CLINICAL TRIALS IN ORGAN TRANSPLANTATION (CTOT)

CTOT-19

Randomized Controlled Trial of Infliximab (Remicade®) Induction Therapy For Deceased Donor Kidney Transplant Recipients

SHORT TITLE: Effects of Inhibiting Early Inflammation in Kidney Transplant Patients

VERSION 9.0/April 27, 2020

Study Sponsor(s): The National Institute of Allergy and Infectious Diseases (NIAID)
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PRINCIPAL INVESTIGATOR **PETER S. HEEGER, MD** Icahn School of Medicine at Mount Sinai Recanati Miller Transplant Institute Annenberg Bldg., 2366B, Room 1243 1 Gustave L. Levy Place New York, NY 10029 **Phone:** 212-241-6324 **Fax:** 212- 987-0389 **Email:** peter.heeger@mssm.edu	**PROTOCOL CHAIR** **DONALD E. HRICIK, MD** Division of Nephrology & Hypertension University Hospitals of Cleveland 11100 Euclid Avenue, Room 8124 Lakeside Building Cleveland, OH 44116 **Phone:** 216-844-8060 **Fax:** 216-844-8060 **E-mail:** Donald.Hricik@uhhospitals.org	**MEDICAL MONITOR** **JONAH ODIM, MD** Transplantation Branch **(TB)** Division of Allergy Immunology & Transplantation **(DAIT)** National Institutes of Allergy & Infectious Diseases **(NIAID)** National Institutes of Health **(NIH)** 5601 Fishers Lane, Rm 6B21, Mail Stop 9827 **US Mail:** Bethesda, MD 20892-9827 **FedEx, UPS:** Rockville, MD 20852 **Direct Line:** 240-627-3540 **Fax Line:** 240-627-3115 **Email address**: odimj@niaid.nih.gov
BIOSTATISTICIAN **KAREN KESLER, PHD** Senior Statistical Scientist Rho Federal Systems 2635 E NC Hwy 54 Durham, NC 27713 **Phone:** 919-595-6244 **Fax:** 919-595-4291 **E-mail:** karen_kesler@rhoworld.com	**PROJECT MANAGER** **HELENA DIOP** Transplantation Branch **(TB)** Division of Allergy Immunology & Transplantation **(DAIT)** National Institutes of Allergy & Infectious Diseases **(NIAID)** National Institutes of Health **(NIH)** 5601 Fishers Lane, Room 6B22 Mail Stop 9827 **US Mail:** Bethesda, MD 20892-9827 **FedEx, UPS:** Rockville, MD 20852 **Direct Line:** 240-627-3497 **Fax Line:** 240-627-3115 **Email address:** dioph@niaid.nih.gov	**SENIOR REGULATORY OFFICER** **JULIA GOLDSTEIN, MD** Office of Regulatory Affairs **(ORA)** Division of Allergy, Immunology & Transplantation **(DAIT)** National Institute of Allergy & Infectious Diseases **(NIAID)** National Institutes of Health **(NIH)** 5601 Fishers Lane, Rm 7B29 Mail Stop 9828 **US Mail**: Bethesda, MD 20892-9827 **FedEx, UPS**: Rockville, MD 20852 **Direct Line**: 240-627-3509 **Fax Line**: 301-480-1537 **Email address**: goldsteinj@nih.gov

National Institute of Allergy and Infectious Diseases

CTOT Clinical Trials In Organ Transplantation

INVESTIGATOR SIGNATURE PAGE	
Protocol: CTOT-19	**Version/Date:** **VERSION 9.0/April 27, 2020**
Title: *Randomized Controlled Trial of Infliximab (Remicade®) Induction Therapy For Deceased Donor Kidney Transplant Recipients*	
Study Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)	
INSTRUCTIONS: *The site Principal Investigator should print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent. After signature, please return the original of this form by surface mail to:* **PPD, Inc. 3900 Paramount Parkway Morrisville, NC 27560 Attn: CTOT DAIT Regulatory Management Center**	

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

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

_____

Site Principal Investigator (Print)

_____ _____

Site Principal Investigator (Signature) **Date**

Protocol Synopsis

Title	Randomized Controlled Trial of Infliximab Induction Therapy For Deceased Donor Kidney Transplant Recipients
Short Title	Effects of inhibiting early inflammation in kidney transplant patients
Clinical Phase	Phase II
Number of Sites	15
Health Authority Applications/ Sponsor Number	NIAID/US IND 124633 NIAID/HC CTA File # HC6-24-C185918, Control #185918
Study Objectives	The objective of the study is to determine the efficacy of intravenous infliximab administered at the time of transplantation, prior to reperfusion, on 2-year kidney transplant survival and function.
Study Design	This is a Phase II, Multicenter, randomized, double blind, placebo-controlled, 2-arm clinical trial of 300 deceased donor kidney transplant recipients. Subjects will be randomized 1:1 to the experimental or control arms (150 subjects per arm).
Primary Endpoint(s)	The difference between the mean 24-month eGFR (modified MDRD) in the experimental vs. control arms.
Secondary Endpoint(s)	**Efficacy Endpoints** **1.** Proportion of subjects with biopsy proven acute cellular rejection (BPAR) within **a)** 6 months and **b)** 2 years of transplant **2.** BANFF grades of first Acute Cellular Rejections (ACR) within 6 months of transplant **3.** Proportion of subjects with biopsy proven acute cellular rejection (BPAR) or borderline rejection within **a)** 6 months and **b)** 2 years of transplant **4.** Proportion of subjects with biopsy proven acute antibody mediated rejection (AMR) within 6 months and 2 years of transplant **5.** Proportion of subjects with biopsy proven acute antibody mediated rejection AMR or suspicious for AMR within **a)** 6 months and **b)** 2 years of transplant **6.** BANFF grades of first AMR within 6 months of transplant **7.** Proportion of subjects with BANFF chronicity scores >or equal 2 on 24-month biopsy **8.** Change in BANFF chronicity scores between implantation and 24 month biopsies

9. eGFR (as measured by both MDRD and CKD-EPI)
- **a)** Change in eGFR between 3 months and 24 months
- **b)** Change in eGFR between post-transplant nadir and 24 months
- **c)** eGFR on days 7, 30, 90, and 180 post-transplant

10. Proportion of subjects with:
- **a)** Death or graft failure within 2 years
- **b)** Only graft failure within 2 years

11. Each of the following:
- **a)** Proportion of subjects that required at least one dialysis treatment within the first week after transplantation
- **b)** Number of dialysis sessions in the first 8 weeks post-transplantation.
- **c)** Duration of DGF defined as time from transplantation to the last required dialysis treatment
- **d)** The incidence of primary non-function (PNF), defined as for dialysis-dependency for more than 3 months or an eGFR 20 ml/min or less at 90 days post-transplant
- **e)** Change from baseline (immediately after surgery) in serum creatinine and serum creatinine concentration at 24, 48, and 72 hours.

12. Days from transplantation until event (ACR, AMR, or hospitalization for infection and or malignancy)

13. Rate of Slow Graft Function (SGF):
- **a)** The proportion of patients with a serum creatinine of more than 3 mg/dL at day 5 post-transplant,
- **b)** Creatinine reduction ratio (CRR) on day 2
- **c)** Creatinine reduction ratio (CRR) on day 5
- **d)** The proportion of patients whose day 5 serum CRR was less than 70%
- **e)** The proportion of patients whose day 2 serum CRR was less than 30%
- **f)** Proportion of subjects who need dialysis after 1 week.

Safety/Complication Endpoints

1. Proportion of subjects with:
- **a)** Any infection requiring hospitalization or resulting in death
- **b)** Mycobacterial or fungal infections

2. Proportion of subjects with CMV viremia that require a change in immunosuppression or anti-viral treatment as per standard of care at the site

3. Proportion of subjects with BK viremia that require a change in immunosuppression or anti-viral treatment as per standard of care at the site

4. Proportion of subjects with malignancy

5. Proportion of subjects with impaired wound healing manifested by wound dehiscence, wound infection, or hernia at the site of the transplant incision.

	Mechanistic Endpoints **1.** Sensitivity, specificity, PPV, and NPV of biomarkers, including PRT, urinary CXCL9, blood genomic profile, and 3 month allograft genomic profile (alone and/or in combination) to predict: **a)** Incident biopsy-proven acute rejection. **b)** Graft loss **c)** Chronic graft injury, as measured by 2-year eGFR **2.** Each of the following: **a)** Inflammatory gene expression profiles **b)** Frequency of donor reactive T-cells **c)** Frequency and function of Treg **d)** Proportion of subjects with de novo DSA within 24 months **e)** Fibrogenic gene expression profiles **f)** Amount of peritubular capillary loss by histology **Ancillary Endpoints** **1.** Percentage of predicted prednisone bottle openings as measured by a medication event monitoring system (MEMS®) in the first 3 months post-transplantation **2.** Standard deviation of the monthly tacrolimus trough levels from 6 months post-transplantation to 2 years post-transplantation
Accrual Objective	300 patients will be enrolled and randomized into the two treatment groups (150 Experimental arm, 150 Control Arm) across approximately 10 participating centers.
Study Duration	5 years (3-year accrual period + 2 year follow up period) • 24 months for primary and majority of secondary endpoints • 5 years with Reduced follow-up to assess patient and graft survival and serum creatinine
Treatment Description	There are two arms/groups in this study, the Experimental Group and the Control Group. Each group will receive the following: • **Experimental Group**: rabbit anti-thymocyte globulin (rATG, Thymoglobulin) is co-administered with anti-TNFa (infliximab/Remicade®) followed by maintenance therapy with tacrolimus, either Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA (or their generic equivalents) and prednisone • **Control group:** rabbit anti-thymocyte globulin (rATG, Thymoglobulin) plus placebo (Sterile normal saline) induction followed by maintenance therapy with tacrolimus, either Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA (or their generic equivalents) and prednisone

