic signs or allergic symptoms develop, or if mucosal involvement develops.

Subjects should permanently discontinue study medications for an isolated Grade 3 or 4 rash, and the subject will be followed off study treatment, on study. Subjects should be treated as clinically appropriate and followed until resolution of the AE.

If the etiology of the rash can be definitely diagnosed as being unrelated to study medications and due to a specific medical event or a concomitant non-study medication, routine management should be performed and documentation of the diagnosis provided.

All cases of rash should also be reported as an adverse event in the EMMES IDES, regardless of grade.

7.1.9 HIV Persistent Virologic Failure

HIV persistent virologic failure defined as a HIV viral load > 1000 copies/mL for more than 90 days that is not the result of an investigator/physician approved interruption in antiretroviral treatment should also be recorded as an adverse event in the EMMES IDES, regardless of grades.

7.2 Pregnancy

If a woman has completed the study or chooses to discontinue from the study before the end of the pregnancy, then site staff should request permission to contact her regarding pregnancy outcomes at the end of pregnancy. If the information is obtained, pregnancy outcomes will be submitted on a CRF at the end of the pregnancy.

The Intrapartum complications and/or pregnancy outcome will be recorded on the CRFs. Pregnancies that occur on study should be reported prospectively to The Antiretroviral Pregnancy Registry. More information is available at www.apregistry.com. Phone: 800-258-4263; Fax: 800-800-1052.

8 Other Medications

8.1 Combination Antiretroviral Therapy (cART)

Unless a change is deemed necessary by the HIV physician, all subjects must be on a stable non-protease inhibitor-based maintenance cART regimen. An integrase inhibitor based regimen consisting of 2 NRTIs and an integrase inhibitor is preferred due to minimal drug interaction but any non-protease inhibitor regimen may be used. See the Manual of Procedures for more detail.

Tenofovir and other nephrotoxic medications should be used cautiously when possible post-transplant.

8.2 Immunosuppression

Maintenance immunosuppressive regimen will be standard of care and may include tacrolimus, mycophenolate mofetil (MMF), and prednisone. Tacrolimus will be the first line calcineurin inhibitor. In the event of toxicity, cyclosporine A and/or sirolimus can be substituted or added. Refer to Manual of Procedures for details on the Risk Evaluation Management Strategy (REMS) for any participant using mycophenolate mofetil as part of maintenance immunosuppression. Induction with an IL-2 receptor inhibitor (anti-CD25 antibody) may be utilized and is preferred, but lympho depletion with thymoglobulin may be used as induction if deemed clinically indicated by the site investigators. Immunosuppressant doses will be modified to obtain routine trough levels standard for kidney transplants. See the Manual of Procedures for more detail

8.2.1 Rejection Treatment Protocol

A biopsy will be performed in all cases of suspected rejection (see the Manual of Procedures for the definition of kidney rejection). Treatment for rejection for > 1 day, including increases in the dose of immunosuppressive medications, cannot be sustained without a biopsy, unless the managing physicians believe biopsy is unsafe. However, therapy may be initiated \leq one day prior to the results of the biopsy if clinically indicated. Treatment of rejection episodes will be according to local site practices and may include sirolimus. Polyclonal anti-lymphocyte preparations have resulted in prolonged reduction in CD4+ counts in HIV infected transplant recipients, and their use should be restricted to treatment for moderate to severe rejection. TOR inhibitor agents may be added to maintenance regimens per site discretion.

8.2.2 Definition of Rejection

The definition for kidney rejection is as defined by the NIH supported Cooperative Clinical Trials in Transplantation:

Type I: mononuclear infiltrate in $>$ or $=$ 5% of cortex, a total of at least three tubules with tubulitis in 10 consecutive high-power fields from the most severely affected areas, and at least two of the three following features: edema, activated lymphocytes, or tubular injury.

Type II: arterial, or arteriolar, endothelialitis with or without the preceding features.

Type III: arterial fibrinoid necrosis or transmural inflammation with or without thrombosis, parenchymal necrosis, or hemorrhage.

8.3 Prophylactic Medications

See Manual of Procedures for recommended prophylaxis guidelines.

9 Study Procedures

9.1 Screening/Baseline Visit

Centers will identify potential study participants which are HIV+ kidney transplant candidates who are listed for transplant. The research study will be explained in lay terms to each potential research participant, and the potential participant will sign an informed consent form before undergoing any study procedures.

During the screening period for study eligibility, the study personnel will review the participant's medical record for previous and current medical history, perform a physical examination, and record the participant's demographic information. Screening tests indicated on the schedule of events with footnote 2 will be recorded if done as standard of care for transplant workup, or if the results are available in the medical record.

The following procedures, assessments, and laboratory measures will be conducted or recorded for the baseline screening visit (after consent has been obtained):

- Symptom & medical review plus physical exam
- Record PPD (or chest x-ray if history of positive PPD)
- Record Vaccination review (Pneumovax, Hepatitis A, Hepatitis B)
- Record Chest x-ray result if available
- Record Safety labs
- Record CD4+ T-cell count
- Record HIV-1 RNA
- Record serologies (CMV Ab, EBV Ab, hepatitis B and C)

The following procedures will be conducted as part of the research protocol:

- Research specimens for mechanistic studies: the collection of all baseline/screening samples are not necessarily drawn at initial screening for the study when all other screening laboratory measures and procedures are performed. All baseline/screening research specimens (blood, urine) should be collected on the day of transplant (day 0, visit 2) if they were not previously collected in the three months prior to transplant, and no more than 13 weeks prior to transplant. If the baseline research samples were not collected previously, or were collected more than 13 weeks prior to day of transplant, they should be collected on the day of transplant, prior to transplant.

9.1.1 Living donors

Living donors must sign an informed consent document prior to any invasive procedures. The living donor's decision to participate or not to participate in this study will not be disclosed to the recipient.

9.1.2 HIV+ donors

Participants who utilize an HIV+ donor must be co-enrolled in an IRB approved research protocol that fulfills the requirements of the DHHS Hope Act Policy which can be found at <https://www.federalregister.gov/documents/2015/11/25/2015-30172/final-human-immunodeficiency-virus-hiv-organ-policy-equity-hope-act-safeguards-and-research-criteria>.

The HIVTR-CCR5 protocol will not be responsible for sample and data collection required by the policy. Sample and data collection and reporting to the DHHS will be the responsibility of each site's IRB approved Hope Act protocol.

9.2 Participant Enrollment

Once a participant has met all study inclusion and exclusion criteria, the participant will be assigned a unique participant number and will be enrolled into the Pre-Transplant (segment A) phase of the study using the EMMES IDES while waiting for organ availability. Enrollment into the Post-Transplant (segment B) phase of the study will occur on the day of transplant.

9.3 Pre-Transplant On-Going Eligibility Monitoring for Segment B

Once eligible participants have been enrolled into the Pre-Transplant (segment A) phase of the EMMES IDES, it is recommended that CD4+ T-cell count and HIV RNA viral load results be regularly obtained from the primary medical provider every 3 months if not being performed at the enrolling center as part of standard of care. This will help ensure documented eligibility if an organ becomes available.

If the participant is no longer eligible for transplant or for segment B of the study, or wishes to withdraw from the study, the reason(s) will be recorded on the appropriate case report form. Participants in segment A may fluctuate between eligibility and ineligibility for segment B, but must meet study eligibility at the time of organ availability.

Participants who withdraw their consent prior to transplant will be terminated from this study with no additional follow-up necessary.

9.4 Randomization and Post-Transplant Study Visits

Segment B study visits will occur at day 0, weeks 1, 2, 4, 8, 13 (month 3), 26 (month 6), 39 (month 9), 52 (year 1), 78 (year 1.5), 104 (year 2), 130 (year 2.5), and week 156 (year 3). At each study visit, a clinical evaluation and physical examination will focus on signs and symptoms suggestive of HIV disease progression, impaired allograft function, and rejection. Clinical evaluation will concentrate on symptoms and examination findings of the oropharynx, respiratory, cardiac, gastrointestinal, skin, lymphatic and nervous system. Monitoring of orthostatic blood pressure should occur during all post-transplant visits to monitor possible hypotension. CD4+ T cell numbers and percent, and quantitative HIV-1 RNA by ultrasensitive bDNA or PCR assays will be determined. All necessary lab work outlined in the Schedule of Events should be performed.

At each study visit, the study coordinator will collect and enter data into the web based data system, including medications, adverse events (AE's)/serious adverse events (SAE's), hospitalizations, infections, and standard of care labs.

Although it is preferred that all post-transplant study visits occur at the transplant center, only the visits on weeks 4, 13, 26, 52, and 104 (when specimen will be collected for mechanistic assays) must occur at the transplant center. If the participant is unable to return to the transplant center for any post-transplant study visit, a complete medical review should be conducted by telephone. In addition, all necessary lab work as outlined in the Schedule of Events should be performed locally at a CLIA-certified laboratory and the results sent to the transplant center for data entry along with the associated normal laboratory reference ranges.

In addition, the following research procedures will be performed:

- **Protocol Biopsy:** A protocol biopsy will be performed at 6 months post-transplant unless the transplant center already performs a 6 month post-transplant biopsy as standard of care. Slides and tissue should be sent to the central repository for central ready and analysis. See the laboratory manual for details.

- **Iohexol clearance:** Samples for iohexol study at week 52 (year 1) will be collected (2, 3 and 4 hours), processed, and shipped to the central repository where they will be stored and then analyzed in batch.
- **Collection of research bloods:** Research blood will be obtained pre-transplant and at weeks 4 (month 1), 13 (month 3), 26 (month 6), 52 (year 1), and 104 (year 2). Research blood will also be collected at the time of kidney rejection. These research specimens will be used primarily for the mechanistic aims of the study. However, some of the samples will be batch tested to address the secondary clinical aims of the trial, including:
 - cystatin C (months 3, 6, 9, years 1, 2, 3)
 - HLA antibodies (pre transplant, month 6, and at time of rejection)
 - KIR typing (pre transplant)
- **Collection of urine:** 50ml – 100ml urine will be obtained at baseline, week 26 (month 6), week 52 (year 1). Urine will also be collected at the time of biopsy for suspected kidney rejection. Urine will be processed and banked at the central repository for future studies to look for HIV DNA and RNA levels if HIVAN is present and funding becomes available.
- **Pharmacokinetic Studies:** Pharmacokinetic studies will be done on the first twenty UCSF participants (or all UCSF participants if accrual is < 20) enrolled in trial at 3 months post- transplant. PK studies will also be repeated on all UCSF participants if the calcineurin inhibitor used for maintenance immunosuppression is changed to cyclosporine (which is allowed only for tacrolimus toxicity). PK studies would be done in the UCSF Parnassus clinical research center. After an overnight fast, participants would be admitted in the morning for a 12 hour PK study. Samples will be collected at 0, 0.5, 1, 2, 3, 4, 6, 8 and 12 hours. Whole blood samples will be frozen for tacrolimus assay and plasma samples for maraviroc assay.

9.5 Visit Windows

Study visits should occur within 2 weeks prior to the defined visit time point, or may occur after the defined visit time point up to the mid-way point between the target date and the subsequent study visit. For example, the week 26 visit may occur up to week 31 [eg $26 + (36 - 26)/2 = 31$].

10 Mechanistic Assays

Mechanistic studies will be performed on blood samples collected pre-transplant, and months 1, 3, 6, 12, 24, and at time of suspected kidney rejection, and on kidney biopsies collected at 6 months and time of suspected rejection. The aims of the mechanistic studies are: to define the impact of maraviroc on immune function and HIV persistence, to characterize the degree of immune dysregulation in HIV+ transplant recipients, and to define immunological parameters associated with rejection. Comparisons will be made between those receiving maraviroc and placebo, and between transplant rejectors and non-rejectors.

The goals of the mechanistic investigations associated with this trial are twofold:

1. To determine the impact of immunosuppression and antiretroviral therapy with CCR5 blockade on HIV persistence in HIV+ recipients.
2. To investigate cellular basis for enhanced graft rejection in HIV (+) recipients and to determine the impact of maraviroc on alloimmune responses in HIV+ recipients.

Assays designed to achieve these goals are described below in this section.

10.1 Samples

All recipients and donor samples will be banked and analyzed in batches.

10.1.1 Recipient Samples:

Peripheral blood mononuclear cells (PBMC) will be processed from heparinized peripheral blood and EDTA samples collected at pre-transplant, 1, 3, 6, 12, and 24 months after transplant and at the time of rejection. The samples will be stored in aliquots as live cells in cryopreservation medium in the vapor phase of liquid nitrogen.

Whole blood samples collected at pre-transplant, month 6, and at the time of rejection for gene expression analysis will be collected directly into Paxgene tubes.

Serum samples will be collected pre-transplant, month 6, and at the time of rejection using serum separation tubes and stored in -80°C freezers in aliquots.

Plasma samples will be collected at pre-transplant, 1, 3, 6, 12, and 24 months after transplant.

Urine samples will be collected at baseline, week 26 (month 6), week 52 (year 1). Urine will also be collected at the time of biopsy for suspected kidney rejection. Samples will be processed into supernatant and pellet and banked at central repository.

Lymph node (LN) samples will be collected during transplant and transferred in sterile organ preservation solution to processing laboratory. LN cells will be released using mechanical disruption. The cells will be cryopreserved in aliquots using the same protocol used for PBMC.

Graft biopsy samples will be collected at 6 months post-transplant, and at the time of all suspected rejection episodes. Two cores will be collected. See the Laboratory Manual for details.

10.1.2 Donor samples:

Splenocytes from cadaveric donors or blood from living donors will also be collected at the time of the transplantation, processed into splenocytes or PBMC, and banked as live cells in liquid nitrogen tanks.

10.2 HIV persistence analyses

Highly sensitive and quantitative assay to measure replication competent HIV in T cell subsets in blood and tissues:

Given the extremely low frequency of reservoir cells in virally suppressed participants, precisely measuring the magnitude of the reservoir is challenging. HIV-1 proviral DNA and cell-associated RNA can be accurately quantified to provide a measurement of the total HIV burden. However, as the majority of the viral genomes detected in resting CD4⁺ T cells are defective, PCR based assays for HIV DNA overestimate the size of the replication competent reservoir (i.e., those variants which can support HIV replication in absence of therapy) [73]. Conversely, standardized viral outgrowth assays (VOA), which measure the cell-associated replication competent HIV pool, provide only minimal estimates of the frequency of resting CD4⁺ T cells harboring replication competent HIV [74]. Also, the VOA approach requires large numbers of freshly collected cells and is both expensive and time consuming [75]. These assays are not amenable to large studies such as the one proposed by this group.

Drs. Nicholas Chomont (consultant in this trial) and his colleagues have developed a novel assay that measures the frequency of cells harboring inducible HIV without the limitations of the VOAs. By combining ultrasensitive detection of multiply-spliced RNA (msRNA) upon stimulation together with a limiting dilution assay, the “Tat/Rev Induced Limiting Dilution Assay (TILDA) quantifies the frequency of cells harboring transcriptionally silent – but nonetheless inducible – viruses. This assay can be performed with less than a million CD4⁺ T cells, as described below.

Cell associated HIV RNA measurements by TILDA: The frequency of cells with HIV multiply spliced (ms) RNAs will be measured upon activation in CD4⁺ T cells by using an assay developed in the laboratory of Dr. Chomont (Fig. 3). While unspliced HIV RNAs are frequently detected in latently infected cells in the absence of viral production, HIV msRNAs (tat/rev) reflect active viral production [76, 77]. These assays will take advantage of this unique feature of msRNAs in a limiting dilution assay to measure the frequency of cells with inducible HIV upon maximal stimulation in CD4⁺ T cells from the blood. Briefly, isolated CD4⁺ T cells will be stimulated for 12 hours with PMA/ionomycin. Serial dilution of the stimulated cells will be distributed in a 96 well plate (4 dilutions, 12 replicates each), lysed, and immediately used for RT-PCR. msRNAs will be quantified by real time PCR [76, 78] and using the maximum likelihood method, the frequency of cells harboring inducible HIV msRNAs in CD4 T cells will be calculated. These experiments will determine if maraviroc leads to a reduction in the size of the latent (but inducible) HIV reservoir.

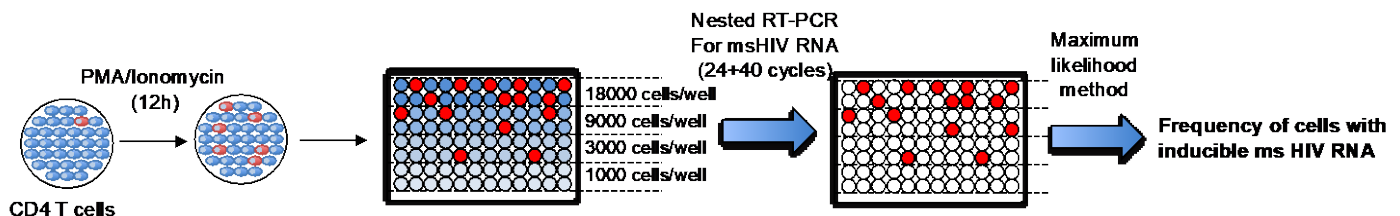


Fig. 3: HIV RNA induction assay. Sorted CD4⁺ T cells are stimulated for 12 hours with PMA/ionomycin. Serial dilutions of the stimulated cells are distributed in a 96-well plate and digested. Multi-spliced HIV RNA are detected in a 2 step real time PCR. The maximum likelihood method is used to calculate the frequency of cells with inducible HIV RNA based on the number of positive wells.

In preliminary studies, the frequency of cells with inducible HIV RNA in CD4⁺ T cells from 9 HIV infected participants who received ART for at least 3 years was measured and compared to frequencies with integrated HIV DNA. The preliminary data indicate that values obtained with the two assays were strongly correlated ($p = 0.002$, **Fig. 4A**). However, the frequency of cells harboring integrated HIV DNA significantly exceeded the frequency of cells producing HIV RNA upon stimulation (median fold = 4.5, **Fig. 4B**). This indicated that the majority of cells harboring integrated HIV DNA do not produce viral RNA upon stimulation. These results confirm that the majority of HIV genomes are not replication competent [73] and reinforce the need for using a functional assay such as our viral induction assay to measure HIV latency.

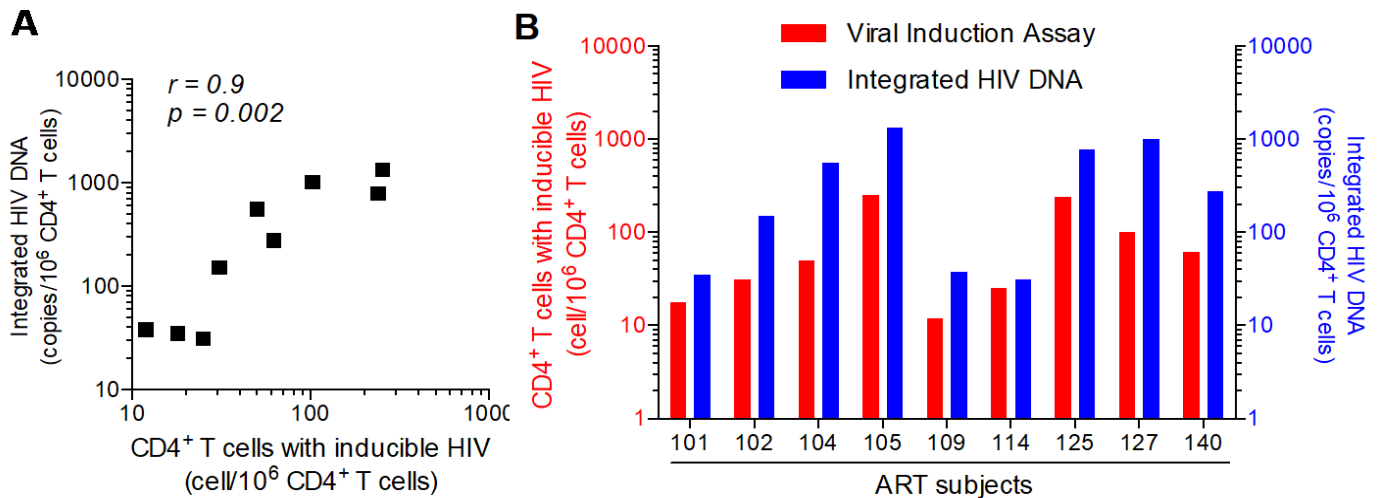


Fig. 4 (A-B): A: the frequency of CD4⁺ T cells with inducible HIV RNA is positively correlated with Integrated HIV DNA. B: However, integrated DNA always exceeds this frequency.

Cell Associated HIV DNA and RNA measurements: The frequency of cells harboring total and integrated HIV DNA will be measured by using our well-established assays that can detect a single copy of the viral genome in 10⁵ cells [79]. While integrated HIV DNA is present in both latently and productively infected cells, the ratio between total and integrated has been recently shown to reflect residual HIV expression and de-novo reverse transcription [80]. Total and integrated HIV DNA will be measured in sorted CD4 T cells to determine the impact of maraviroc on this outcome measure. The frequency of cells expressing HIV RNA will be measured from isolated enriched CD4 T cells or pelleted cells from urine for single copy sensitivity qPCR assays using the AllPrep DNA/RNA kit (Qiagen, Ventura CA) as specified by the manufacturer. Cellular RNA will be normalized to cell equivalents based on qPCR using GAPDH mRNA expression (Life Technologies, Grand Island NY). Total cellular RNA is quantified with a qPCR TaqMan assay using LTR-specific primers F522-43 (5' GCC TCA ATA AAG CTT GCC TTG A 3'; HXB2 522-543) and R626-43 (5' GGG CGC CAC TGC TAG AGA 3'; 626-643) coupled with a FAM-BQ probe (5' CCA GAG TCA CAC AAC AGA CGG GCA CA 3) on a StepOne Plus Real-time PCR System (Applied Biosystems Inc, Foster City CA) using external quantitation standards prepared from full length NL4-3 virion RNA normalization to NIH Virology Quality Assurance HIV-1 RNA standards. [81]

Quantifying virion HIV-1 in blood plasma: Virion HIV-1 RNA will be quantified from blood plasma using the Abbott RealTime HIV-1 test modified for ultrasensitive detection. [82] Briefly, up to 30 mL plasma is layered over 0.6% OptiPrep Density Gradient medium cushions (Sigma Aldrich, St. Louis MO) and centrifuged for 3 hours at 4° C at 54,000 or 48,000 x g using 10 or 30 mL ultracentrifugation tubes, respectively. Pelleted virions are resuspended in 1.0 mL Abbott lysis buffer, followed by RNA extraction and viral load qPCR using the m2000/rt2000 system Abbott RealTime HIV-1 assay. Low copy detection frequencies for large volume measurements are described [83]. For medium volume (6.0 mL)

plasma input volumes, a probit analysis including repeat measurements of VQA virion RNA standards diluted to target copy numbers at 0, 1, 2, 5, 10, and 40/mL demonstrated 100% specificity (0/20 detected at 0 cps/mL). Detection frequencies for standards with estimated 1-40 copies/mL are as follows: 6/20 (30%) for 1 copy/mL; 11/20 (55%) for 2 copies/mL; 19/20 (95%) for 5 copies/mL; 18/20 (90%) for 10 copies/mL; and 20/20 (100%) for 40 copies/mL.

Viral genotype-based tropism: HIV-1 coreceptor usage preference will be determined using ultra-deep sequencing of the V3 region of the HIV-1 envelope, followed by implementation of established bioinformatic prediction tools. Ultra deep sequencing is critical when characterizing HIV-1 drug resistance or tropism in clinical samples, to enable sensitive detection of circulating minority variants. Dr. Pillai (co-investigator on this study) has extensive experience in HIV-1 sequencing methodology and sequence-based prediction of viral tropism. In this study, CD4+ T cells will be negatively selected from PBMCs using the EasySep Human CD4+ T cell Enrichment Kit (StemCell Technologies), to enrich the cellular population for HIV-1 DNA. Genomic DNA will be extracted from CD4+ T cells using the Qiagen AllPrep kit. The C2-V3 region of the HIV-1 envelope will be amplified using nested PCR as previously described [84]. Illumina Nextera DNA sample kits will be used to prepare sequencing libraries and incorporate multiplex identifier barcodes that will allow pooling of 96 samples in a single deep sequencing run. Sequencing will be performed on the Illumina MiSeq instrument in the Pillai Lab (2 x 250 read length). Multiple sequence alignments of the C2-V3 region will be generated using hidden Markov models, and amino acid sequences of the V3 loop region will be used to predict the coreceptor usage of each V3 loop variant using established bioinformatics prediction tools including Wetcat [85] and Geno2pheno [86]. For each clinical sample, the frequencies of predicted CXCR4-tropic and CCR5-tropic clones will be reported.

CCR5 genotyping: The presence of the CCR5 delta-32 mutation will be determined in both transplant recipients and donors by implementing a PCR assay in the Pillai Lab as previously described [87]. Genomic DNA will be extracted from PBMCs using the Qiagen DNA Blood Mini Kit, according to the manufacturer's instructions. PCR amplification of a region of the CCR5 locus will be performed with the following primers: forward primer, 5'-GCTCTCTCCCAGGAATCATCTTTAC-3'; reverse primer 5'-TTGGTCCAACCTGTTAGAGCTACTG-3'. The thermocycler program is as follows: 95°C, 10 min, followed by 40 cycles of 95°C for 30 s, 62°C for 1 min, and 72°C for 1 min. The PCR yields a product of 356 bp for the wildtype CCR5 allele and a product of 324 bp for the CCR5 delta-32 deletion mutant. Gel visualization of PCR products is performed to differentiate between wild type homozygosity, CCR5 delta-32 heterozygosity, and CCR5 delta-32 homozygosity.

In Situ Hybridization (ISH). Detection of low copy number HIV infection in tissues by immunohistochemistry (IHC) or immunofluorescence (IF) microscopy is hampered by low sensitivity of these methodologies. To assess low copy number HIV infection in formalin-fixed paraffin-embedded kidney biopsies, the Laszik lab has adapted the highly sensitive RNAScope® ISH platform using probes targeting multiple HIV1 clade B, A, and D strains on the coding sequence, including gag, pol, env, nef, tat, rev, vif and vpr. This novel ISH assay is based on a protocol of signal amplification and background suppression and Dr. Laszik (co-investigator on this study) has significant experience detecting low copy number cytokines in kidney biopsies using this technology. The HIV ISH signal will be quantitated from representative fields of the cortex and medulla in the glomerular, tubular, interstitial, and vascular compartments using computer-assisted digital image analysis. The HIV ISH sections will be co-stained with multiplex IF using a panel of podocyte, endothelial, tubular, interstitial and inflammatory cell markers for precise quantitation of the HIV signal within various cellular compartments of the kidney. Preliminary results of HIV ISH show strong and specific hybridization signal in the podocytes from a patient with HIV nephropathy. The HIV signal is unequivocally localized to the podocytes by the HIV positive cells co-expressing synaptopodin on an ISH section co-stained with IF.