Inclusion Criteria	**1.** Adult (>18 years of age) male and female recipients (all races and ethnicities) **2.** Subject must be able to understand and provide consent **3.** Recipients of deceased donor kidney transplants (including re-transplants) **4.** Negative crossmatch, actual or virtual, or a PRA of 0% on historic and current sera as determined by each participating study center. **5.** Donor kidneys from deceased donors and donors after cardiac death (DCD) with Kidney Donor Profile Indices (KDPI) ranging from ≥20 to <95 **6.** Female participants of childbearing potential must have a negative pregnancy test upon study entry **7.** Subjects must have a negative test result for latent tuberculosis (TB) infection (PPD, QuantiFERON, ELISPOT) . Subjects who have a negative test result for latent TB infection within 1 year of transplant date are eligible for enrollment and no further action is required. Subjects who have a negative test for latent TB infection that is greater than 1 year old are eligible for enrollment but are required to have a repeat test prior to transplantation. Samples for testing latent TB infection can be obtained during the hospital admission for the transplant but must be collected prior to the initiation of immunosuppression and prior to transplant. The results of this repeat test will determine the next step. • If the test is **negative**, no further action is required • If the test is **positive**, **lost**, **indeterminant** or **unavailable** **the subject must be treated for latent TB infection** (if the subject is enrolled, received study drug/placebo and transplanted). o Treatment for latent TB infection is required for all in this group. o The treatment regimen (including the specific drug, dose and duration of treatment) will be determined by the local Infectious Disease team. • If the subject has **no latent TB testing available**, then the subject **cannot be enrolled** Subjects may be enrolled if they have not had a test for latent TB infection within one year of the transplant if samples for the test are collected prior to the administration of immunosuppressive drugs and prior to transplant. ***Refer to Section 8.1.1 for additional information.***
Exclusion Criteria	**1.** Inability or unwillingness of a participant to give written informed consent or comply with study protocol **2.** Recipients of living donor transplants **3.** Presence of other transplanted solid organs (heart, lung, liver, pancreas, small intestines) or co-transplanted organ **4.** HIV+ recipients **5.** EBV IgG negative recipients

	6. Hepatitis B surface antigen positive kidney transplant recipients **7.** Hepatitis B core antibody positive kidney transplant recipients **8.** Hepatitis B negative kidney transplant recipients that receive transplant from Hepatitis B core antibody positive donor **9.** Hepatitis C virus positive (HCV+) patients who are either untreated or have failed to demonstrate sustained viral remission for more than 12 months after anti-viral treatment **10.** Recipients with a previous history of invasive fungal infection **11.** Recipients with a previous history of active TB disease **12.** Recipients with a positive test for latent TB infection (PPD, QuantiFERON, ELISPOT), regardless of previous therapy. **13.** Any severe infection at the time of transplantation. Severe infection determination will be made by the local site investigator. **14.** Severe congestive heart failure (NYHA functional class III or higher) **15.** Subjects with a known hypersensitivity to any murine/ mouse proteins. **16.** Subjects with any history of receiving any anti-TNF products **17.** Subjects in whom rATG or infliximab might not be tolerated **18.** Subjects with less than 3000/mm3 WBC **19.** Subjects with less than 100,000/mm3 platelets counts **20.** Subjects with systolic blood pressure <100 mm/Hg **21.** Subjects with symptomatic orthostatic hypotension or currently requiring Midodrine for blood pressure support. **22.** Subjects from or who have traveled to endemic areas with a history of active histoplasmosis or with a chest x-ray consistent with previous active histoplasmosis (no serological testing required). Endemic regions determined by site based on local standard of care **23.** Subjects currently or formerly residing in regions of the US that are highly endemic for coccidioidomycosis, and who have a positive serologic test for coccidioidomycosis. Endemic regions determined by site based on local standard of care. * * *(Subjects currently or formerly residing in regions of the US that are highly endemic for coccidioidomycosis, and who have a negative pre-transplant serologic test for coccidioidomycosis are eligible for enrollment only if they receive fluconazole 200 mg/day for the duration of the study. Serologic testing for coccidioidomycosis is required for subjects that currently or formerly resided in regions that are highly endemic for coccidioidomycosis only, per the site's standard of care).* **24.** Recipients are excluded if the local site decides to treat the recipient with fluconazole because of diagnosis or suspicion of fungal infection in the donor. **25.** Subjects that receive IVIG treatment within 3 months of transplant or planned IVIG treatment peri-transplant. **26.** Use of an investigational agent within 4-weeks prior to study entry

Study Stopping Rules	Satisfaction of any of the following stopping rules at any time during the post-transplant (treatment) follow-up will trigger an *ad hoc* DSMB Safety Review. • Any single occurrence of a life-threatening or fatal AE that is possibly, probably, or definitely related to either the investigational agent (infliximab/infliximab placebo) or a study mandated procedure. **<u>Across both treatment arms</u>:** • Incidence of PTLD of 1% or more subjects • Incidence of Adverse Events for tuberculosis active disease of 1% or more subjects • Incidence of invasive fungal infection of 3% or more subjects • Incidence of coccidioidomycosis of 1% or more subjects • Incidence of histoplasmosis of 1% or more subjects • Incidence of death of 10% or more subjects **<u>Within either treatment arm</u>:** • Incidence of infection of any type requiring hospitalization of 40% or more subjects • Incidence of graft loss of 20% or more subjects • Incidence of BPAR (Banff Grade 1 or higher) or AMR based on local read of 25% or more subjects

Study Contacts: Participating Centers

University Hospitals of Cleveland Donald Hricik, MD University Hospitals of Cleveland Division of Nephrology 11100 Euclid Avenue Room 8124 Lakeside Building Cleveland OH 44106-5048 Phone: (216) 844 8060 Fax: (216) 844 5204 Dhricik@Aol.Com	**Mount Sinai School of Medicine** Madhav Menon, MD Icahn School of Medicine at Mount Sinai Recanati Miller Transplant Institute Annenberg Building, Floor 23 1468 Madison Avenue New York, NY 10029 Phone: (212) Fax: (212) 987-0389 Madhav.Menon@Mssm.Edu	**Yale University** Richard Formica, Jr., MD Yale University School of Medicine Department of Medicine Division of Nephrology FMP 107, PO Box 208029 New Haven CT06520-8029 Phone: (203) 785-4184 Fax: (203) 785-7068 Richard.Formica@Yale.Edu
University of Manitoba David Rush, MD, FRCPC, FACP, FASN University of Manitoba Health Sciences Centre, 820 Sherbrook Street, GE421 Winnipeg, MB Canada R3A 1R9 Phone: (204) 787-3437 Fax: (204) 787-3326 Drush@Exchange.Hsc.Mb.Ca	**University of Manitoba** Peter Nickerson, MD University of Manitoba, Health Sciences Centre, Transplant Immunology Laboratory 777 William Avenue Winnipeg, Manitoba, Canada R3A 1R9 Phone: (204) 789-1125 Fax: (204) 783-6780 Peter.Nickerson@Med.Umanitoba.Ca	**University of California, Los Angeles** Suphamai Bunnapradist, MD UCLA 200 UCLA Medical Plaza Los Angeles, CA 90055 Phone: (310) 794-8516 Bunnapradist@Mednet.Ucla.Edu
Cleveland Clinic Emilio Poggio, MD The Cleveland Clinic Glickman Urological Institute 9500 Euclid Ave, Q7-103 Cleveland OH 44195 Phone: (216) 444-5383 Fax: (216) 444-8372 Poggioe@Ccf.Edu	**University of Michigan** Randall Sung, MD University of Michigan 2926D Taubman Center 1500 E. Medical Center Drive Ann Arbor, MI 48109-5331 Phone: (734) 936-8363 Fax: (734) 232-1915 Rssung@Med.Umich.Edu	**Washington University** Tarek Alhamad, MD, MS, FACP, FASN Medical Director, Pancreas Transplant Assistant Professor of Medicine Division of Nephrology Washington University in St. Louis/ Barnes Jewish Hospital 660 S. Euclid Avenue. Cb 8126 St. Louis, Mo 63110 Phone: (314) 362-8259 Fax: (314) 362-2713 Talhamad@Wustl.Edu
Emory University Kenneth Newell, MD 1364 Clifton Road Suite E426 Atlanta, GA 30322 Phone: (404) 727-2489 Fax: (404) 712-2999 KANEWEL@EMORY.EDU	**University of California San Francisco** Flavio Vincenti, MD Professor of Clinical Medicine Transplant Services University of California At San Francisco 505 Parnassus Avenue, Rm #M884 San Francisco, CA 94118-0780 Phone: (415) 353-1322 FLAVIO.VINCENTI@UCSFMEDCTR.ORG	**University of Maryland** Jonathan S. Bromberg, MD, PhD Professor of Surgery & Microbiology and Immunology Vice Chair for Research University of Maryland School of Medicine 22 S. Greene St., S8B06 Baltimore, MD 21201 Office: (410) 328-6430 FAX: (410) 328-0401 JBROMBERG@SOM.UMARYLAND.EDU

University of Alabama Roslyn B. Mannon, MD Professor of Medicine and Surgery University of Alabama At Birmingham 1900 University Boulevard THT 643 Birmingham, Al 35294 Phone: (205) 934-7220 Fax: (205) 975-0102 RMANNON@UABMC.EDU	**Toronto General Hospital** Kathryn Tinckam MD, MMSc, FRCPC, FAST Toronto General Hospital – Peter Munk Building 11th Floor, Room 187 585 University Ave, Toronto, On Canada M5g 2n2 Phone: (416) 340-4800 Ext.8225 Fax Number: (416) 340-3252 KATHRYN.TINCKAM@UHN.CA	**University of Wisconsin** David Foley, MD 600 Highland Ave. H4/766 CSC Madison, Wi 53792 United States Phone: (608) 263-3343 FOLEY@SURGERY.WISC.EDU
Johns Hopkins University Daniel C. Brennan, MD Professor, Medical Director & Co-Director of The Comprehensive Transplant Center 1830 East Monument St., Suite 416 Baltimore, MD 21287 Phone: 410-955-7658 Fax Number: 410-367-2259 dbrenna4@jhmi.edu		

Study Contacts: Core Laboratories

ADMINISTRATIVE CORE Peter Heeger, MD Icahn School of Medicine at Mount Sinai Recanati Miller Transplant Institute Annenberg Building, Floor 23-04, Box 1243 1 Gustave L. Levy Place New York, NY 10029 **Phone:** (212) 241-6324 **Fax:** (212) 987-0389 Peter.heeger@mssm.edu	**CELLULAR/IMMUNOLOGY CORE** Peter Heeger, MD Icahn School of Medicine at Mount Sinai Recanati Miller Transplant Institute Annenberg Building, Floor 23-04, Box 1243 1 Gustave L. Levy Place New York, NY 10029 **Phone:** (212) 241-6324 **Fax:** (212) 987-0389 Peter.heeger@mssm.edu	**HUMORAL ANTIBODY CORE** Peter Nickerson, MD University of Manitoba, Health Sciences Centre, Transplant Immunology Laboratory 777 William Avenue Winnipeg, Manitoba, Canada R3A 1R9 **Phone:** (204) 789-1125 **Fax:** (204) 783-6780 peter.nickerson@med.umanitoba.ca
GENOMICS CORE Barbara Murphy, MD Icahn School of Medicine at Mount Sinai Recanati Miller Transplant Institute Annenberg Building, Floor 23-04, Box 1243 1 Gustave L. Levy Place New York, NY 10029 **Phone:** (212) 241-6324 **Fax:** (212) 987-0389 barbara.murphy@mssm.edu	**HISTOLOGY CORE** Ian Gibson, MD University of Manitoba Canadian Blood Services 777 William Avenue Winnipeg, MB R3E 3R4 Canada **Phone**: (204) 787-1791 **Fax**: (204) 783-6780 igibson@exchange.hsc.mb.ca	**MOLECULAR IMMUNOLOGY CORE** Robert Fairchild, PhD Cleveland Clinic Foundation NB3-59 Lerner Research Institute 9500 Euclid Avenue Cleveland, OH 44195 **Phone:** (216) 444-3146 **Fax**: (216) 444-8372 FAIRCHR@CCF.ORG
GFR CORE Emilio Poggio, MD The Cleveland Clinic Glickman Urological Institute 9500 Euclid Ave, Q7-103 Cleveland OH 44195 **Phone**: (216) 444-5383 **Fax:** (216) 444-8372 poggioe@ccf.edu		

Table of Contents

Glossary of Abbreviations

ACR	Acute Cellular Rejection
AE	Adverse Event
AHG	Anti-human Globulin
ALP	Alkaline Phosphatase
ALT (SGPT)	Alanine Aminotransferase
AMR	Antibody Mediate Rejection
ANCOVA	Analysis at Covariance
Anti -TNFa-mAb	Anti-Tumor Necrosis Factor Alpha-monoclonal antibody
APC	Antigen Presenting Cells
AR	Acute Rejection
AST (SGOT)	Aspartate Aminotransferase
ATG	Anti-Thymocyte Globulin (Thymoglobulin®)
AUC	Area Under the Curve
BPAR	Biopsy Proven Acute Rejection
BID	Twice Daily
BKV	BK Polyoma Virus
BMP7	Bone Morphogenic Protein 7
CBC	Complete Blood Count
CD4, 8, etc.	Cluster of Differentiation 4, 8, etc.
CDC	Complement Dependent Cytotoxicity Assay
CFR	Code of Federal Regulations
CFR	Code of Federal Regulations
CI	Confidence Interval
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration equation
CMH	Cochran-Mantel-Haenszel
CMV	Cytomegalovirus
CNI	Calcineurin Inhibitors
CRF	Case Report Form
CRO	Clinical Research Organization
CRR	Creatinine Reduction Ratio
CS	Corticosteroids
CTCAE	Common Terminology Criteria for Adverse Events
CTOT	Clinical Trials in Organ Transplantation

CV	Cardiovascular Disease
DAIT	Division of Allergy, Immunology, and Transplantation
DGF	Delayed Graft Function
DNA	Deoxyribonucleic Acid
DSA	Donor Specific Antibody
DSMB	Data Safety Monitoring Board
EBV	Epstein Barr Virus
EC	Ethics Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme Linked Immunosorbent Assay
ELISPOT	Enzyme Linked Immunospot
EMT	Epithelial-Mesenchymal Transition
ESRD	End Stage Renal Disease
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GLP	Good Laboratory Practice
H&E	Haematoxylin and Eosin
HLA	Human Leukocyte Antigen
HPLC	High Performance Liquid Chromatography
HTN	Hypertension
ICH	International Conference on Harmonization
IF/TA	Interstitial Fibrosis/Tubular Atrophy
IgG	Immunoglobulin G
IL	Interleukin
IND	Investigational New Drug
INF-γ	Interferon gamma
IoR	Investigator of Record
IR Injury	Ischemia Reperfusion Injury
IRB	Institutional Review Board
ITT	Intent-to-Treat
IV	Intravenous
MDRD	Modification of Diet in Renal Disease

MESF	Molecular Equivalents of Fluorescence
MFI	Mean Fluorescent Intensity
MHC	Major Histocompatibility Complex
MI	Multiple Imputation
MMF	Mycophenolate Mofetil (CellCept®)
MOP	Manual of Procedures
MPA	Mycophenolate Acid (Myfortic®)
mRNA	Messenger RNA
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NR	Not Reported
OI	Opportunistic Infections
OR	Operating Room
PAS	Periodic Acid-Schiff
PBL	Peripheral Blood Lymphocytes
PBMC	Peripheral Blood Mononuclear Cells
PCA	Principal Components Analysis
PCR	Polymerase Chain Reaction
PP Sample	Per Protocol Sample
PHI	Personal Health Identifiers
PI	Principal Investigator
PNF	Primary Non-Function
PRA	Panel Reactive Antibody
PRT	Panel Reactive T-cell
QOD	Every Other Day
qRT-PCR	Quantitative Real Time Polymerase Chain Reaction
RA	Rheumatoid Arthritis
rATG	Rabbit Anti-Thymocyte Globulin (Thymoglobulin®)
RMSE	Root Mean Squared Error
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristics
ROS	Reactive Oxygen Species
RR	Relative Risk
RTE	Recent Thymic Emigrants

SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
sCr	Serum Creatinine
SD	Standard Deviation
SGF	Slow Graft Function
SOE	Schedule of Events
SOP	Standard Operating Procedure
SRTR	Scientific Registry of Transplant Recipient
TAC	Tacrolimus
TB	Tuberculosis
TB	Transplantation Branch
TNFa	Tumor Necrosis Factor Alpha
TNFR: Fc	Tumor Necrosis Factor Receptor: Fc Fusion Protein
UNOS	United Network for Organ Sharing
URI	Upper Respiratory Infection
UTI	Urinary Tract Infection
WBC	White Blood Cell
WHO	World Health Organization

Study Definitions Page

Acute Cellular Rejection	Based on BANFF 2007 Scoring Criteria. Grade 1A or higher. **1. Normal** **2. Borderline changes**: This category is used when no intimal arteritis is present, but there are foci of mild tubulitis (t1, t2, or t3) with minor interstitial infiltration (i0, or i1) or interstitial infiltration (i2, i3) with mild (t1) tubulitis. **3. T-cell mediated rejection** *(may coincide with categories 2 and 5 and 6).* ***Acute T-cell-mediated rejection (Type/Grade):*** **IA** Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of moderate tubulitis (t2) **IB** Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of severe tubulitis (t3) **IIA** Cases with mild-to-moderate intimal arteritis (v1) **IIB** Cases with severe intimal arteritis comprising >25% of the luminal area (v2) **III** Cases with 'transmural' arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation (v3) ***Chronic active T-cell mediated rejection*** 'Chronic allograft arteriopathy' (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima) **4. Interstitial fibrosis and tubular atrophy**, no evidence of any specific etiology (May include nonspecific vascular and glomerular sclerosis, but severity graded by tubulointerstitial features). **Grade I** Mild interstitial fibrosis and tubular atrophy (<25% or cortical area) **Grade II** Moderate interstitial fibrosis and tubular atrophy 26-50% of cortical area) **Grade III** Severe interstitial fibrosis and tubular atrophy/ loss (>50% of cortical area) **5. Other -**Changes not considered to be due to rejection-acute and/or chronic; may include isolated g, cg, or cv lesions and coincide with categories 2, 3, 4, and 5.
Acute Rejection	Based on BANFF 2007/2013 Scoring Criteria. Any episode of rejection that meets the criteria for ACR and/or ABMR.

Antibody Mediated Rejection (ABMR) Based on BANFF 2013 Criteria	**Acute/Active ABMR** (all three features must be present for diagnosis)[1, 2] **1.** Histologic evidence of acute tissue injury, including one or more of the following: • Microvascular Inflammation (g>0[3] and/or ptc>0) • Intimal or transmural arteritis (v>0)[4] • Acute thrombotic microangiopathy, in the absence of any other cause • Acute tubular injury, in the absence of any other apparent cause **2.** Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following: • Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d>0 by IHC on paraffin sections) • At lease moderate microvascular inflammation (Ig + ptc≥2)[5] • Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated[6] **3.** Serologic evidence of donor-specific antibodies (DSAs) (HLA or other antigens) These lesions may be clinically acute, smoldering or subclinical. Biopsies showing two of the three features except those with DSA and C4d without histologic abnormalities potentially related to ABMR or TCMR (C4d staining without evidence of rejection) may be designated as "suspicious" for acute/active ABMR. **Chronic, active ABMR; all three features must be present for diagnosis**[1,7] **1.** Morphologic evidence of chronic tissue injury, including one or more of the following: • Transplant glomerulopathy (TG) (cg>0)[8], if no evidence of chronic thrombotic microangiopathy • Severe peritubular capillary basement membrane multi-layering (requires EM)[9] • Arterial intimal fibrosis of new onset, excluding other causes[10] **2.** Evidence of current/recent antibody interaction with vascular endothelium, including at least one of the following: • Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d>0 by IHC on paraffin sections) • At least moderate microvascular inflammation ([g+ptc]≥2)[5] • Increased expression of gene transcripts in the biopsy tissue indicative of endothelial injury, if thoroughly validated[6] **3.** Serologic evidence of DSAs (HLA or other antigens) **C4d staining without evidence of rejection; all three features must be present for diagnosis**[11] **1.** Linear C4d staining in peritubular capillaries (C4d2 or C4d3 by IF on frozen sections, or C4d>0 by IHC on paraffin sections) **2.** g=0, ptc=0, cg=0 (by light microscopy and by EM if available), v=0; no TMA, no peritubular capillary basement membrane multi-

layering, no acute tubular injury (in the absence of another apparent cause for this)

3. No acute cell-mediated rejection (Banff 97 type 1A or greater) or borderline changes

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cg, Banff chronic glomerulopathy score; EM, electron microscopy; ENDAT, endothelial activation and injury transcript; g, Banff glomerulitis score; GBM, glomerular basement membrane; IF, immunofluorescence; IHC, immunohistochemistry; ptc, peritubular capillary; TCMR, T cell–mediated rejection; v, Banff arteritis score.