10.3 Alloimmune analyses

10.3.1 Overview

The goal of the alloimmune mechanistic assays is to define immunological parameters associated with rejection, and to determine the impact of maraviroc on immunological profiles.

Our hypothesis for enhanced rejection in HIV+ recipients is that HIV+ patients have higher frequencies of allo-reactive memory T cells generated as a result of cross-reactivity between alloantigens and chronic viral antigens. Experiments in animal models of transplantation demonstrate that multiple viral infections can induce the generation of memory allo-reactive T cells as a result of cross-reactivity between alloantigen- and pathogen-reactive T cells [24-30]. Cross-reactivity between human alloantigen and viral antigen-reactive T cell lines and clones has also been demonstrated [31]. These viro-allo cross reactive memory T cells have a lower threshold for activation, can directly traffic to grafts, and are capable of expressing effector functions upon first encounter with donor antigens, all of which can contribute to enhanced graft rejections and resistance to tolerance induction. Decades of chronic HIV and co-pathogen infection has the potential to expand viral reactive T cells and skew the immune repertoire, leading to increase in VACR memory T cells. Furthermore, HIV infection can exacerbate this condition by depleting T cells. Although T cell counts recover in most patients once HIV viremia is controlled by antiviral therapy, T cell receptor repertoire in patients with long-term HIV infection is dramatically narrower than age-matched HIV- patients. Depending on the donor allotype, a particular HIV+ recipient may have a narrow TCR repertoire depleted of donor-reactive T cells, whereas another recipient may have a narrow repertoire greatly enriched of donor reactivity due to the expansion of cross reactive memory T cells. The study will directly test this hypothesis by comparing frequencies of memory T cells and cross reactive T cells in samples collected pre-transplant in rejectors and non-rejectors.

Memory T cells express high levels of CCR5, which facilitate CD4 and CD8 T cell cooperation and activation. In rodent models of transplantation, CCR5 blockade has been shown to be beneficial for graft survival in some settings [11-15], but may exacerbate rejection in others [88-90]. The difference seems to reside in the mechanism of graft rejection – CCR5 blockade was effective at blocking Th1-mediated rejection, but enhanced Th2 and antibody-mediated rejection. Another possible mechanism proposed for exacerbation of rejection by CCR5 blockade is the inhibition of Treg infiltration of the grafts [88, 91]. It is important to note that these rodent experiments used CCR5 blockade as single therapy [88-90] and the outcome is likely to be very different if it is combined with additional immunosuppressive agents as proposed in this trial. Effect of maraviroc in kidney transplantation in patients is unknown at present time. Patients with CCR5d32 have less graft rejection suggesting that CCR5 plays a role in alloimmune response in humans and CCR5 blockage may be effective at improving transplant outcome [16, 17]. A recently concluded study in GVHD suggests that maraviroc is effective at restricting leukocyte trafficking to target tissues [18]. By comparing alloimmune profile in participants enrolled in the standard of care and maraviroc arms of this trial, the study will be able to determine the impact of maraviroc on alloimmune responses in humans with direct clinical relevance.

10.3.2 Assays

10.3.2a Cellular assays

10.3.2a1 Multiparameter flow cytometric analysis (MFC): HIV+ patient not only progressively lose CD4+ T cells; most of the immune cell subsets in patients with chronic HIV infection are dysregulated. These changes can be detected using MFC. These studies will use 4 MFC panels listed in Table 1 to assess Ccr5 expression among leukocyte subsets, frequencies and numbers of various subsets of regulatory cells, memory T cells, and B cell subsets in the peripheral blood and LN. The data will be analyzed in conjunction with CBC+differential collected in clinical labs at the same time point to calculate the total number of each cells subset. In addition to enumeration of immune cell subsets, we have

designed the MFC panels to simultaneously analyze chemokine receptor expression on various immune cells to determine the impact of maraviroc on their numbers and level of chemokine receptor expression (Table 1)

Table 1. MFC panels

Panels	Markers	Rationale
Leuk-Ccr5	CD3, 14, 16, 19, 45, 56, HLA-DR, Ccr5	Enumerate T cells, B cells, subset of monocytes, dendritic cells, and subsets of NK cells and determine their Ccr5 expression
T cell-Ccr5	CD3, 4, 8, 25, 127, 73, FOXP3, HELIOS, Ccr5	Enumerate subsets of T cells and their expression of Ccr5
Tmem	CD3, 4, 8, 28, 45RA, 57, Ccr7, PD1	Frequencies of naïve, memory, effector, exhausted T cells
ChemR	CD3, 4, 8, 25, 127, Ccr4, Ccr6, Cxcr3,	Enumerate T cells that express various chemokine receptors found on Th1 (Cxcr3+), Th2 (Ccr4+), and Th17 (Ccr6+) cells
B cell	CD10, 19, 20, 21, 27, 95	Frequencies of immature, transitional, naïve, activated, memory, and plasmablast B cells.

The investigators from the UCSF core Immunology lab (under the direction of Qizhi Tang, PhD) have used the Tmem panel to determine the frequencies of naïve (CD45RA+CCR7+), memory (CD45RA-CCR7+), and effector (CD45RA-CCR7-) T cells [92] in pre-transplant samples collected from the participants in the HIVTR trial (Fig 5A). Our analysis demonstrates that end-stage renal disease is associated with increased effector CD4 T cells in both of HIV+ and HIV- age-matched patients and HIV+ rejectors and non-rejectors had similar frequencies of effector T cells (Fig. 5B).

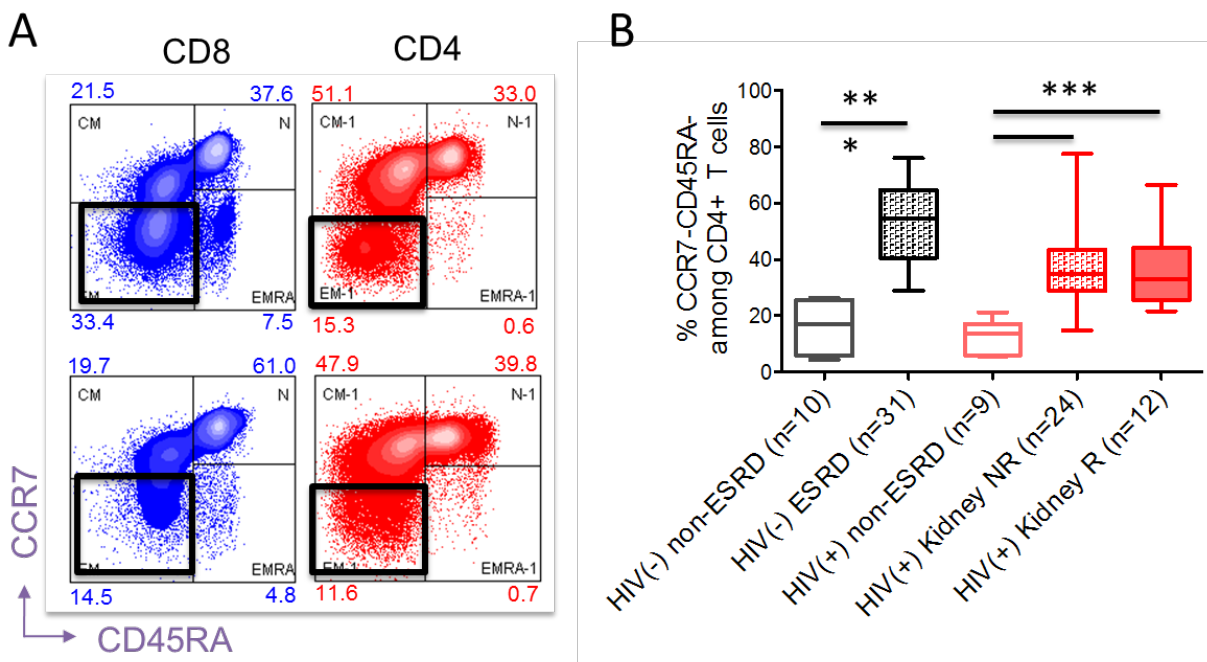


Fig 5 (A-B). Flow cytometric analysis of memory and effector T cells. (A) Frozen PBMCs are thawed and stained with the Tmem panel of fluorochrome-conjugated antibodies. The cells are then analyzed on a BD Fortessa flow cytometer. CCR7 and CD45RA expression patterns of CD3+CD8+ (blue) and CD3+CD4+ (red) from a representative HIV(+) and an age-matched HIV(-) sample are shown on top. (B) Summary data of results for CD4+ T cells using samples HIV- controls without end stage renal disease (non-ESRD), HIV- controls with ESRD, and HIV+ non-ESRD, and pretransplant samples from HIVTR rejectors and non-rejectors.

Our Leuk-Ccr5 panel demonstrates that Ccr5 is primarily expressed on subset of T cells, monocytes, and dendritic cells (Fig 6A). Flow cytometric analyses can have significant experiment-to-experiment variations, therefore samples to be directly compared, such as those collected from the same patient before and after maraviroc treatment should be analyzed at the same time in one experiment. The core immunology investigator Qizhi Tang plans to do this by cryopreserving all PBMC samples and thawing and analyzing an entire time series from pre-transplant to 1 year after transplant in batch. We have determined that Ccr5 expression pattern is not affected by cryopreservation (Fig 6B), making batch analysis of time series possible.

These MFC assays will be performed on PBMC samples collected at all time points and LN samples collected pre-transplant.

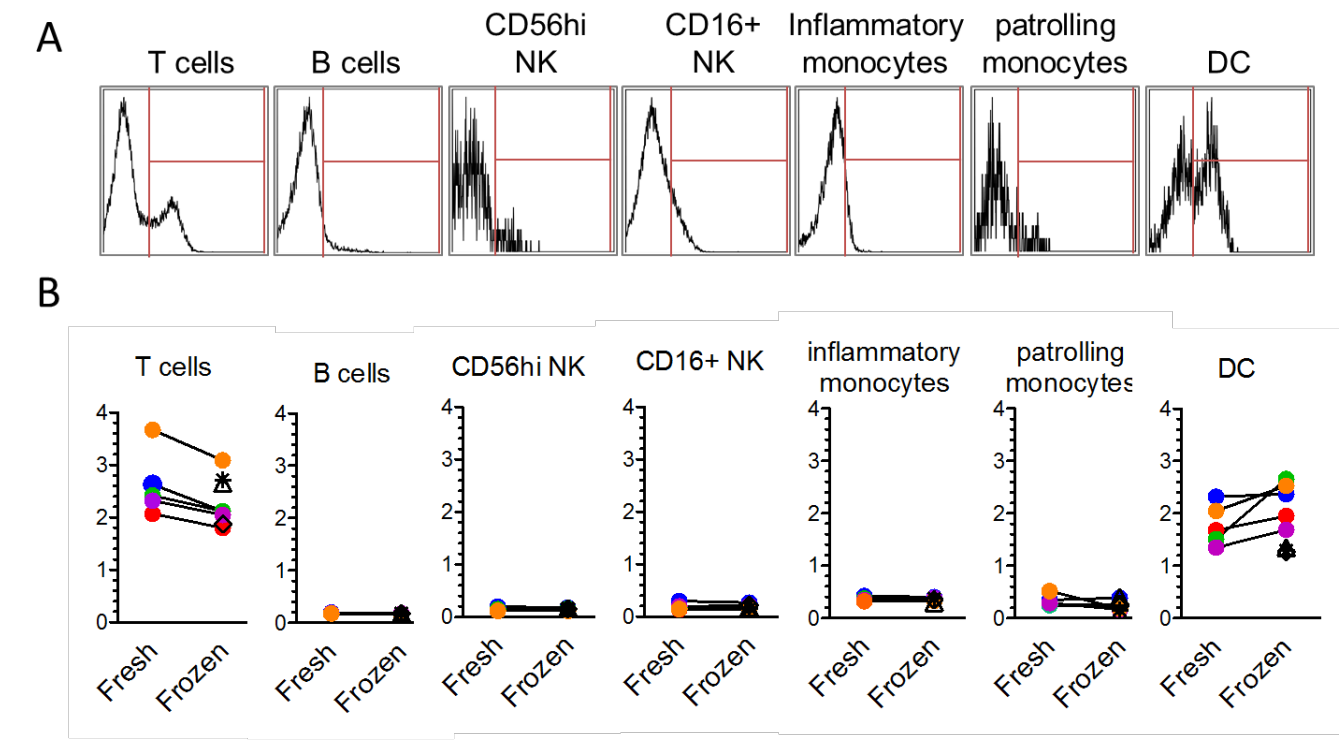


Fig 6 (A-B). Flow cytometric analysis of Ccr5 expression on various leukocyte subsets. (A) Example of Leuk-Ccr5 panel result obtained using PBMC collected from a normal donor. The result shows expression of Ccr5 on a subset of T cells and dendritic cells and largely absence of expression on B cells, monocytes and NK cells. (B) Cryopreservation does not affect the quantification of Ccr5 expression on leukocyte subsets.