1. *For all ABMR diagnoses, it should be specified in the report whether the lesion is C4d-positive (C4d2 or C4d3 by IF on frozen sections; C4d>0 by IHC on paraffin sections) or without evident C4d deposition (C4d0 or C4d1 by IF on frozen sections; C4d0 by IHC on paraffin sections).*
2. *These lesions may be clinically acute, smoldering or subclinical. Biopsies showing two of the three features, except those with DSA and C4d without histologic abnormalities potentially related to ABMR or TCMR (C4d staining without evidence of rejection; see footnote 11, below) may be designated as "suspicious" for acute/active ABMR.*
3. *Recurrent/de novo glomerulonephritis should be excluded.*
4. *It should be noted that these arterial lesions may be indicative of ABMR, TCMR or mixed ABMR/TCMR. "v" lesions are only scored in arteries having a continuous media with two or more smooth muscle layers.*
5. *In the presence of acute TCMR, borderline infiltrates or evidence of infection, ptc $\geq$2 alone is not sufficient to define moderate microvascular inflammation and g must be $\geq$1.*
6. *At present the only validated molecular marker meeting this criterion is ENDAT expression (4), and this has only been validated in a single center (University of Alberta). The use of ENDAT expression at other centers or other test(s) of gene expression within the biopsy as evidence of ABMR must first undergo independent validation as was done for ENDAT expression by Sis et al (4).*
7. *Lesions of chronic, active ABMR can range from primarily active lesions with early TG evident only byEM(cg1a) to those with advanced TG and other chronic changes in addition to active microvascular inflammation. In the absence of evidence of current/recent antibody interaction with the endothelium (those features in the Second Section), the term active should be omitted; in such cases DSA may be present at the time of biopsy or at any previous time post-transplantation.*
8. *Includes GBM duplication by EM only (cg1a) or GBM double contours by light microscopy.*
9. *$\geq$7 layers in one cortical peritubular capillary and $\geq$5 in two additional capillaries (17), avoiding portions cut tangentially.*
10. *While leukocytes within the fibrotic intima favor chronic rejection, these are seen with chronic TCMR as well as chronic ABMR, and are therefore helpful only if there is no history of TCMR. An elastic stain may be helpful as absence of elastic lamellae is more typical of chronic rejection and multiple elastic lamellae are most typical of arteriosclerosis, although these findings are not definitive.*
11. *The clinical significance of these findings may be quite different in grafts exposed to anti-blood-group antibodies (ABO-incompatible allografts), where they do not appear to be injurious to the graft (18,19) and may represent accommodation. However, with anti-HLA antibodies such lesions may progress to chronic ABMR (20) and more outcome data are needed.*

BANFF Chronicity Scores	Change in IFTA (Ci+Ct)≥ 2 between baseline and 24 months post-transplant
Biopsy Proven Acute Rejection (BPAR)	Banff grade of greater than or equal to 1A with or without clinical symptoms.
BK Viremia	The protocol definition of BK Viremia will be based on Center-dependent definition of a viral load resulting in a change of immunosuppression.
Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) **Equation**	GFR = 141 × min (sCr /κ, 1)α × max(sCr /κ, 1)-1.209 × 0.993Age × 1.018 [if female] × 1.159 [if black]
Clinically Indicated Biopsy	Any biopsy performed for a clinically indicated reason as determined by the local investigator
Cold Ischemia Time	Sites will defer to their SOC practices
Congestive Heart Failure	The NYHA functional Class II or higher criteria will be used to determine congestive heart failure for this protocol.
Creatinine Reduction Ratio (CRR)	([Cr1 – CrX] × 100) / Cr1, where Cr1 and CrX represent serum creatinine on day one and day X post-transplant respectively
Cytomegalovirus (CMV) Viremia	Based on Center-dependent definition of a viral load resulting in a change of immunosuppression
Delayed Graft Function (DGF)	Determined locally. The need for at least one dialysis treatment in the first week after transplantation. Duration of DGF is defined from transplantation to the last required dialysis treatment
Estimated Glomerular Filtration Rate (eGFR)	Determined centrally. Will be measured by both the modified MDRD and CKD-EPI equations
Graft Loss/Failure (Other than Primary (Graft) **Non-Function)**	The need for post-transplant dialysis for more than 56 days. Date of dialysis is the day of the 1st dialysis treatment.
Impaired Wound Healing	Manifested by wound dehiscence, wound infection, or hernia at the site of transplant incision
Intent-to-Treat Sample	All randomized subjects who receive the Remicade®/Placebo infusion. This sample will be used for efficacy and safety summaries/analyses.

Invasive Fungal Infection	Defined by the presence of a fungus in blood (determined by fungal blood culture), normally sterile site (other than mucous membrane; determined by culture) or in tissue (determined either by histology or by tissue culture). **Exclusions include:** • Uncomplicated cystitis resulting from Candida or non-Candida yeast • Cutaneous, nail, or mucosal (e.g. thrush) infections resulting from Candida or non-Candida yeast or from cutaneous pathogens such as tinea pedis, trichophyton, epidermopyhton, and microsporum species
Malignancy	Determined locally. Any malignancy including squamous cell carcinoma/skin cancers and PTLD
Modification of Diet in Renal Disease (MDRD) **Equation**	GFR (mL/min/1.73 m2) = 175 × (sCr)-1.154 × (Age)-0.203 × (0.742 if female) × (1.212 if African American)
Per Protocol (PP) **Sample**	All randomized subjects who receive the Remicade®/Placebo infusion who are compliant with their medications and do not have any major protocol deviations.
Primary Non-Function	Dialysis-dependency for more than 3 months or an eGFR 20 ml/min or less at 90 days post-transplant
Protocol Mandated Procedures	Any procedure performed solely for the purpose of this research study (not site-specific standard of care)
Randomized	A subject 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 the two treatment groups.
Screening Sample	All subjects who are enrolled in the study. This sample will be used for disposition summaries.
Serious Infectious Complications	Any infection requiring hospitalization or resulting in death
Slow Graft Function (SGF)	Defined as one of the following: • A serum creatinine of more than 3mg/dL at day 5 post-transplant • Serum creatinine reduction ratio less than 70% at post-operative day 5 • Serum creatinine reduction ratio less than 30% at 48 hours post-transplant **Rate of SGF** is defined as the proportion of subjects with serum creatinine of more than 3mg/dL at Day 5 post-transplant and/or creatinine reduction ratio (CRR) on Day 2 and Day5

Study Therapy Regimen	The investigational therapeutic regimen and all protocol required therapies
Tuberculosis (TB) **Active Disease**	Clinical evidence of active tuberculosis with positive tuberculosis cultures as determined by the clinical site.
Tuberculosis (TB) **Exposure Positivity/Latent TB Infection**	For this protocol, tuberculosis exposure positivity is defined as subjects with a positive test for tuberculosis exposure (PPD, QuantiFERON, ELISPOT). Tuberculosis exposure is not the same as active tuberculosis disease.
Warm Ischemia Time (Recipient)	Time from allograft removal from the ice bucket or perfusion pump in the recipient operating room to vascular unclamping (following completion of anastomoses), and reperfusion
Warm Ischemia Time (Donor)	**a)** Time from extubation to pronouncement of death **b)** Time from pronouncement of death to cross-clamp

1. Study Hypotheses/Objectives

1.1. Hypotheses

Ischemia followed by reperfusion (I-R) of a kidney transplant results in up-regulated TNFa production within the allograft. The I-R induced TNFa plays a crucial role in amplifying allograft inflammation by enhancing activation, expansion and graft infiltration of donor-reactive T cells and B cells (the latter of which also produce donor-reactive antibodies), while simultaneously inhibiting regulatory T cell induction and function. Together with early TNFa-mediated induction of a fibrogenic molecular profile in the graft, these early post-transplant, TNFa-dependent processes have prolonged, detrimental, pro-inflammatory consequences that injure the transplanted organ. This hypothesis predicts that peritransplant TNFa blockade (initiated intra-operatively, prior to graft reperfusion) will have a profound effect by preventing amplification of the downstream pro-inflammatory and pro-fibrogenic responses, thereby reducing costly injury in deceased donor grafts. As a consequence, blocking TNFa at the time of transplantation will improve both short-term and long-term outcomes in recipients of deceased donor kidney transplants.

1.2. Primary Objective

The objective of the study is to determine the efficacy of adding intravenous infliximab administered at the time of transplantation (prior to reperfusion) to rATG and standard 3-drug immunosuppression on 2-year kidney transplant function.

1.3. Secondary Objective(s)

Secondary Objectives
Efficacy Objectives To determine the efficacy of intravenous infliximab administered at the time of transplantation (prior to reperfusion) plus rATG followed by TAC, MMF, and prednisone on **1.** Incidence of biopsy-proven acute cellular rejection **2.** Severity of acute cellular rejection based on Banff criteria **3.** Incidence of biopsy-proven acute cellular rejection or borderline rejection **4.** Incidence of biopsy-proven acute humoral rejection **5.** Incidence of biopsy-proven acute humoral rejection or suspicious for AMR **6.** Severity of acute humoral rejection based on Banff criteria **7.** Incidence of chronic allograft injury **8.** Progression of chronic allograft injury **9.** Change in renal function over the 24 months of the study **10.** Allograft survival rate **11.** Incidence of delayed graft function **12.** Time to events (ACR, AMR, graft failure, or hospitalization for infection and/or malignancy) **13.** Incidence of Slow Graft Function (SGF)

Safety /Complication Objectives

To determine the complication rate of intravenous infliximab administered at the time of transplantation (prior to reperfusion) plus rATG followed by TAC, MMF/MPA, and prednisone as measured by:

1. Incidence of serious infectious complications
2. Incidence of CMV viremia
3. Incidence of BK viremia
4. Incidence of malignancy
5. Incidence of impaired wound healing

Mechanistic objectives

1. To define the utility of previously identified, noninvasive biomarkers as risk assessment, diagnostic, and predictive testing strategies for outcomes (acute rejection, graft loss, and eGFR at 2 years) in higher risk recipients of deceased donor kidney allografts including (alone or in some combination)
   a) Panel of reactive T cell (PRT) assays
   b) Urinary chemokine and urinary PCR assays
   c) Gene expression profiles in peripheral blood
   d) Gene expression profiles in graft tissue

2. To test the effects of induction therapy with ATG plus anti-TNFa mAb on graft inflammation, donor reactive immunity and graft pathology including
   a) Effects of intraoperative anti-TNFa mAb on post-transplant inflammation
   b) Effects of intraoperative anti-TNFa mAb on donor-reactive effector T cells
   c) Effects of intraoperative anti-TNFa mAb on regulatory T cells
   d) Effects of intraoperative anti-TNFa mAb on donor specific antibody
   e) Effects of intraoperative anti-TNFa mAb on fibrogenic gene profiles in the allograft
   f) Effects of intraoperative anti-TNFa mAb on peritubular capillary loss

Ancillary objectives

1. To determine the prevalence of non-adherence to immunosuppressive therapy as measured by a medication event monitoring system (MEMS®) for prednisone in the first 3 months post-transplantation and as measured by the standard deviation of the monthly tacrolimus troughs from 6 months post-transplantation to 2 years post-transplantation in both infliximab and control groups.

2. Background and Scientific Rationale

2.1. Background

Kidney Graft and Patient Survival

Despite impressive reduction in rates of acute kidney graft rejection (AR), the widespread use of calcineurin inhibitors and accompanying immunosuppressive medication, including induction therapy, has had only a modest impact on prolonging transplant survival, particularly in recipients of deceased donor organs. In fact, the average life span of kidney allograft recipients may be decreasing, with death with a functioning graft [50% due to cardiovascular (CV) disease] as the most common cause of graft loss (1, 2). Multiple factors are implicated in post-transplant mortality but the influence of renal dysfunction on CV status is an important suspected etiology. Chronic immune-mediated injury to the graft is common, and post-transplant kidney function is strongly associated with both the incidence and risk of CV death, independent of other known risk factors (3).

Current standard of care immunosuppression for kidney transplantation

Standard of care immunosuppression for deceased donor kidney transplantation in most North American centers, consists of induction therapy with rabbit anti-human thymocyte globulin (rATG or Thymoglobulin®) and triple maintenance therapy with tacrolimus, either mycophenolate mofetil or enteric-coated mycophenolate sodium, and corticosteroids (SRTR Annual Report 2010). rATG is a polyclonal antibody derived from immunization of rabbits with human thymocytes. The final product includes antibodies against CD2, CD3, CD4, CD8, CD11A, CD18, CD25, CD44, CD45, HLA-DR, and HLA class I heavy chains, and is effective in preventing cellular immune responses against a variety of antigenic stimuli (4). In a randomized trial of 72 kidney transplant recipients, rATG was superior to horse anti-thymocyte globulin resulting in lower rates of acute rejection (4% versus 25%) and producing more sustained lymphopenia (5). Two randomized trials have compared the overall effectiveness of rATG to anti-interleukin 2 receptor antibodies in patients deemed to be “high risk” (6, 7). The inclusion criteria for these two studies differed, but are broadly encompassed by those of the current protocol. In each study, ATG proved to be superior to anti-interleukin 2 receptor antibodies in preventing acute rejection, supporting the use of ATG as standard of care for this high-risk population.

In the above-described studies of ATG in high-risk kidney allograft recipients, DGF rates were less than 1%, raising the possibility that polyclonal antibodies may prevent DGF. A randomized study of 58 deceased donor kidney transplant recipients managed with either intraoperative or postoperative rATG found significantly less DGF in the group receiving intraoperative treatment (8). Interestingly, the incidence of DGF was lower for rATG-treated patients in the study by Noel et al (7), although not in the larger study by Brennan et al (6). rATG does not completely mitigate the development of DGF and its role in mollifying IR injury remains unclear, supporting the need to test additional agents. Adhesion molecules, cytokines, chemokines and their receptors contribute to IR injury and their blockade by a polyclonal antibody may mitigate the development of DGF. This theoretical benefit of polyclonal agents forms the basis for their initial administration intra-operatively, prior to revascularization of the allograft.

Ischemia reperfusion injury and kidney transplantation

Increasing evidence implicates inflammatory events related to ischemia and reperfusion (IR) injury occurring immediately after transplantation of deceased donor organs as impacting short- and long-term survival. Delayed graft function, the forme fruste of post-transplant IR injury, results in acute renal failure manifested by post-transplantation oliguria and requires dialytic support. DGF

is associated with increased allograft immunogenicity, elevated risk of acute rejection episodes, and decreased long-term survival (9, 10). The incidence of DGF averages 30% of all deceased donor kidney transplants (9, 10). The development of DGF post-transplant strongly correlates with poorer kidney graft survival and with the subsequent development of chronic allograft nephropathy or IF/TA (11, 12). One study of 126 cadaveric kidney transplant recipients demonstrated that while patients with immediate graft function had a 90% 5-year graft survival, those with DGF for more than 9 days had a 5-year graft survival of 50% (11). A meta-analysis found that patients who experienced DGF had a higher risk of acute rejection [RR 1.38 (95% CI 1.29–1.47), $P < 0.001$] and worse long-term renal function (13). In addition, DGF is associated with prolonged hospitalization, higher transplantation costs and adverse effects on the rehabilitation of transplant recipients (14). Prolonged ischemic times are associated with increased incidence of delayed kidney graft function (10, 15). Longer cold ischemic storage times for kidney grafts are also associated with increased incidence of acute rejection episodes and the development of graft fibrosis and arteriopathy (10, 16, 17). Thus, DGF is both an outcome following kidney transplantation and a predictor for long-term graft function.

Mechanistic studies of IR injury and associated inflammation in animal models and humans (reviewed in (10)) indicate that reperfusion of an ischemic organ, whether or not it causes delayed graft function, induces an intense inflammatory response in the tissue. This inflammation is initiated by the production of reactive oxygen species (ROS) that activate the vascular endothelium of the tissue to express adhesion molecules, produce complement components (18-21), and release a variety of pro-inflammatory cytokines (22, 23), including IL-1, IL-6, TNFa, and pro-inflammatory chemokines and complement. The released pro-inflammatory molecules and cytokines, including TNF a activate the vascular endothelium to mobilize selectins and von Willebrand factor to the luminal membrane and upregulate surface expression of integrin ligands. TNFa also initiates the activation of interstitial dendritic cells and their emigration from tissue sites of inflammation to the peripheral lymphoid organs draining the inflammatory sites where they activate donor antigen-reactive T cells to become effector T cells. Together, these processes provide an immunogenic, pro-inflammatory environment that facilitates the induction and function of adaptive immune responses ultimately resulting in graft failure.

Animal studies performed by multiple groups have shown that blocking TNFa signaling (including studies of subjects with TNFR polymorphisms), among other early pro-inflammatory mediators (e.g. complement activation) can significantly attenuate tissue injury (24-27). While each of these pathways contributes to inflammation, acute injury and graft dysfunction, it is possible to significantly dampen the effects by blocking one pathway. These data show synergistic interactions of the overlapping pro-inflammatory mechanisms, and highlight the concept that inhibiting one relevant mechanism can dampen the entire vicious cycle of inflammation and have important protective consequence. The published findings strongly implicate TNFa as a key driver of early post-transplant inflammation that contributes to late allograft injury and loss. Thus, the overarching goal of the proposed study is to test the efficacy of blocking post-transplant TNFa on early and late outcomes following deceased donor kidney transplantation.

2.2. Rationale for Selection of Investigational Product or Intervention

Following the recognition that TNFa plays a crucial role in the pathogenesis of rheumatoid arthritis (RA), multiple TNFa blocking agents were developed and tested in humans (28). In 2014, five TNF blocking agents are FDA approved for human use: infliximab, a mouse/human chimeric anti- TNFa mAb (administered IV, half-life of 8-10d); adalimumab and golimumab, fully humanized anti-TNF mAbs (administered SC, half-lives 7-20 d); certolizumab, which is a PEGylated anti- TNFa Fab fragment (administered SC half-life 14 d); and etanercept, a TNFR2:Fc fusion protein that binds

TNFa and lymphotoxin family members (administered SC half-life 4 d). Approved indications for TNFa blockade include treatment of RA, Crohn's disease, ankylosing spondylitis, and psoriasis.

Accumulating data indicate strong safety profiles for each of these agents, but chronic TNFa blockade is associated with increased risks for infection including reactivation of tuberculosis (TB) and other invasive fungal infections. There is controversial evidence indicating that chronic TNFa blockade can inhibit wound healing [current recommendations are that patients on chronic TNFa blockade stop the treatment prior to surgery (28, 29)]. TNFa blocking agents have been tested as chronic therapies in large trials to treat heart failure without efficacy, and with some evidence of paradoxical worsening of heart disease (30). These latter results, possibly due to chronic inhibition of anti-inflammation (protective) TNFR2 signaling have resulted in a relative contraindication for chronic TNFa blockade in RA patients with heart failure. Autoimmune hepatitis has been described as an uncommon complication of chronic TNFa blockade (see package insert).

In our proposed studies we will restrict administration of anti- TNFa mAb to a single perioperative dose. We will use infliximab over other TNFa blockers because: **a)** it is FDA approved for use in humans (albeit not in transplant recipients) and it is effective in RA and Crohn's disease; **b)** it is the only agent administered IV such that high levels can be achieved if given in the OR; **c)** its relatively short half-life (8-10 days) so as to block TNFa during and after reperfusion injury for only up to several weeks post-transplant, thereby increasing the likelihood of blocking post-transplant inflammation without significantly increasing long term infectious risk; and d) its cost is affordable.