10.3.2a2 Alloreactive T cell frequency assay (ATF): Transplants induce vigorous immune responses because of the exceptionally high frequencies of alloantigen-reactive T cells. Transplant immunology research in the past decade revealed that allo-reactive Tregs capable of suppressing rejection are also present at high frequency and the overall immune response toward a transplanted organ depends on the balance between the rejection-prone conventional T (Tconv) cells and the tolerance-prone Tregs. The ATF assay measures the frequency of donor-reactive Tconv and Tregs. This assay uses highly potent CD40L-stimulated B cells (sBc) from donors to induce proliferation of donor-reactive T cells from recipients. Because of the high expression of HLA and costimulative CD80 and CD86 molecules, the sBcs can activate Tconv and Tregs. An established CFSE dilution method is used to record the division history of the donor-reactive T cells, which can be analyzed using flow cytometry (along with markers to identify CD4, CD8 and Tregs) and used to calculate the frequency of the reactive T cells (Fig 7A).

The UCSF Immunology lab has used this assay to determine pre-transplant donor-reactive T cell frequencies in HIV+ kidney transplant recipients. Our preliminary analysis of 8 rejectors and 8 non-rejectors shows that the rejectors have significantly higher donor-reactive CD8 T cells before transplant and donor-reactive CD4 Tconv and Tregs were the same between the two groups (Fig 7B).

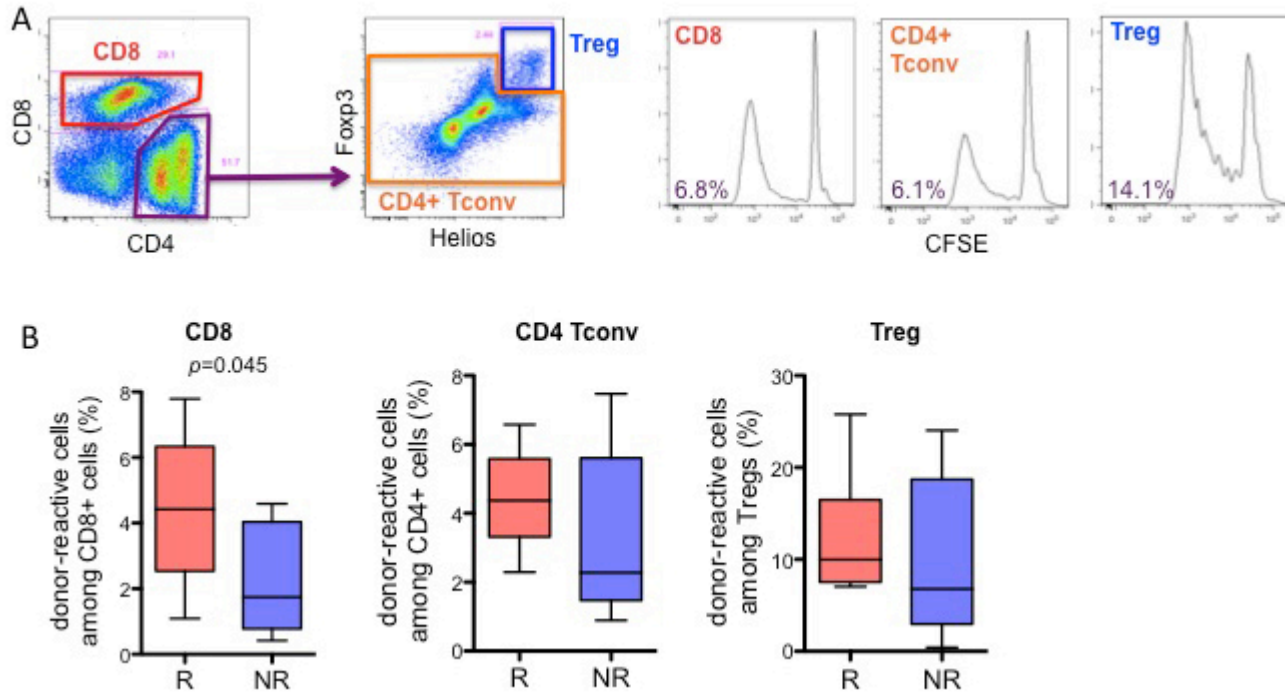


Fig 7 (A-B) Alloreactive T cell frequency assay (ATF). (A) Recipient PBMCs are labeled with CFSE and stimulated with donor sBc for 64 hours. The culture is harvested and stained for CD3, CD4, CD8, Fopx3 and Helios and analyzed on a flow cytometer. The CFSE profiles of CD8, CD4+ Tconv, and Tregs are used to calculate the frequencies of donor-reactive T cells in each of the subset (numbers in purple). (B) Comparison of Donor-reactive T cell frequencies using PBMC sample collected pretransplant from HIV+ kidney transplant recipients who had acute cellular rejection (R, n=8) or not (NR, n=8).

This assay will be used to measure the frequencies of donor-reactive Tregs, Tconv CD4+ T cells and CD8⁺ T cells in recipient PBMC samples collected at all time points and LN samples collected pre-transplant.

10.3.2a3 Alloreactive memory T cell assay (AMT): Donor reactive memory T cells pose a major threat to transplanted grafts because they have a lower activation threshold and are resistant to immunosuppression. We hypothesize that HIV+ transplant recipients have higher memory alloreactive T cells due to heterologous immunity generated through their anti-viral T cells. A hallmark of memory T cells is their ability to produce effector cytokines shortly after antigen encounter. The alloreactive effector T cell assay will measure frequencies of alloantigen stimulated cytokine production after a short stimulation with either a panel of allogeneic sBcs (panel-reactive effector T cell assay [93]) or donor sBcs (donor-reactive effector T cell assay). Cytokine secretion will be blocked during stimulation to permit the detection of intracellular IFN γ and IL-4 using flow cytometry. The cells will also be stained for CD3, CD4, CD8, CD45RO, CD19/CD14/CD16 (dump), and viability. As a positive control, each sample will also be stimulated with PMA and ionomycin to determine the cytokine production potential of the cells. Negative control will be cells incubated alone without stimulants (Fig 8).

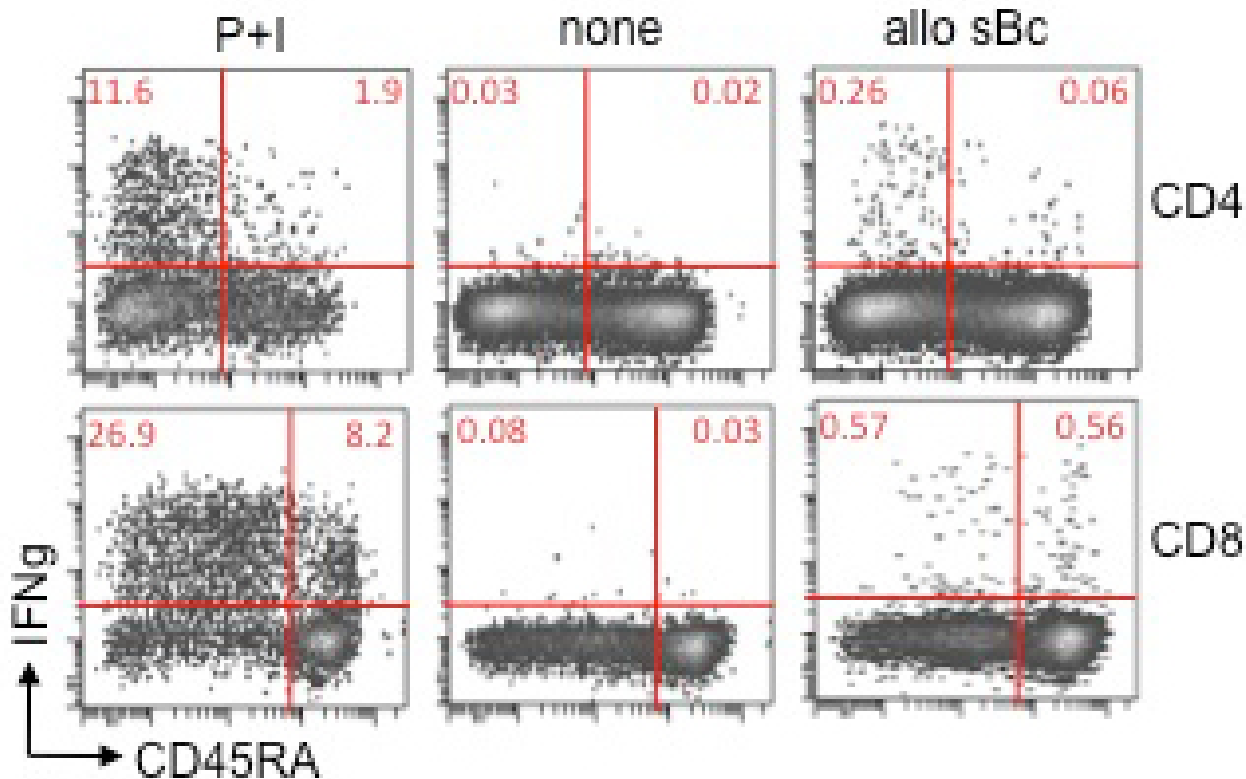


Fig 8. Alloreactive memory T cell assay. Frozen PBMCs are thawed and incubated with allogeneic sBcs (right), PMA and ionomycin (left, positive control), or no stimulants (middle, negative control) in the presence of brefeldin A. The cells are then stained for CD3, CD4, CD8, CD45RA, CCR7, and IFNg before flow cytometric analysis. Result from a healthy blood bank donor is shown. Red numbers indicate percentages of the cells in the quadrant.

This assay will be used to measure the frequencies of panel- and donor-reactive IFNg-producing effector cells in recipient PBMC samples collected at all time points and LN samples collected pre-transplant.

10.3.2a4 Cross reactive T cell assay (CRT): We have developed an approach to identify T cells with allo and viral dual reactivities (Fig 9). Our approach takes advantage of the potent allostimulatory activity of the sBc. We found that after one round of allogeneic sBc stimulation of PBMC and 10-day expansion in IL-2, virtually 100% of the T cells in culture are reactive to the sBcs (data not shown). We then restimulated the alloreactive T cell lines with various pools of viral peptides. Cells reactive to viral antigens were then identified using cell surface activation marker CD98 and dilution of a proliferation dye CTV. Fig 9 shows an example of identification of T cells in an HIV+ patient that are cross-reactive between donor alloantigens and an HIV-Pol peptide pool. Similarly we have also identified allo-reactive T cells that can recognize HIV-Gag, HIV-Nef, CMV, EBV, and Flu peptide antigens.

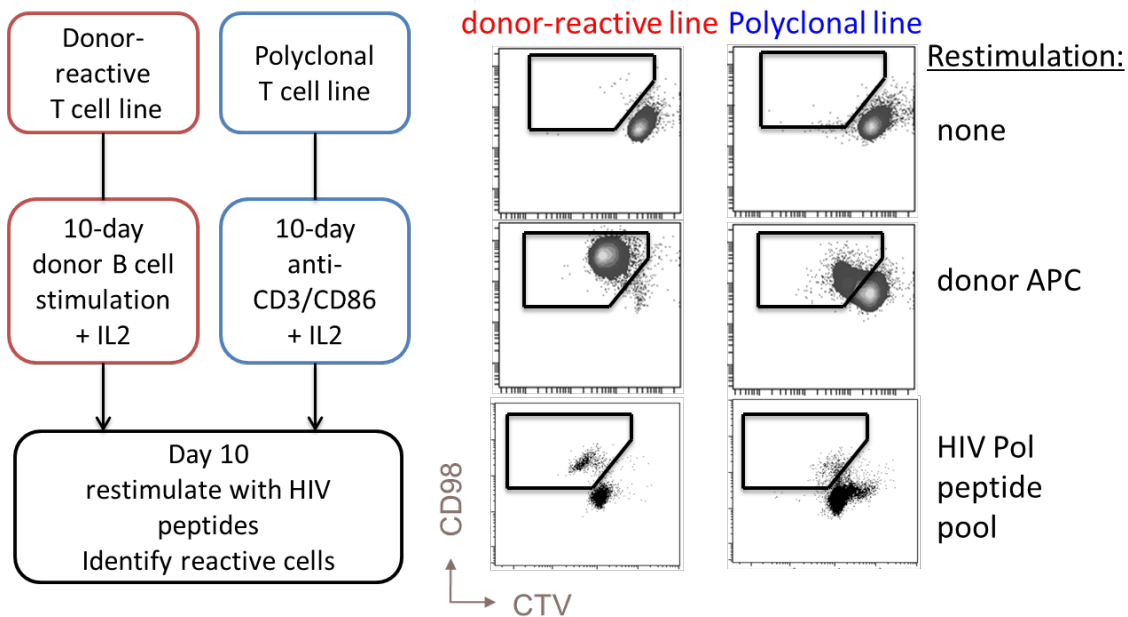


Fig 9. T cell cross-reactivity assay. (A) experiment protocol. Frozen PBMCs are thawed and expanded with anti-CD3 and CD86-expressing feeder cells (polyclonal line) or allogeneic sBCs (donor-reactive line) for 10 days. The cells are then labeled with cell tracker violate (CTV) and restimulated with a pooled overlapping library of peptides from HIV Pol protein. Expression of CD98 and dilution of CTV on day 3 post restimulation were determined using flow cytometry. (B) A representative experiment using sample from an HIVTR rejector. Black box highlights cells that are activated by the restimulation. The presence of HIV pol peptide activated cells in the donor-reactive line indicates cross-reactivity.

We will use this assay to measure the frequencies of cross reactive T cells in recipient PBMC and LN samples collected before transplant and at various time points after transplant.