2.3. Preclinical Experience

Anti- TNFa mAb inhibits post-transplant inflammation and prolongs allograft survival in rodents. Experiments performed in mice by the Fairchild lab [Co-I, member of this consortium] demonstrated that a single dose of anti- TNFa mAb at the time of allograft reperfusion has multiple protective consequences (24). It attenuates many components of the early post-transplant inflammation including production of neutrophil and macrophage chemoattractants and the infiltration of these leukocyte populations as well as donor-reactive memory CD8 T cells into the allograft within the first 48 hours post- transplant. These CD8 memory T cells reactive to donor class I MHC molecules produce IFNy within 24 hours of allograft reperfusion (24) and are important contributors to the establishment of an inflammatory milieu in the allograft that facilitates the infiltration of effector T cells primed in the spleen into the allograft. Peri-transplant administration of a single dose of anti-TNFa antibody also has a profound effect in inhibiting alloreactive CD4 and CD8 T cell priming. Interstitial dendritic cell emigration from tissue inflammatory sites is promoted by TNFα (31, 32)suggesting this as a mechanism accounting for the decreased effector CD4 and CD8 T cell priming in the anti-TNFa treated recipients. TNFa upregulates class I/II MHC (31, 33) so that neutralization in allograft recipients may also decrease the immunogenicity of the graft dendritic cells as well as the antigenicity of the graft itself.

Administration of anti-TNFa antibodies with CyA, anti-CD40L or anti-CD4 mAb prolonged heart and small bowel survival in rodent models that was associated with delayed T cell infiltration into the allografts (33-37). Brouard and colleagues extended these mechanistic findings to show that anti- TNFa mAb administered to MHC mismatched rat heart transplant recipients, at the time of the transplant: a) decreased graft inflammation and extended graft survival and b) strikingly prevented induction of anti-donor MHC IgG antibodies (38). The latter occurred without detectable effects on Th1 or Th2 cytokines suggesting a specific effect on inhibiting T cell help to B cells, a possible mechanism that warrants further testing. TNF blockade with a TNFR:Fc fusion protein was tested with and without cyclosporine in a small number of cynomolgus monkey kidney transplant recipients (39). Acute rejection was delayed from 5 to 11 days with TNF

blockade alone and to ~35 days in animals treated with CYA plus TNF blockade (10 days in CYA alone treated animals). No significant off target effects were noted in this short-term study. Together, the preclinical data support the conclusion that blocking TNFa at the time of the transplant has profound effects on immediate post-transplant inflammation, and on subsequent T and B cell alloimmunity and graft outcome, supporting the need to test the effects of early TNFa blockade in human transplant recipients, as proposed herein.

2.4. Clinical Studies

Studies of human heart transplant biopsies showed elevated TNFa mRNA and protein expression early post-transplant and unrelated to rejection grade or serum levels (40) consistent with the concept that TNFa is produced in the graft as part of the IR injury response. TNFa blocking agents have been tested, to our knowledge, in 2 reports of human solid organ transplant recipients. Kirk and Mannon used chronic therapy with infliximab plus Campath 1H in an attempt to induce kidney transplant tolerance in a small number of patients (41). While the therapy was deemed safe, it was not efficacious in the protocol employed: all patients ultimately developed rejection. The Bogaev group used etanercept as chronic therapy to prevent ventricular hypertrophy in 49 heart transplant recipients (42), again demonstrating safety when administered with other immunosuppressants, and showing some efficacy in preventing ventricular hypertrophy. No wound healing problems were reported. Importantly, none of the studies have attempted to use TNFa blockade as solely an induction therapy to dampen inflammation and its downstream consequences, as we propose herein. Etanercept has also been used in association as induction therapy (given on day 0, 3, 7 and 10), with and without concomitant ATG, in islet transplant recipients with evidence of efficacy and without significant infectious complications (43-45). Finally, the group from Berlin (Schnachtner et al, WTC 2014 Abstract #1365) recently reported their experience combining an anti-TNF antibody with a T cell-depleting antibody in 40 kidney re-transplant recipients who were “sensitized” based on the presence of donor-reactive T cells prior to transplantation. Compared to a control group of 40 “non-sensitized” re-transplant patients treated with conventional immunosuppression, the group treated with anti-TNF actually had significantly better 5-year graft survival (p=0.02). There were no differences in acute rejection and, notably, no differences in septic complications or in rates of CMV, BK, or EBV viremia. Together, the preclinical data, the established safety profile of the class of drugs in general and in small numbers of transplant patients support the protocol in which we will test the additive efficacy of TNFa blockade with infliximab, in addition to rATG induction plus standard 3 drug maintenance immunosuppression for deceased donor kidney transplant recipients.

3. Study Design

3.1. Description of Study Design

This is a phase II, multicenter, randomized, double blind, and placebo-controlled, 2-arm study of 300 deceased donor kidney transplant recipients randomized 1:1 to the experimental and control arms (150 patients per arm). Subjects in the control arm will be treated with rATG plus placebo (sterile normal saline) induction followed by tacrolimus, a mycophenolic acid derivative (either MMF or enteric coated MPA), and corticosteroids. Subjects in the experimental arm will receive infliximab plus rATG induction followed by tacrolimus, a mycophenolic acid derivative (either MMF or enteric coated MPA), and corticosteroids. Please refer to the study therapy regimen below.

Please refer to Section 8-Study Procedures for additional details regarding dosing and administration.

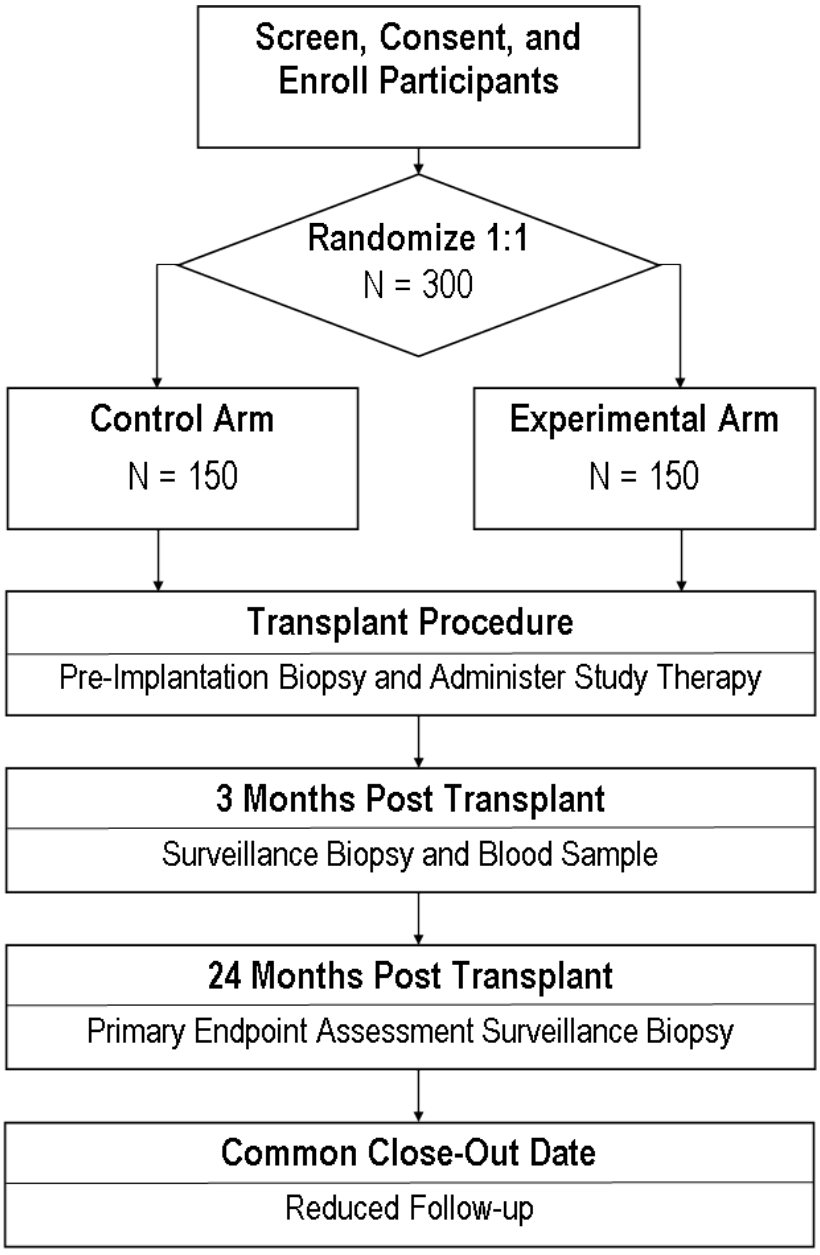
Study Therapy Regimen	
Experimental Arm	• **Remicade®** (Infliximab) • **Thymoglobulin®** (Anti-Thymocyte Globulin Rabbit) • **Tacrolimus** (Prograf®) or one of the following: o Generic equivalent o An equivalent once-daily formulation of Tacrolimus • **Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA** (or their generic equivalents • **Methylprednisolone** or generic equivalent
Control Arm	• **Placebo** (Normal Saline for Injection) • **Thymoglobulin®** (Anti-Thymocyte Globulin Rabbit) • **Tacrolimus** (Prograf®) or one of the following: o Generic equivalent o An equivalent once-daily formulation of Tacrolimus • **Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA** (or their generic equivalents) • **Methylprednisolone** or generic equivalent

Estimated GFR (eGFR) will be calculated using the MDRD formula based on serum creatinine performed centrally at the Cleveland Clinic, Cleveland, OH, GFR core laboratory, (Poggio PI). Pathology analyses of graft tissue will be performed at the Pathology core lab at University of Manitoba at Winnipeg (Gibson, PI). Anti-HLA antibody testing will occur at the core HLA lab at the University of Manitoba at Winnipeg (Nickerson, PI). Mechanistic studies will be performed at 2 additional core laboratories at the Cleveland Clinic, Cleveland OH (Fairchild, PI) and the Icahn School of Medicine at Mount Sinai, NY, NY (Heeger, PI).

Medication Adherence

Non-adherence to immunosuppressive medications is known to be associated with inferior allograft outcomes. Hence, short- and long-term adherence to immunosuppressives will be monitored during the study. During the first 3 months post-transplantation, prednisone adherence will be monitored using a medication event monitoring system (MEMS®). Subjects will be asked to transfer their prednisone to a MEMS® bottle for the 3-month duration after transplant. Long-term tacrolimus adherence will be quantified using the standard deviation of the monthly tacrolimus troughs from 6 months post-transplantation to 2 years post-transplantation.