10.3.2b Serum HLA antibodies (HLA)

10.3.2b1 HLA antibodies: Prior alloantigen exposure can lead to the formation of alloreactive memory T cells and B cells and presence of anti-HLA antibodies in the serum that can contribute to graft injury. In addition, lymphopenia in HIV-infected patients has been shown to drive homeostatic proliferation of B cells and increase the frequencies of transitional B cells that produce cross-reactive antibodies. We will determine if B cell dysregulation in HIV+ patients also leads to increased alloantibodies before and after transplant. We will measure antibodies to class I and class II HLA using Luminex single antigen coated beads (LABScreen; One Lambda, Inc., Canoga Park, CA). Donor specificity of HLA alloantibodies will be determined by comparing the specificities with the HLA typing of the donor.

In addition of anti-HLA antibodies, B cell dysregulation can also lead to increase of antibodies to other specificities. Particularly, non-HLA antibodies have been shown to mediate chronic graft injuries [94]. We will bank extra serum aliquots for future exploratory analysis of non-HLA antibodies.

10.3.2c Histological analysis of biopsy samples using multiplex immunofluorescence (mIF) and in situ hybridization (ISH)

Standard histopathology will be used to determine tissue integrity and the degree of inflammation.

Quantitative mIF stains will be performed on formalin fixed paraffin-embedded tissue to detect collagen 3, leukocyte common antigen (LCA), and various inflammatory cell subpopulations including CD68+ macrophages, the CD4, CD8, CD20, NK, CXCL9/CXCL10/CCL5, PD1, PD1L1, PD1L2, FoxP3, Th17, and T-bet+ subsets of lymphocytes (Fig 10). C4d deposition will also be analyzed to detect antibody-mediated rejection. Quantitative image analysis will be used to calculate the inflammatory cell density per sq. mm for each subset and the proportion of the collagen 3+ interstitial compartment in the non-glomerular renal cortex. In situ hybridization (ISH) using a novel signal amplification and

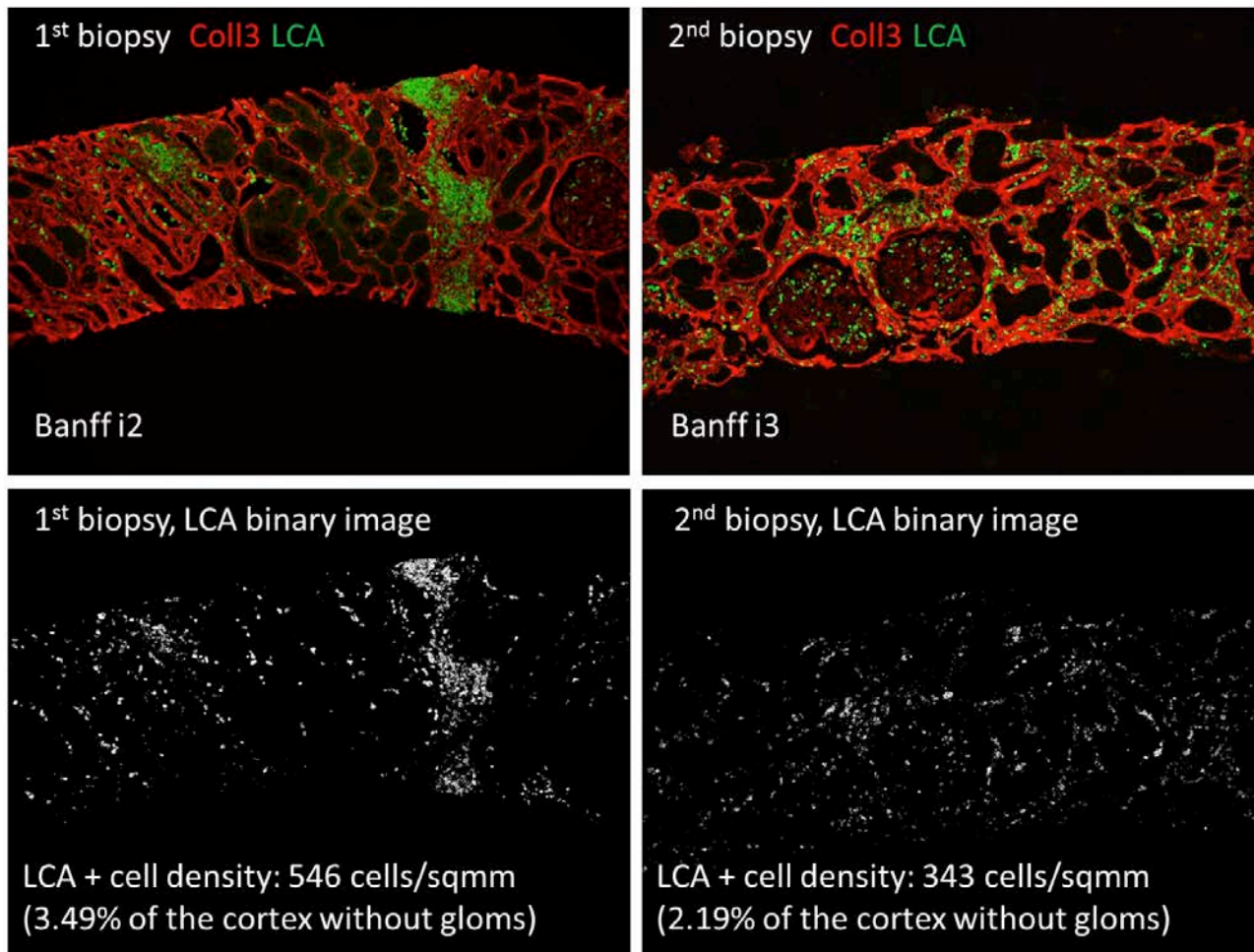


Fig 10. Quantitative measurement of the inflammatory cell load in the cortex by computer-assisted image analysis on IF stained sections. The pictures show two consecutive biopsies from the same patient, both stained with collagen 3 (red) and leukocyte common antigen (LCA, green). This example shows 37.2% reduction of the inflammatory cell load (LCA+ cells) in the 2nd biopsy

background suppression protocol patented by Advanced Cell Diagnostics (Hayward, CA) allows the visualization of single mRNA molecules per cell. For markers that no reliable IHC protocol exists, such as IL-10, ISH can be a good alternative to visualize local IL-10 production and is also the preferred methodology to detect IL-6, IL-17 and GATA3. The ISH will be quantitated by computer assisted image analysis (Tissue Studio, Definiens) and combined with multiple IF stains (cytokeratin for the tubules, CD31 for endothelial cells, leukocyte common antigen for lymphocytes, CD68 for macrophages) for precise signal localization within various compartments of the kidney.

Whole genome gene expression profiling: RNA deep sequencing (RNA-Seq) will be utilized as our primary method for defining the mechanism of graft rejection in HIV+ patients and the impact of maraviroc on the rejection mechanism. Transcript sequences are mapped back to a reference genome and transcriptome. Reads that map back to the reference are quantified to assess the level of gene expression, with the number of mapped reads being the measure of expression level for that gene or genomic region. Because RNA-Seq provides direct access to the sequence, it has several technical advantages over microarray: Junctions between exons can be assayed without prior knowledge of the gene structure, RNA editing events can be detected, and knowledge of polymorphisms can provide direct measurements of allele-specific expression. RNA-Seq can detect expressed regions of the genome that correspond to yet to be annotated genes. RNA-Seq can quantify transcript isoforms that result from alternative splicing, making possible the study of transcript isoform diversity and abundance.

Identification of gene transcripts will be carried out by deep sequencing of the cDNA libraries reverse transcribed from poly(A)-tailed RNA isolated from graft biopsies. We will use the Illumina GAIIX (Illumina Inc., San Diego, CA) platform for this analysis. We will use approximately 5 ug of total RNA per library preparation. We will amplify the poly(A)-tailed RNA isolated prior to library preparation by Ambion's MessageAmp II aRNA Amplification Kit (Austin, TX). For each sample, 100 ng of amplified polyA+ RNA will be used, fragmented, converted to double stranded cDNA and subjected to RNA-Seq. We plan to barcode and sequence 5 samples in one lane on our Illumina HiSeq 2000 machine. This currently produces about 30M reads per sample. Since ~60% yield unique reads, we will obtain 18M mapped reads per sample. This is adequate for accurate monitoring of gene expression for most genes. Prior work with embryonic stem cells suggests that we can obtain information on approximately 80% of detectable genes with this depth. These figures are consistent with the results of others. Moreover, we will generate 76b paired-end reads to monitor splicing isoforms, though we may only cover approximately ~40% of the known variants at this depth. The results obtained from both arms of the patients in this trial will be compared to each other and will be compared with historical RNAseq data on HIV- rejection biopsies published by others and that in the Sarwal lab.

10.3.3 Alloimmune assay data analysis plan

Endpoints: Mechanistic endpoints will be analyzed using assays described above. Specific measurements, both categorical and quantitative, to be collected from the assays are as described in Table 2. Other planned assays are for exploratory assessment and any statistically significant results from these tests will be reported as requiring independent verification and will provide a basis for further studies.

Table 2 Endpoints measured by the assays

Assays	Endpoints
MFC/Leuk-CCR5, T cell-Ccr5	MFI of CCR5 on subsets of T cells, B cells, subsets of monocytes, dendritic cells, and subsets of NK cells
MFC/leuk-CCR5, T cell-Ccr5	Percentages and absolute numbers of subsets of T cells, B cells, monocytes, and NK cells in circulation
MFC/Tmem	Percentages and absolute numbers of T cell subsets including naïve, memory, effector and exhausted CD4, and CD8

MFC/B cells	Percentages and absolute numbers of B cell subsets including transitional, naïve, memory, and terminally differentiated B cells
ATF	Frequencies of donor-reactive CD4, CD8, and regulatory T cells at baseline and various time points after transplant.
AMT (donor)	Frequencies of donor antigen stimulated IFN γ -producing CD4 and CD8 T cells at baseline and various times after transplant.
AMT (PMA+I)	Frequencies of mitogen-stimulated Th1 (IFN γ -producing), Th2 (IL-4-producing), and Th17 (IL17-producing) CD4 and CD8 T cells at baseline and various time points after transplant.
CRT	Frequencies of T cells with cross-reactivity between donor alloantigens and HIV, CMV, and EBV antigens at baseline and at various time points after transplant
HLA	Anti-HLA antibodies, including donor-specific antibodies, in serum at baseline and various times after transplant; DSA MFI
mIF/ISH	Histological analysis for evidence of cellular and antibody mediated rejection mechanisms
RNA-seq	Gene expression profiling

Treatment groups and comparators:

To define immunological parameters associated with rejection, participants will be further divided into 4 groups: HIV+ standard immunosuppression rejectors and non-rejectors, HIV+ maraviroc rejectors and non-rejectors. Results obtained using pre-transplant samples will be compared across all four groups. Results obtained from samples collected at the time of rejection (blood and graft) will be compared among HIV+ rejectors on standard immunosuppression and HIV+ rejectors on maraviroc to determine if rejections in the two groups have distinct or similar profiles.

To determine the impact of maraviroc on immunological profiles, comparisons will be made between HIV+ patients receiving standard immunosuppression and those on maraviroc-containing regimen.

Data integrity checks: The first step of data analysis will be to perform data integrity checks, including checks for unrealistic outliers in assay measurements and missing data. Data that are not within a plausible clinical range will be individually checked for potential causes e.g., data entry errors; if no plausible explanation is found, they will be treated as missing data.

Descriptive analyses: Descriptive analyses will be used to summarize participant characteristics of our study populations across treatment groups. Dichotomous variables will be summarized as proportions with 95% confidence intervals. Continuous variables will be summarized using means, standard deviations, and 95% confidence intervals if they are symmetric and unimodal. Otherwise, they will be summarized using the median and the interquartile range. Simple t-, ANOVA, chi-squared, or Fisher's exact test, as appropriate, will be used to compare quantitative measures across treatment groups.

Statistical modeling: Statistical analysis will be performed primarily via ANOVA, chi-squared, or Fisher's exact test, as appropriate, and when warranted (in the case of repeated measurements on the same participants), using the linear mixed-effect model. In the case of mixed effects model, the random-effects we consider are the intercept term for the 2-timepoint analyses and intercept plus slopes modeled for 3-timepoints. Fixed effects will include intercept, slope, treatment group and treatment group by time interactions. We will also consider the need for heterogeneous residual variance magnitude between groups (as has been observed in some of our assays). A major strength of this linear mixed-effects modeling approach is the ability to model all of the data available for a person, regardless of length of follow-up, number and spacing of evaluations, and/or missing outcome data at some time points (assuming non-informative missingness). An additional important advantage is the enhanced power obtained by using reliable measures of the outcome at multiple time points.

Statistical testing for hypotheses: In the case of linear mixed effects models, hypothesis testing will use the linear mixed-effect model to determine whether the inclusion of a treatment group variable and its interaction with time significantly improves the model fit for the assay response variables (conditional t-tests within linear mixed effects model fits). Primary focus will be on detection of a statistically significant time by treatment group interaction. We will also examine any effects in terms of restricted maximum likelihood point estimates and 95% confidence intervals. As far as the primary hypotheses are concerned, we will use a nominal significance threshold of $\alpha = 0.05$. However, we will make adjustments for multiple comparisons when multiple pairwise assessments need to be conducted among groups.

Assessing normal assumption: Many of the statistical procedures we will use, including the linear mixed-effects model, assume that responses follow a normal distribution. We will use standard diagnostic methods such as quantile-quantile plots and direct tests of normality to examine the appropriateness of this assumption. If we detect severe departures from normal distributions, we will either transform the outcome to produce better approximations to normal distributions or apply non-linear mixed-effects modeling approaches.

Outliers: We will examine plots of data and residuals from fitted models to detect potential outlying and influential points. If we detect such points, we will flag and check them with the sites for accuracy. If any outliers are not in error, then we will assess the effect of these points on the analysis by rerunning the analysis without the points. If the two sets of analyses differ substantially, we will report the results separately.

Missing data: Every attempt will be made to determine the reason for any missing data, in particular for missing observations caused by loss to follow up. Individuals with missing data on key variables will be compared to those with complete data to assess for potential bias due to non-random missing data. If a participant's observation is deemed missing at random (i.e. missing follow-up for reasons unrelated to the outcome) then we can continue to apply models "as is" with the missing observation simply left out of the analysis. When missing outcome data is deemed informative (i.e. not missing at random), we will perform sensitivity analyses. The sensitivity analysis will examine multiple approaches to imputing the missing data (e.g. simple carry last one forward, or advanced methods such as Expectation Maximization), as well as outcomes corresponding to different extreme case scenarios for the missing data mechanism.

10.4 Pharmacokinetic Analysis

10.4.1 Pharmacokinetic studies

PK studies will be performed on the first 20 participants enrolled at UCSF at month 3 post-transplant, and will be repeated on all UCSF participants in the unlikely event that the calcineurin inhibitor used for maintenance immunosuppression is changed to cyclosporine (which is allowed only for tacrolimus toxicity). In the previous HIV-transplant studies, drug regimens have been modified in response to drug side effects, low drug levels, or rejection episodes.

Participants will be admitted to the UCSF Clinical Research Center for the pharmacokinetic studies. According to previously published protocols, participants are admitted after an overnight fast, and after drawing trough blood samples, participants take their morning cART, maraviroc or placebo and CNI. [70] Blood samples are drawn at 0, 0.5, 1, 2, 3, 4, 6, 8 and 12 hours. No food is allowed for the first three hours after the study was started, and all participants eat a 30% fat meal at the same times relative to taking their medication. Medications known to be strong CYP3A and P-glycoprotein inhibitors (e.g., ketoconazole or fluconazole) are not given until after the pharmacokinetic study had been completed. In general, PK studies are initiated 12 hours after the prior doses of potential concomitant ARV inhibitors/inducers and other prescribed medications.

10.4.2 Analysis of blood samples

Blood samples are frozen at -80°C until analyzed.

Whole blood samples are analyzed for tacrolimus (or cyclosporine or sirolimus) by a validated HPLC/MS assay in combination with automated online sample preparation (LC/LC-MS) (Hewlett-Packard; Palo Alto, CA). Method validation has been described in detail by Christians et al.[95]

We have developed an updated methodology for analysis of maraviroc. API4000 LC/MS/MS trace from injection of 20 pmol maraviroc. The method is sensitive down to injections of 1 pmol. Toronto Research Chemicals offers d6-maraviroc, the ideal internal standard, at 175/mg. The MS method uses the MS/MS transition m/z 514.3 \rightarrow 390.1. The Sciex API4000 instrumental parameters (in positive ion electrospray mode) are: declustering potential = 56 V, collision energy = 29 V, collision cell exit potential = 22 V, entrance potential = 10.5 V, collision gas = 12, curtain gas = 16, ion source gas1 = 30, ion source gas2 = 40, ionspray voltage = 5500 V, temperature = 500 C.

The LC method is carried out with a Shimadzu Prominence LC system including a pair of LC20-AD pumps, a SIL-20AC HT autosampler and a CBM-20A controller. It uses a Phenomenex Kinetex C18 50 x 4.6 mm (5 μm , 100 \AA) column and the following gradient at a flow rate of 0.5 ml/min: 0-1 min, 10% B; 1-4 min, linear ramp to 100% B; 4-6 min, 100% B; 6-6.5 min, linear ramp to 10% B; 6.5-10 min, 10% B, where A = 15% methanol:water, B = 100% methanol, and both A and B contain 0.1% formic acid, 0.1% acetonitrile and 160 mg/L ammonium acetate. A similar method has been published by a group from Tandem Laboratories and Pfizer. [96]

10.4.3 Pharmacokinetic analysis

Individual whole blood concentration-time data are used for pharmacokinetic parameter estimation using noncompartmental methods (WinNonlin software, Professional Edition, version 5.0; Pharsight, Mountain View, CA). Pre-dose sample times of less than time zero are assigned values of zero. Samples below the limit of quantitation of bioanalytical assays that occurred before the first quantifiable concentration was achieved are assigned a concentration of zero to prevent overestimation of initial area under the curve (AUC). Values below the limit of quantitation at all other time points are treated as missing data. Maximum concentration (C_{max}) and time at maximum concentration (T_{max}) are taken as the observed values.

AUCs are calculated over the dosing interval using the linear-log trapezoidal method and adjusted for both drug dose and patient weight. Dividing these values into the dose yielded measures of weight adjusted apparent oral clearance (CL/F). Terminal half-lives during the dosing interval [representing the multiple dosing operational half-life, are used to calculate the apparent oral volume of distribution: $V/F = (\text{CL}/F) \times (t_{1/2,op}/0.693)$].[97]

10.5 APOL1

Donor samples will be collected and stored to determine APOL1 polymorphisms retrospectively.

11 Biospecimen Storage

Biological specimens from this study will be sent to the central repository, Precision Bioservices, where they will be sent in batch to the core mechanistic labs for analysis. Details can be found in the Laboratory Manual.

Biological specimens obtained under this protocol may be used in future assays to reevaluate biological responses as additional research tests are developed over time. These specimens will be collected at time points already scheduled for the core mechanistic studies, in order to allow specimens to be stored for use in new assays that have yet to be optimized or conceived. Appropriate informed consent will be obtained for both the collection and storing of samples. The specimens from these evaluations may be stored beyond the funding period. During the funding period, samples will be identifiable, which means samples will be coded with a participant ID number that could be directly linked to the participant and the participant's medical record.

Study participants will be informed that they may be approached about additional clinical evaluations or studies that have received the full approval of the NIAID as new evaluations are identified. If additional evaluations are determined to be desirable, this protocol (and other appropriate study documents, e.g., the informed consent and the statistical analysis plan) will be amended and submitted to the appropriate regulatory authorities, ethics committees, and IRBs for approval. Each participant's signature will be obtained on the revised informed consent form before additional evaluations are performed. The specimens from these evaluations may be stored up to the end of the contract—approximately 5 years, or longer if the contract is extended.

12 Criteria for Participant and Study Completion and Premature Study Termination

12.1 Participant Completion

Participants will have completed the study at the week 156 (year 3) study visit, or once the study has been closed. All participants will be followed for at least 52 weeks (1 year) post-transplant depending on enrollment date (4 years of accrual).

12.2 Participant Stopping Rules and Withdrawal Criteria

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

12.2.1 Treatment discontinuation

1. Participant meets criteria to discontinue study treatment (MVC or placebo)
2. The investigator no longer believes continuing study treatment is in the best interest of the participant.
3. Participant refuses to continue study treatment.
4. Treatment is held or discontinued for clinical reasons for greater than 60 days.

12.2.2 Study discontinuation

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).
3. For those on the waiting list, confirmed or predicted failure to receive transplant by the end of year 4
4. Study closed by sponsor or FDA
5. Despite being randomized and receiving study treatment, participant did not receive a transplant as expected.

12.3 Participant Replacement

Participants who withdraw or are withdrawn post-transplant will not be replaced.

12.4 Study Stopping Rules

The study may be discontinued at any time by the EC/IRB, NIAID, the pharmaceutical supporter(s) or designee, the FDA, or other government entities as part of their duties to ensure that research participants are protected.

12.4.1 Continuous Monitoring of Specific Events

In addition, the incidence of specific safety-related events of particular concern will be continuously monitored throughout the study to determine if any of their observed subject-based incidence rates exceed a threshold incidence rate of concern pre-specified for each particular event.

These events and their corresponding thresholds of concern are:

1. During year 1 of follow-up, death due to any reason except accidental death above 10%.
2. During year 1 of follow-up, graft loss due to any reason except accidental death above 16%.
3. During year 1 of follow-up, site-reported treated acute rejection above 35%

These rules will be implemented by the Emmes data center continuously monitoring the occurrence of any of these events to determine if the currently observed rate of each exceeds the corresponding threshold rate of concern with a pre-specified level of confidence. The stopping rule will be considered to be met if the lower one-sided 95% exact confidence limit on the observed incidence rate exceeds the corresponding threshold level of concern for that event. If any stopping rule is met, enrollment and randomization will be halted pending expedited DSMB review.

The following two tables describe the threshold numbers of events (n) out of selected numbers of subjects randomized to each treatment arm (N) which, if equaled or exceeded, would satisfy the stopping rule for each event of particular concern.

Table 3: Thresholds for Meeting the Stopping Rule for Death in the First Year of Follow-up

Number of Subjects with Event (n)	Number of Subjects Randomized (N)	Observed Incidence Rate (%)	Lower 95% Confidence Limit (%)
4	10	40.0	15.0
5	20	25.0	10.4
7	30	23.3	11.5
8	40	20.0	10.4
10	50	20.0	11.3
11	60	18.3	10.6
12	70	17.1	10.2
14	80	17.5	10.9
15	90	16.7	10.6
16	100	16.0	10.3
17	110	15.5	10.1
19	120	15.8	10.6
20	130	15.4	10.4

Table 4: Thresholds for Meeting the Stopping Rule for Graft Loss in the First Year of Follow-up

Number of Subjects with Event (n)	Number of Subjects Randomized (N)	Observed Incidence Rate (%)	Lower 95% Confidence Limit (%)
5	10	50.0	22.2
7	20	35.0	17.7
9	30	30.0	16.6
11	40	27.5	16.3
13	50	26.0	16.1
15	60	25.0	16.1
17	70	24.3	16.1
19	80	23.8	16.1
21	90	23.3	16.2
23	100	23.0	16.3
25	110	22.7	16.3
27	120	22.5	16.4
29	130	22.3	16.4

Table 5: Thresholds for Meeting the Stopping Rule for Treated Acute Rejection

Number of Subjects with Event (n)	Number of Subjects Randomized (N)	Observed Incidence Rate (%)	Lower 95% Confidence Limit (%)
7	10	70.0	39.3
12	20	60.0	39.4
16	30	53.3	37.0
20	40	50.0	36.1
24	50	48.0	35.7
28	60	46.7	35.6
32	70	45.7	35.5
37	80	46.3	36.7
40	90	44.4	35.6
44	100	44.0	35.6
48	110	43.6	35.6
52	120	43.3	35.7
55	130	42.3	35.0

13 Safety Monitoring and Reporting

13.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. All grade 3 adverse events or higher will be reported in the EMMES IDES database. Adverse Event reporting is only required post-transplant. In addition, adverse events that are classified as serious according to the definition of health authorities must be reported promptly (per section 13.5 Expedited Adverse Event Reporting to DAIDS) to the sponsor following Version 2.0 of the DAIDS EAE manual which is available on the RSC website at <https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids>.

Severity of AEs will be graded according to the “Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events” (Corrected Version 2.1, July, 2017).

Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*, *ICH Guideline E-6: Guideline for Good Clinical Practice*, 21CFR Parts 312 and 320.

13.2 Definitions

13.2.1 Adverse Event (AE)

Any untoward or unfavorable medical occurrence associated with the participant’s participation in the research, whether or not considered related to the participant’s participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice) (from OHRP “Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)” <http://www.hhs.gov/ohrp/sites/default/files/ohrp/policy/advevntguid.pdf>).

For this study, an adverse event will include any untoward or unfavorable medical occurrence associated with, but not limited to:

- Worsening (change in nature, severity, or frequency) of conditions present at the onset of the study
- Intercurrent illnesses
- Drug reactions or interactions of antiretroviral agents, immunosuppressant agents, or other concomitant medications used on study
- Infections
- Events related or possible related to maraviroc or concomitant medications
- Abnormal laboratory values (significant shifts from baseline within the range of normal that the investigator considers to be clinically important)
- Clinically significant abnormalities in physical examination, vital signs, weight, and/or tests and procedures
- Surgical complications of kidney transplantation

13.2.2 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the Investigator Brochure or is not listed at the specificity, severity or rate of occurrence that has been observed.

“Unexpected” also refers to adverse events or suspected adverse reactions that are mentioned in the Investigator Brochure or package insert as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation (21 CFR 312.32(a))

13.2.3 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or Sponsor [DAIDS], it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death.
2. A life-threatening event: An AE or SAR is considered “life-threatening” if, in the view of either the investigator or Sponsor, its occurrence places the participant 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.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Elective hospitalizations or hospital admissions for the purpose of conduct of protocol mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

13.3 Grading and Attribution of Adverse Events

13.3.1 Grading Criteria

Severity of AEs will be graded according to the “Division Of AIDS Table For Grading The Severity Of Adult And Pediatric Adverse Events” (Corrected Version 2.1, July 2017).

Adverse Events not found in the Toxicity Table will be assessed for severity and classified into one of the categories below:

Grade 1 (Mild): Event requires minimal or no treatment and do not interfere with the participant’s daily activities.

Grade 2 (Moderate): Event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Grade 3 (Severe): Event interrupts a subject’s usual daily activity or functioning and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Grade 4 (Potentially Life threatening): Events causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death.

Grade 5 (Death)

13.3.2 Causality Assessment

Causality (likelihood that the event is related to the study agent) will be assessed from the time study dosing begins until 30 days following the last dose considering the factors listed under the following categories:

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship
OR
- good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship
OR
- definitely due to an alternative etiology

Note: Other factors (e.g., dechallenge, rechallenge) should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The Investigator may revise the causality assessment as additional information becomes available.