CTOT-19 Study Flow Diagram



3.2. Primary Endpoint

The primary endpoint is the difference between the mean 24-month eGFR (modified MDRD) in the experimental vs. control groups.

3.3. Secondary Endpoint

The secondary endpoints include efficacy, safety and mechanistic endpoints. See table below.

Secondary Endpoints

Efficacy Endpoints

1. Proportion of subjects with biopsy proven acute cellular rejection (BPAR) within
   - **a)** 6 month and
   - **b)** 2 years of transplant
2. BANFF grades of first Acute Cellular Rejections (ACR) within 6 month of transplant
3. Proportion of subjects with biopsy proven acute cellular rejection (BPAR) or borderline rejection within
   - **a)** 6 month and
   - **b)** 2 years of transplant
4. Proportion of subjects with biopsy proven acute antibody mediated rejection (AMR) within 6 months and 2 years of transplant
5. Proportion of subjects with biopsy proven acute antibody mediated rejection AMR or suspicious for AMR within
   - **a)** 6 months and
   - **b)** 2 years of transplant
6. BANFF grades of first AMR within 6 months of transplant
7. Proportion of subjects with BANFF chronicity scores >or equal 2 on 24-month biopsy
8. Change in BANFF chronicity scores between implantation and 24 month biopsies
9. eGFR (as measured by both MDRD and CKD-EPI)
   - **a)** Change in eGFR between 3 months and 24 months
   - **b)** Change in eGFR between post-transplant nadir (lowest eGFR in first 6 months) and 24 months
   - **c)** eGFR on days 7, 30, 90, and 180 post-transplant
10. Proportion of subjects with:
    - **a)** Death or graft failure within 2 years
    - **b)** Only graft failure within 2 years
11. Each of the following:
    - **a)** Proportion of subjects that required at least one dialysis treatment within the first week after transplantation
    - **b)** Number of dialysis sessions in the first 8 weeks post-transplantation.
    - **c)** Duration of DGF defined as time from transplantation to the last required dialysis treatment
    - **d)** The incidence of primary non-function (PNF), defined as dialysis-dependency for more than 3 months or an eGFR 20 ml/min or less at 90 days post-transplant
    - **e)** Change from baseline (immediately after surgery) in serum creatinine and serum creatinine concentration at 24, 48, and 72 hours.
12. Days from transplantation until event (ACR, AMR, or hospitalization for infection and or malignancy)

13. Rate of Slow Graft Function (SGF):
- **a)** The proportion of patients with a serum creatinine of more than 3 mg/dL at day 5 post-transplant,
- **b)** Creatinine reduction ratio (CRR) on day 2
- **c)** Creatinine reduction ratio (CRR) on day 5
- **d)** The proportion of patients whose day 5 serum CRR was less than 70%
- **e)** The proportion of patients whose day 2 serum CRR was less than 30%
- **f)** Proportion of subjects who need dialysis after 1 week.

Safety/Complication Endpoints

1. Proportion of subjects with:
- **a)** Any infection requiring hospitalization or resulting in death
- **b)** Mycobacterial or fungal infections

2. Proportion of subjects with CMV viremia that require a change in immunosuppression or anti-viral treatment as per standard of care at the site

3. Proportion of subjects with BK viremia that require a change in immunosuppression or anti-viral treatment as per standard of care at the site

4. Proportion of subjects with malignancy

5. Proportion of subjects with impaired wound healing manifested by wound dehiscence, wound infection, or hernia at the site of the transplant incision.

Mechanistic Endpoints

1. Sensitivity, specificity, PPV, and NPV of biomarkers, including PRT, urinary CXCL9, blood genomic profile, and 3-month allograft genomic profile (alone and/or in combination) to predict:
- **a)** Incident biopsy-proven acute rejection.
- **b)** Graft loss
- **c)** Chronic graft injury, as measured by 2-year eGFR

2. Each of the following:
- **a)** Inflammatory gene expression profiles
- **b)** Frequency of donor reactive T-cells
- **c)** Frequency and function of Treg
- **d)** Proportion of subjects with de novo DSA within 24 months
- **e)** Fibrogenic gene expression profiles
- **f)** Amount of peritubular capillary loss by histology

Ancillary Endpoints

1. Percentage of predicted prednisone bottle openings as measured by a medication event monitoring system (MEMS®) in the first 3 months post-transplantation

2. Standard deviation of the monthly tacrolimus trough levels from 6 months post-transplantation to 2 years post-transplantation

3.4. Stratification, Randomization, and Blinding/Masking

Subjects will be randomized 1:1 through a computer program administered by the data coordinating center to either the treatment (infliximab) or control (saline) arm in a blinded fashion and using a standard randomization process. Only the study pharmacist at each center will be unblinded. Randomization will be stratified by enrollment site.

3.4.1. Blinding

This is a double-blinded study; therefore, medication assignments will be unknown to the study participants as well as to the site clinical personnel. Only the site research pharmacist will have access to the unblinded randomization assignment and schedule for that site. In the event that the subject undergoes a life-threatening infusion reaction, then the study subject and the treating physician will be unblinded to treatment assignment. Safety events that do not occur during an infusion of the study drug, but that in the opinion of the investigator cannot be adequately treated without knowledge of the group assignment can also be cause for unblinding. IND safety reports will be reported to the FDA, DSMB, and IRBs in an unblinded fashion.

3.4.2. Unblinding Authorization

Emergency unblinding of a participant for safety purposes is to be handled through the site Investigators, the NIAID medical monitor, and the site pharmacist. Whenever possible the NIAID medical monitor should be notified prior to and involved in the decision to unblind. In all cases, including when the medical monitor is not notified prior to the decision to unblind, prompt notification and reporting is required. Except in the case of a life-threatening infusion reaction, a request to unblind a study subject should be based in the belief that knowledge of the treatment assignment is necessary in order to treat the patient appropriately. In the case of a life-threatening infusion reaction every effort should be made to contact the NIAID medical monitor prior to the unblinding, however ultimately the site Investigator must act in the study subject's best interest.

3.4.3. Reporting Unblinding Events

Any unblinding event will require a full written report. The report should include 1) a brief description of the medical events which led to the decision to unblind, 2) the name of medical monitor who was notified and the date and time of notification, and 3) the justification for unblinding the subject. If the unblinding occurred in a situation other than the infusion of the study drug, the report should indicate how knowledge of the treatment assignment was expected to influence therapeutic decisions. All unblinding information will be reported to the DSMB and will be included in the final study report to the FDA.

During site visits, the site monitor must verify that the DAIT NIAID medical monitor of the trial was notified and that a written account (described above) was completed.

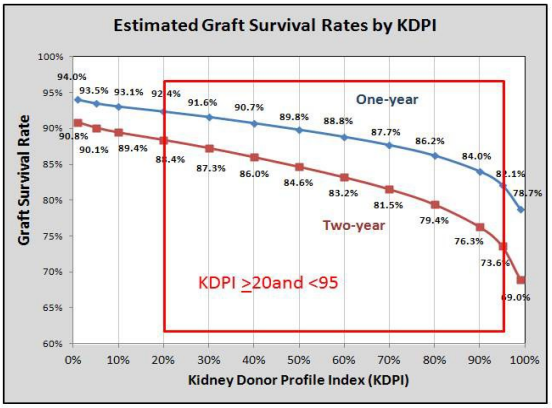
4. Selection of Participants and Clinical Sites/Laboratories

4.1. Rationale for Study Population

Our intention is to recruit patients at moderately high risk for DGF, acute rejection and, poorer long-term graft function and survival. This strategy is based on the notion that an effective intervention in a moderately high-risk population will is more likely to improve outcomes than in low risk patients. On the other hand, limiting the study to very high-risk patients could hamper enrollment and reduce the generalizability of the study's results. Study enrollment will be limited to male and female adult recipients of all races and ethnicities that received HLA mismatched deceased donor kidney-only transplants. The inclusion criteria are based on the Kidney Donor Profile Index. In this new allocation scheme KDPI <20 will be the best kidneys which we want to avoid for this study. By setting KDPI ≥ 20, and by restricting our study to adults, we expect mean KDPI to be 50-60 and average CIT >20hrs. Excluding KDPI >95 will exclude the highest risk kidneys and those most likely to go to oldest (e.g. octogenarians) recipients, a population that differs from the majority of transplants recipients and which we do not feel are appropriate for this study due to a) higher infectious risks from over immunosuppression) and b) are least likely to benefit because of poor kidneys.

KDPI

KDPI variables
- Donor age
- Height
- Weight
- Ethnicity
- Hx HTN
- Hx DM
- Cause of Death
- Serum creatinine
- HCV status
- DCD status



4.2. Inclusion Criteria

Individuals who meet all of the following criteria are eligible for enrollment as study participants:

1. Adult (>18 years of age) male and female recipients (all races and ethnicities)
2. Subject must be able to understand and provide consent
3. Recipients of deceased donor kidney transplants (including re-transplants)
4. Negative crossmatch, actual or virtual, or a PRA of 0% on historic and current sera as determined by each participating study center.
5. Donor kidneys from deceased donors and donors after cardiac death (DCD) with Kidney Donor Profile Indices (KDPI) ranging from ≥20 to <95
6. Female participants of childbearing potential must have a negative pregnancy test upon study entry

7. Patients must have a negative test result for latent tuberculosis (TB) infection (PPD, QuantiFERON, ELISPOT).

   Subjects who have a negative test result for latent TB infection within 1 year of transplant date are eligible for enrollment and no further action is required.

   Subjects who have a negative test for latent TB infection that is greater than 1 year old are eligible for enrollment but are required to have a repeat test prior to transplantation. Samples for testing latent TB infection can be obtained during the hospital admission for the transplant but must be collected prior to the initiation of immunosuppression and prior to transplant. The results of this repeat test will determine the next step.