13.4 Collection and Recording of Adverse Events

13.4.1 Collection Period

Adverse events will be collected from the time of transplant until a participant completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

13.4.2 Collecting Adverse Events

Adverse events (including SAEs) may be discovered through any of these methods:

- Observing the participant.
- Interviewing the participant [e.g., using a checklist, structured questioning, diary, etc.] .
- Receiving an unsolicited complaint from the participant.
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in section 13.3 Grading and Attribution of Adverse Events.

13.4.3 Recording Adverse Events and Serious Adverse Events

Throughout the study, the investigator will record all adverse events and serious adverse events as described previously (13.2 Definitions) on the appropriate electronic case report form in the EMMES IDES regardless of the relationship to study therapy regimen or study procedure.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the participant prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

13.4.4 Adverse Events and Serious Adverse Events Exempt from Reporting

- Elevated creatinine in the first week post-transplant if they are within expected ranges.
- Decline in CD4+ T-cell counts during the first year following thymoglobulin treatment for rejection.
- Elective hospitalizations or hospital admissions for the purpose of conduct of protocol mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

13.4.5 Special Adverse Events requiring reporting

Only grade 3 or higher adverse events must be recorded in the EMMES IDES. However, all cases of the following should be recorded in the EMMES IDES, regardless of grade:

- Postural hypotension
- Allergic reaction
- Rash
- HIV persistent virologic failure defined as an HIV viral load >1000 copies/mL for more than 90 days that is not the result of an investigator/physician approved interruption in antiretroviral treatment.

13.4.6 Reporting of Adverse Events to IRBs/IECs

All investigators shall report adverse events, including expedited reports, in a timely fashion to their respective IRBs/IECs in accordance with applicable regulations and guidelines. All Safety Reports to the FDA shall be distributed by the sponsor or designee to all participating institutions for site IRB/IEC submission.

13.5 Expedited Adverse Event Reporting to DAIDS

Requirements, definitions and methods for expedited reporting of Adverse Events (AEs) are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the RSC website at <https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids>.

The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, must be used for expedited AE reporting to DAIDS. In the event of system outages or technical difficulties, expedited AEs may be submitted using the DAIDS EAE Form. This form is available on the DAIDS RSC website at <https://rsc.niaid.nih.gov/clinical-research-sites/paper-eae-reporting>.

For questions about DAERS, please contact NIAID CRMS Support at CRMSSupport@niaid.nih.gov. Please note that site queries may also be sent from within the DAERS application itself.

For questions about expedited reporting, please contact the DAIDS RSC Safety Office at DAIDSRSCSafetyOffice@tech-res.com.

For questions about expedited reporting, please contact DAIDS RSC (DAIDSRSCSafetyOffice@tech-res.com).

13.5.1 Expedited Reporting Requirements for this Study

- The SAE Reporting Category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study.
- The study agents for which expedited reporting are required are: maraviroc (MVC) and placebo.

13.5.2 Expedited AE Reporting Period

- The expedited AE reporting period for this study is per Version 2.0 of the EAE Manual.
- After the protocol-defined AE reporting period, unless otherwise noted, only SUSARs as defined in Version 2.0 of the EAE Manual will be reported to DAIDS if the study staff become aware of the events on a passive basis (from publicly available information).

13.6 Pregnancy Reporting

All pregnancies during study enrollment should be reported in the EMMES IDES.

All pregnancies should also be registered in “The Antiretroviral Pregnancy Registry” by a health care provider from the enrolling center (<http://www.apregistry.com/>).

All pregnancy complications that result in a congenital abnormality, birth defect, miscarriage, and medically indicated abortion will be reported to the sponsor thru DAERS as outlined in Version 2.0 of the DAIDS EAE Manual.

13.7 Reporting of Other Safety Information

An investigator shall promptly notify the site IRB as well as the Sponsor when an “unanticipated problem involving risks to participants or others” is identified, which is not otherwise reportable as an adverse event.

14 DSMB Review

The DSMB will be chartered to perform independent oversight for the trial through regular review of enrollment and safety information provided in tables, listing and figures generated from the clinical database.

14.1 Planned DSMB Reviews

The Data and Safety Monitoring Board (DSMB) shall review safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs.

14.2 Ad hoc DSMB Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for *ad hoc* reviews. The DSMB will review any event that potentially impacts safety at the request of the protocol chair or DAIDS/DAIT/NIAID. In addition, the following events will trigger an *ad hoc* comprehensive DSMB Safety Review:

- Any death in the study which is considered possibly, probably or definitely related to study treatment regimen.
- Non-skin cancer including post-transplant lymphoproliferative disease (PTLD).

After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

14.3 Temporary Suspension of enrollment/drug dosing for ad hoc DSMB Safety Review

A temporary halt in study enrollment or drug dosing at all participating clinical sites may be implemented pending expedited review of all pertinent data by the institutional review board (IRB), the National Institute of Allergy and Infectious Diseases (NIAID), and the NIAID Data Safety Monitoring Board (DSMB) if any unexpected fatal or life-threatening AE related to the study treatment occurs and the study team and DAIT/DAIDS medical officers advise to do so.

A temporary halt in enrollment/drug dosing will be implemented if an ad hoc DSMB safety review is required for any unexpected fatal or life-threatening AE related to the study treatment.

15 Clinical Site Monitoring

Site monitors under contract to the National Institute of Allergy and Infectious Diseases (NIAID) will visit participating clinical research sites to review participants records, including consent forms, CRFs, medical records (e.g., physicians' progress notes, nurses' notes, individuals' hospital charts), and laboratory records to ensure protection of study participants, compliance with the EC/IRB approved protocol/amendments, and accuracy and completeness of records. The monitors will inspect sites' regulatory files to ensure that local regulatory requirements, in addition to U.S. Federal regulations, are being followed. They will also inspect sites' pharmacies to review product storage and management.

16 Statistical Considerations and Analytical Plan

16.1 Overview

This trial is a prospective, multi-center, double-blind phase II study of kidney transplantation in HIV+ individuals assessing the safety and efficacy of maraviroc (Arm 1) or placebo (Arm 2) given at transplant with an antiretroviral drug regimen that does not include protease inhibitors. Analysis of the primary endpoint will be based on outcomes measured at 52 weeks post-randomization, while analyses of most secondary outcomes will be done on measurements taken at months 3, 6, 9, years 1, 2 and/or 3 post-randomization. The primary clinical objective is to evaluate CCR5 blockade as a strategy to improve long term kidney function following transplantation in the HIV positive recipient. In addition, this study has a large number of secondary clinical and mechanistic objectives.

16.2 Measures to Minimize Bias

Donor type (deceased versus live) is an important confounding factor known to impact graft function, inflammation and survival outcomes post kidney transplantation, and recent approval regarding the use of HIV+ donors, although not observed in the South Africa experience, could yield differences in outcomes compared to use of HIV- donors. Outcome differences may also arise due to known and unknown center-specific differences. Hence, participants will be randomized in a blinded fashion to one of the two treatment arms in a 1:1 ratio within each center-by-donor type stratum using a web-based randomization system with donor type categories being HIV negative deceased, HIV negative live, and HIV positive deceased. The randomization schedule will not incorporate other stratification variables. The maraviroc and placebo pills will be identical in appearance to maintain the masking of study participants. The masking of study and lab investigators to individual participant assignments will be maintained until the end of the study to avoid any bias in treatment or assessment. During the study, unblinded data listings can be provided to DSMB members for any scheduled or ad hoc reviews of safety data. To minimize bias, central read of biopsy slides by the core renal pathologist will be implemented for histological endpoint assessments.

For the primary and secondary endpoints on graft function, missing data can be of concern and a source of bias in the case of graft failures. Hence, we will consider graft failures as informed drop-outs in these analyses. Appropriate imputations will be implemented as explained for the primary endpoint below. For the secondary endpoints on graft function, similar imputations will be performed at the time of graft failure.

16.3 Endpoint Assessments

16.3.1 Primary Analysis of Primary Efficacy Endpoint

The primary efficacy endpoint is defined in section 3.2. The primary efficacy endpoint will be the 52-week GFR as measured by iohexol clearance. Mean GFR of maraviroc group (Arm 1) will be compared to the mean GFR of the placebo reference group (Arm 2) via a Wilcoxon rank-sum test. Informed drop-outs will consist only of graft failures prior to week 52, and will be incorporated to the analysis by imputing the worst GFR value observed at that time point in the study or a value of 10 ml/min/1.73m² (10 being the average estimated GFR at the time of dialysis initiation in the U.S.), whichever is lower. Note that graft failure will be defined as the first observation of any of the following events: 1) death with a non-functioning graft, 2) retransplantation, or 3) initial return to chronic dialysis. Results will be reported with p-value associated with the Wilcoxon rank-sum test and the estimated mean treatment difference with two-sided 95% confidence interval. All tests of significance will be two-tailed at the 0.05 level.

16.3.2 Supportive Analyses of Primary Efficacy Endpoints

We will conduct 2 sensitivity analyses for the primary efficacy endpoint: (1) include only the completers in the analysis (i.e., exclude all dropouts and treatment discontinuations prior to week 52); (2) impute the worst GFR value observed at that time point in the study or a value of 10 ml/min/1.73m², whichever is lower, for deaths with graft function that occur prior to week 52. The sensitivity analyses will be included as additional secondary analyses.

16.3.3 Primary Safety Endpoint

The primary safety endpoint will be the incidence of graft loss and toxicities \geq Grade 3 and/or permanent treatment discontinuation within the first 52 weeks post-transplant. Cumulative incidence in maraviroc group (Arm 1) will be compared to the cumulative incidence in the placebo reference group (Arm 2) via a log-rank test. Results will be reported with p-value associated with the log-rank test and the Kaplan-Meier estimates for each treatment group with two-sided 95% confidence intervals. All tests of significance will be two-tailed at the 0.05 level.

16.3.4 Secondary Clinical Endpoints

The secondary clinical endpoints are defined in section 3.3. The endpoints are to be computed and summarized by treatment and, in a few cases, by day/week and treatment. With the exception of specific AEs and lab parameters, proportions or means will be estimated for each treatment arm and tested for treatment differences through the use of a statistical model. The particular model to be used depends on the scale of measurement of the endpoint. The accompanying table lists each endpoint and its measurement scale (continuous, dichotomous, and ordinal). All tests of significance in all analyses will be made at the 0.05 level.

Dichotomous measures of prevalence and incidence will be analyzed with log-binomial regression models, wherein treatment effects will be estimated as relative risk ratios; the reference group will be the Arm 2 participants. The log-binomial model (rather than logistic regression) is applied for prevalence and incidence analyses because model-based estimates of relative risk (RR) are both more desirable and appropriate for non-Poisson events that are expected to occur more commonly than in 10% of the cases. In such cases, logistic regression-based odds ratio (OR) estimates are poor estimators of the relative risk [98-101]. It may be appropriate to treat some of the dichotomous outcomes as censored data; if this is the case, we will obtain RR estimates by fitting Cox regression models to the data [102]. The single ordinal secondary outcome variable (Banff cell-mediated AR score) will be analyzed in a proportional odds model [103] which may include additional covariates and interaction terms, as appropriate.

For continuous outcomes, we will test for differences in response means among the treatment arms by fitting normal theory analysis of variance (ANOVA) or analysis of covariance (ANCOVA) models to the original scores or to some normalizing-variance stabilizing transformation of them. For some endpoints, these models may be fit separately by day or week post-randomization (i.e., time will be treated as a by-variable and no repeated measures analysis will be performed). Repeated measures models will be fit to the slopes of eGFR vs. time regressions. The latter will be estimated in a mixed effects repeated measures model in which both treatment-group means (with 95% confidence limits) of the individual participant slopes and the individual participant slopes themselves will be estimated and tested for equality. Distributions of the individual slopes will be examined graphically and compared among the treatment groups.

Table 6: Summary of Proposed Analyses for Primary and Secondary Clinical Response Variables

Response type	Response	Measurement Scale	Summary Statistics	Models to test for treatment effects
Primary Efficacy Endpoints	Mean GFR at week 52 (based on iohexol clearance)	continuous	Mean and/or geometric mean + 95% CI, and median [IQR]	Wilcoxon rank-sum test
Secondary/sensitivity analyses for primary efficacy endpoint	Mean GFR at week 52, with dropouts removed	continuous	Mean and/or geometric mean + 95% CI, and median [IQR]	Wilcoxon rank-sum test
	Mean GFR at week 52, with a GFR imputation for deaths and graft failures	continuous	Mean and/or geometric mean + 95% CI, and median [IQR]	Wilcoxon rank-sum test
Primary Safety Endpoint	Cumulative incidence of graft loss, toxicities \geq Grade 3 per the DAIDS toxicity table and/or permanent treatment discontinuation	continuous	Kaplan Meier estimates by treatment group	Logrank test for a treatment group difference

Inflammation Endpoints	Mean CD45 gene expression count (PTPRC) at week 26	continuous	Mean and/or geometric mean + 95% CI, and median [IQR]	Wilcoxon rank-sum test
	Mean CD45 quantitative IHC at week 26	continuous	Mean and/or geometric mean + 95% CI, and median [IQR]	Wilcoxon rank-sum test
	Mean tCRM score at week 26	continuous	Mean and/or geometric mean + 95% CI, and median [IQR]	Wilcoxon rank-sum test
	Mean uCRM score at weeks 26 and 52	continuous	Mean and/or geometric mean + 95% CI, and median [IQR]	Wilcoxon rank-sum test
Renal Function and Injury Endpoints	Indicator of week 52 eGFR<60 mL/min/1.7 m ² (based on CKD-EPI, cystatin C, and CKD-cystatin C equations)	dichotomous	Proportion + 95% CI	Log-binomial or Poisson regression
	Mean eGFR at week 52, 104 and 156 based on CKD-EPI, cystatin C, and CKD-cystatin C Eqs.	Continuous	Mean and/or geometric mean + 95% CI	Mixed Effects Repeated Measure ANCOVA
	Slope of eGFR vs. time Regression over week 13 – week 52, for each treatment arm, based on CKD-EPI, cystatin C, and CKD-cystatin C Eqs.	Continuous	Slope Mean and std. error or mean + 95% CI	Mixed effects Repeated Measures ANCOVA
Histologic Evidence of Rejection and Graft Dysfunction Endpoint	Cumulative Incidence of biopsy proven Acute Rejection (AR) within the first 52, 104 and 156 weeks	continuous	Kaplan Meier estimates by treatment group	Logrank test for a treatment group difference in cumulative incidence
Severity of Rejection and Anti-Donor Reactivity Endpoints	Banff score of the 1 st observed AR within 52 weeks post-transplant	Ordered categories (3 levels)	Proportion + 95% CI	Proportional Odds model
	Highest Banff score observed within 52 weeks post-transplant	Ordered categories (3 levels)	Proportion + 95% CI	Proportional Odds model
	Incidence of humoral rejection within the first 52 weeks and overall	dichotomous	Proportion + 95% CI	Log-binomial or Poisson regression
	Prevalence of anti-donor antibodies at week 52	dichotomous	Proportion + 95% CI	Log-binomial or Poisson regression
Safety Profile Endpoints	Death within the first 52 weeks and overall	continuous	Kaplan Meier estimates by treatment group	Logrank test for a treatment group difference
	Graft loss within the first 52 weeks and overall	continuous	Kaplan Meier estimates by treatment group	Logrank test for a treatment group difference
	Cumulative incidence of opportunistic infections or neoplasms within the first 52 weeks and overall	continuous	Kaplan Meier estimates by treatment group	Logrank test for a treatment group difference in cumulative incidence
	Cumulative incidence of non-opportunistic infections requiring	continuous	Kaplan Meier estimates by treatment group	Logrank test for a treatment group difference in

	hospitalization within the first 52 weeks and overall			cumulative incidence
HIV persistence	HIV msRNA	continuous	Mean estimate + 95% CI	Mixed Effects Repeated Measure ANCOVA
	HIV DNA	continuous	Mean estimate + 95% CI	Mixed Effects Repeated Measure ANCOVA

16.3.5 Secondary Mechanistic Endpoints

The primary mechanistic endpoints are defined in section 3.4. The mechanistic assays and planned comparisons/analyses are detailed in Section 10.

16.3.6 Descriptive Analyses

Summary descriptive statistics for baseline and demographic characteristics will be provided for all enrolled participants. Demographic data will include age, race, sex, body mass index; these data will be presented in the following manner:

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

The number and percentage of participants receiving immunosuppressive medications and certain concomitant medications or therapies of interest will be presented.

16.3.7 Safety Analysis

All adverse events (AEs) and serious adverse events (SAEs) will be classified by body system and preferred term according to the Medical Dictionary for Regulatory Activities (MedDRA). AEs and SAEs will be summarized as the frequency of each event by treatment group.

Frequency tables by treatment group and category of event (e.g., serious, related to study therapy, causing the discontinuation of study therapy) and by the DAIDS Toxicity Table grade will be presented. Selected laboratory values will also be summarized by treatment group using the mean and standard deviation of the change from baseline at scheduled visits.

16.4 Interim Analyses

No interim analyses are planned for this study. Unspecified interim analyses are only conducted if they will not adversely affect the integrity of the study or for safety reasons. The nature of the planned and ad hoc DSMB reviews is detailed in Section 14.

16.5 Sample Size Considerations

Primary efficacy endpoints: The primary efficacy endpoint is mean glomerular filtration rate (GFR) calculated for each treatment group using iohexol clearance at 52 weeks. The expected mean \pm SD GFR level at week 52 post-transplant for the control group (Arm 2) was based on the data observed in HIV-TR and other post-transplant studies and assumed to be 60 \pm 20 ml/min per 1.73 m². Using the same SD for the maraviroc group (Arm 1) and group sample sizes of 65 with a 8% drop-out rate at 1 year, we would achieve about 80% power to detect a difference of 10.6 ml/min per 1.73 m² or more in mean GFR levels between the two groups (with a significance level of 0.05 using a two-sided Wilcoxon rank-sum test assuming that the actual distribution is normal). Average drop in GFR in patients on calcineurin inhibitors is 2ml/min per 1.73 m² per year [104, 105]; hence, we have targeted detection of around a 10 ml/min per 1.73 m² difference

between the two treatment groups that would be equivalent to about 5 years of difference in the life of the renal allograft. Note that drop-out rate is assumed to be 13%, but 5% is expected to be informed drop-outs that will be incorporated to the primary analyses. Hence, the adjusted drop-out rate used for this calculation is equivalent to 8%.

Secondary endpoints: Based on expected rates of normal, borderline changes and acute cellular rejection in the HIV+ kidney transplant recipients at 26 weeks from previous studies, the expected mean \pm SD CD45 gene expression count (PTPRC mRNA in log₂ scale) for the control group (Arm 2) was assumed to be 7.9 ± 1.0 . Using the same SD for the maraviroc group (Arm 1) and group sample sizes of 30 (assuming enrollment of 60 HIV+ cases in the study with follow-up to week 26 or further), we would achieve about 80% power to detect a difference of 0.8 or more in mean CD45 gene expression count between the two groups (with a significance level of 0.05 using a two-sided Wilcoxon rank-sum test with normal actual distribution assumption).

Another secondary endpoint of interest is the incidence of treated acute rejection at 52 weeks post-transplant. We expect 1-year cumulative incidence of rejection for the placebo group to be around 0.30, based on the rate observed in the HIVTR study. By assuming a loss-to-follow-up rate of 0.13 and proportional hazard rates, a two-sided log rank test achieves at least 80% power at a 0.05 significance level to detect a reduction of 0.21 or more in the rejection rate in the maraviroc group. Sample size calculations were performed using PASS 2008 software.

16.6 Missing Data Conventions

Except for the sensitivity analyses described for the primary endpoint in Section 16.3.2, no data imputation is planned. Analyses of all secondary clinical and mechanistic endpoints will be carried out on the observed, non-missing data. However, summary statistics will include counts of missing data, summarized by treatment arm.

17 Identification and Access to Source Data

Each clinical research site is responsible for maintaining accurate clinical research records and documentation in compliance with DAIDS policies, ICH E6 GCP, and all other applicable regulatory and institutional requirements. Essential documents are clinical research records, source documents, or files that permit the evaluation of both the conduct of a clinical trial and the quality of the data produced. Source data are all information, original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. All source documents and source data are considered essential documents.

As part of participating in a NIAID (DAIDS)-supported and/or –sponsored clinical trial, the site investigators and site staff will permit authorized representatives of the sponsor(s), DAIDS, the FDA, the OHRP, and other local, US, and international regulatory entities, other state and local health authorities, and pharmaceutical or device companies and their commercial partners, and the local Institutional Review Board to examine (and when required by applicable law, to copy) clinical research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

18 Ethical Considerations and Compliance with Good Clinical Practice

18.1 Statement of Compliance

This clinical study will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the NIAID DSMB. Any amendments to the protocol or to the consent materials will also be approved by the NIAID DSMB before they are implemented.

18.2 Informed Consent

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol informed consent forms approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) WILL be reviewed and approved by the DAIDS PRO and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approval(s) for an amendment, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICFs **WILL NOT** be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

18.3 Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The principal investigator or designee listed on the Investigator of Record form (or FDA 1572 if applicable) will review the consent and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants (or their legally acceptable representative) will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in participants' primary language. A copy of the signed consent form will be given to the participant.

The consent process will be ongoing. The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study. Study participants will be re-consented if new information affecting participant safety is made available.

18.4 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing personal health identifiers (PHI) to the study sponsor or their representatives.

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Appendix 1. Schedule of Events

Years:	Year 0	Year 1										Years 2 - 3 every 6 m ¹
	Visit 1		Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visits 11 - 14
Weeks:	Screen	Enroll Pre-TX Segment A	Day 0 Segment B	Week 1	Week 2	Week 4	Week 8	Week 13	Week 26	Week 39	Week 52	Week 53-156
Informed Consent	X											
Symptom & Medical Review plus Physical Exam	X		X					X	X	X	X	X
STANDARD OF CARE ASSESSMENTS												
CLINICAL												
Documentation of HIV infection	X											
Monitor orthostatic blood pressure for hypotension				X	X	X	X	X	X	X	X	X
PPD (or chest x-ray if history of positive PPD)	X ²											
Vaccination Review (Pneumovax, Hep A and B)	X ²											
Pregnancy Test			X ³									
RADIOLOGY												
CXR	X ²											
SAFETY LABS												
CBC-diff	X ²		X	X	X	X	X	X	X	X	X	X
Renal/Electrolytes/glucose/albumin	X ²		X	X	X	X	X	X	X	X	X	X
LFTs	X ²		X	X	X	X	X	X	X	X	X	X
Fasting Lipid Panel (Chol, LDL, HDL, TGL)	X ²		X								X	Y2
Immunosuppressant levels				X	X	X	X	X	X	X	X	X
HIV LABS												
CD4+ T-cell count	X ²	X ⁴	X		X	X	X	X	X	X	X	X
HIV-1 RNA (bDNA or PCR)	X ²	X ⁴	X		X	X	X	X	X	X	X	X
RPR/VDRL	X ²											
Toxoplasmosis Quantitative ⁵	X ²											
General Serology												
CMV Ab	X ²		X ²									
HepBSAg	X ²		X ²									

Years:	Year 0	Year 1										Years 2 - 3 every 6 m ¹
	Visit 1		Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visits 11 - 14
Weeks:	Screen	Enroll Pre-TX Segment A	Day 0 Segment B	Week 1	Week 2	Week 4	Week 8	Week 13	Week 26	Week 39	Week 52	Week 53-156
HepBSAb	X ²		X ²									
HepB core Ab	X ²		X ²									
HepB DNA	X ²		X ²									
HCV Ab	X ²		X ²									
HCV RNA	X ²		X ²									
EBV Ab	X ²		X ²									
RESEARCH												
Specimen (Study Participants)												
Recipient Blood collected and shipped to central repository for mechanistic studies	X ⁵		X ⁶			X		X	X		X	Y2
Recipient urine collected and shipped to central repository for banking for future analysis	X ⁵		X ⁶						X		X	
Cystatin C samples collected and shipped to central repository for analysis								X	X	X	X	Y2, Y3
Recipient Lymph Nodes collected and shipped			X									
Iohexol study samples collected, processed and shipped to central repository											X	
Specimen (Donors)												
Living donor blood collected and shipped			X									
Deceased donor spleen collected and shipped			X									
Biopsy (Study Participants)												
Protocol biopsy (or SOC if applicable per site). Slides and tissue to be sent to repository.									X			
Pharmacokinetics (UCSF ONLY)												
Maraviroc pK								X				

1. every 6 months except where indicated
2. Record if done as standard of care. CD4+ T-cell count and HIV-1 RNA are required for eligibility and may be obtained from primary provider if not done locally

3. Must be completed no more than 30 days prior to day 0.
4. Recommend collecting every 12 weeks from primary provider if not done locally since the protocol requires a result no more than 26 weeks prior to transplant, although not required. Only pre-transplant results used for study eligibility/enrollment into segment B are required.
5. Baseline samples must be drawn not more than 13 weeks prior to day of transplant (day 0). Urine is not required in subjects with anuria. Do not draw baseline blood samples at screening if subject is not expected to be transplanted and randomized within the next 13 weeks. If it is not possible to obtain these baseline research blood samples within window, they should be drawn on the day of admission for transplant and prior to study drug administration and transplant. Drawing research samples on the day of transplant can be difficult logistically since samples need to be collected, processed and shipped to the repository, and transplant may occur with no study team present, so all attempts should be made to obtain baseline samples prior to day 0 if possible.
6. If the screening samples were not previously drawn, or were drawn more than 13 weeks prior to day of transplant (day 0), redraw all screening blood and/or urine samples for mechanistic studies on the day of admission for transplant and prior to study drug administration and transplant.

Appendix 2. Schedule of Events (Donor)

Donor Type	Day of Transplant
Deceased Donor	Spleen tissue, 1cm ³
Living Donor	50 cc blood NaHep or ACD

Appendix 3. Schedule of Events Associated with Suspected Rejection

	Suspected Rejection
BIOPSY	
Biopsy slides sent to repository for central review.	X
Biopsy tissue: 2 cores sent to repository	X
BLOOD	
Collected prior to treatment for rejection.	X
URINE	
Recipient urine collected and shipped to central repository for banking for future analysis. Collected prior to treatment for rejection.	X

Appendix 4. Reduced Follow-up Schedule for Participants who Prematurely Discontinue Study Treatment

Safety Labs	<ul style="list-style-type: none"> • At time of study drug discontinuation. • If study treatment discontinued due to drug toxicity, should have follow-up safety evaluations as deemed necessary by local investigators. • Every 6 Months Post Discontinuation if available as standard of care.
Phone call to assess patient survival, graft survival, and medical diagnosis (see the Manual of Procedures for details)	Every 6 Months Post Discontinuation
Medical Record Review	Every 6 Months Post Discontinuation
Serious Adverse Events	Recorded as discovered by the study site