   - If the test is **negative**, no further action is required
   - If the test is **positive**, **lost**, **indeterminant** or **unavailable** the subject must be treated for latent TB infection (if the subject is enrolled, received study drug/placebo and transplanted).
     - Treatment for latent TB infection is required for all in this group.
     - The treatment regimen (including the specific drug, dose and duration of treatment) will be determined by the local Infectious Disease team.
   - If the subject has **no latent TB testing available**, then the **subject cannot be enrolled**

   Refer to Section 8.1.1 for additional information.

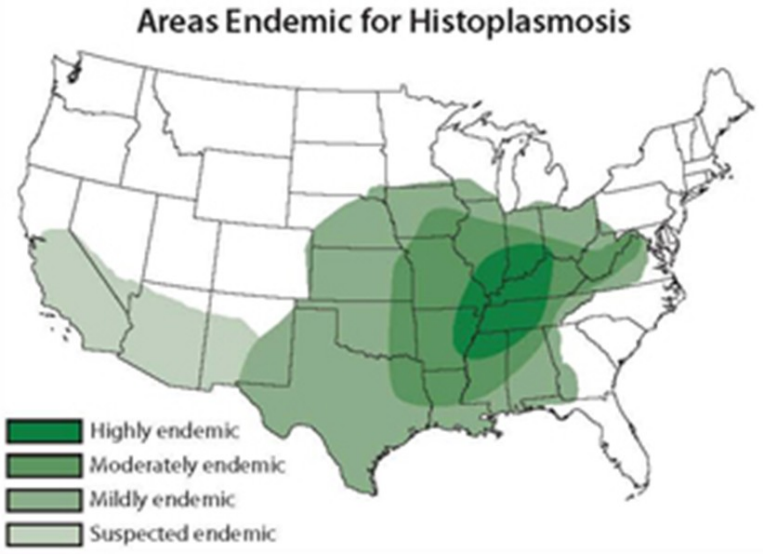
4.3. Exclusion Criteria

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

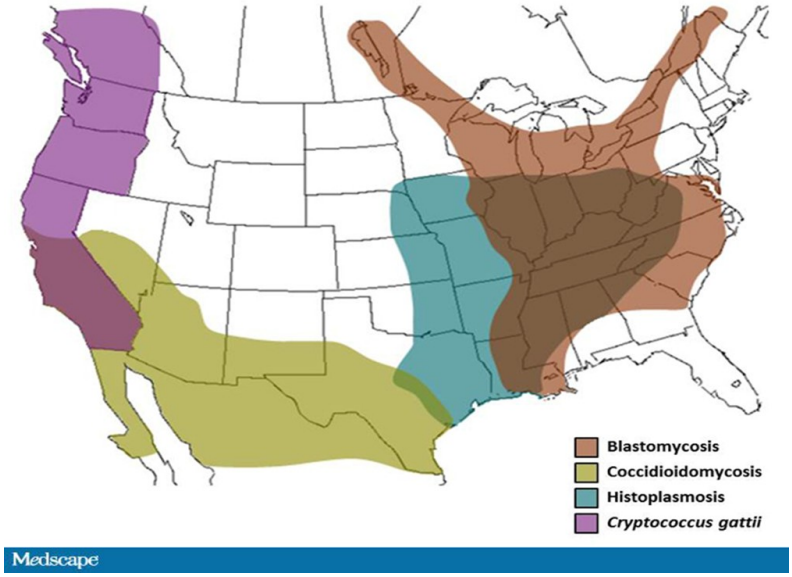
1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol
2. Recipients of living donor transplants
3. Presence of other transplanted solid organs (heart, lung, liver, pancreas, small intestines) or co-transplanted organ
4. HIV+ recipients
5. EBV IgG negative recipients
6. Hepatitis B surface antigen positive kidney transplant recipients
7. Hepatitis B core antibody positive kidney transplant recipients
8. Hepatitis B negative kidney transplant recipients that receive transplants from Hepatitis B core antibody positive donor
9. Hepatitis C Virus positive (HCV+) patients who are either untreated or have failed to demonstrate sustained viral remission for more than 12 months after anti-viral treatment
10. Recipients with a previous history of invasive fungal infection
11. Recipients with a previous history of active TB disease
12. Recipients with a positive test for latent TB infection (PPD, QuantiFERON, ELISPOT), regardless of previous therapy. (Refer to Section 8 for additional information)
13. Any severe infection at the time of transplantation. Severe infection determination will be made by the site investigator
14. Severe congestive heart failure (NYHA functional class III or higher)
15. Subjects with a known hypersensitivity to any murine/ mouse proteins.
16. Subjects with any history of receiving any anti-TNF products

17. Subjects in whom rATG or infliximab might not be tolerated
18. Subjects with less than 3000/mm^3 WBC
19. Subjects with less than 100,000/mm^3 platelets counts
20. Subjects with systolic blood pressure <100 mm/Hg
21. Subjects with symptomatic orthostatic hypotension or currently requiring Midodrine for blood pressure support
22. Subjects from or who have traveled to endemic areas with a history of active histoplasmosis or with a chest x-ray consistent with previous active histoplasmosis (no serological testing required). Endemic regions determined by site based on local standard of care.
23. Subjects currently or formerly residing in regions of the US that are highly endemic for coccidioidomycosis, and who have a positive serologic test for coccidioidomycosis. Endemic regions determined by site based on local standard of care.*
    * *(Subjects currently or formerly residing in regions of the US that are highly endemic for coccidioidomycosis, and who have a negative pre-transplant serologic test for coccidioidomycosis are eligible for enrollment only if they receive fluconazole 200 mg/day for the duration of the study. Serologic testing for coccidioidomycosis is only required for subjects that currently or formerly resided in regions that are highly endemic for coccidioidomycosis, per the site's standard of care.)*
24. *Recipients are excluded if the local site decides to treat the recipient with fluconazole because of diagnosis or suspicion of fungal infection the donor.*
25. *Subjects that receive IVIG treatment within 3 months of transplant or planned IVIG treatment peritransplant.*
26. Use of an investigational agent within 4-weeks prior to study entry

Figure1. Guide to Areas Endemic for Histoplasmosis and Coccidioidomycosis



Areas Endemic for Coccidioidomycosis



4.4. Selection of Clinical Sites/Labs

Patients will be enrolled at 15 sites within our consortium (US and Canada). Thirteen domestic US sites: Barnes/Jewish Hospital/Washington University St Louis Mo, University Hospitals Case Medical Center, Cleveland OH, Cleveland Clinic, Cleveland, OH, Emory University Hospital, Atlanta GA, Mount Sinai Hospital NY, NY, University of California Los Angeles, Los Angeles, CA, University of California San Francisco, San Francisco, CA, University of Michigan Medical Center, Ann Arbor, MI, Yale New Haven Hospital, New Haven, CT, University of Alabama, Birmingham, AL , University of Wisconsin, Madison, WI, the University of Maryland, Baltimore, MD and Johns Hopkins University, Baltimore, MD. University of Manitoba, Winnipeg, in Manitoba, Canada and Toronto General Hospital in Ontario, Canada are the only international sites participating in this protocol. Additional clinical sites may be recruited if enrollment is lower than anticipated.

The core laboratories that will be used in the study are the following: the Cleveland Clinic, Cleveland, OH, GFR core laboratory (Emilio Poggio, PI) will be the serum creatinine core, pathology analyses of graft tissue will be performed at the Pathology core lab at University of Manitoba at Winnipeg (Ian Gibson, PI). Anti-HLA antibody testing will occur at the core HLA lab at the University of Manitoba at Winnipeg (Peter Nickerson, PI). Mechanistic studies will be performed at 2 additional core laboratories at the Cleveland Clinic, Cleveland OH (Robert Fairchild, PI) and the Icahn School of Medicine at Mount Sinai, NY, NY (Peter Heeger, PI).

5. Known and Potential Risks and Benefits to Participants

5.1. Risks of Investigational Product, Remicade® (Infliximab)

According to the package insert, the most common side effects of infliximab (occurring in > 10% of patients) are infections, infusion-related reactions, headache, and abdominal pain. In clinical trials, infections occurred in 36% of patients receiving infliximab versus 25% of patients receiving placebo. The most common infections were upper respiratory tract infections. Rare but serious infections include reactivation of latent tuberculosis, invasive fungal infections, and reactivation of hepatitis B. Approximately 18% of patients experience infusion reactions, including fever and chills (3%) and either chest pain, hypotension or hypertension (1%). Rare anaphylactic reactions have been reported. Other rare but serious side effects include malignancy (including lymphoma and non-melanoma skin cancers), hepatotoxicity, worsening of pre-existing heart failure, cytopenias, demyelination syndrome, and a lupus like syndrome (approximately 20% of patients develop new anti-dsDNA antibodies but symptoms of lupus are rare). Most of the side effects reported above occurred in patients receiving multiple doses of infliximab. Subjects will receive one dose of infliximab in this study. The presence of active infection is a contraindication to administering the drug.

Adverse effects during administration of Remicade® (infliximab) have included flu-like symptoms, headache, dyspnea, hypotension, transient fever, chills, gastrointestinal symptoms, and skin rashes. Anaphylaxis might occur at any time during REMICADE® infusion. Approximately 20% of REMICADE®-treated patients in all clinical trials experienced an infusion reaction compared with 10% of placebo-treated patients. Prior to infusion with REMICADE®, pre-medication will be administered to participants. Pre-medication will include antihistamines (anti-H1 +/- anti-H2) and acetaminophen.

During infusion, mild to moderate infusion reactions may improve following slowing or suspension of the infusion, and upon resolution of the reaction, reinitiating at a lower infusion rate and/or therapeutic administration of antihistamines, acetaminophen, and/or corticosteroids. For patients that do not tolerate the infusion following these interventions, REMICADE® should be discontinued.

During or following infusion, patients who have severe infusion-related hypersensitivity reactions should be discontinued from further REMICADE® treatment. The signs and symptoms of the reaction should dictate the management of severe infusion reactions.

REMICADE® at doses >5 mg/kg should not be administered to participants with moderate to severe heart failure. Participants in this protocol will receive one 3mg/kg dose of REMICADE®. In a randomized study evaluating REMICADE® in patients with moderate to severe heart failure (New York Heart Association [NYHA] Functional Class III/IV), REMICADE® treatment at 10 mg/kg was associated with an increased incidence of death and hospitalization due to worsening heart failure [see product labeling: Warnings and Precautions and Adverse Reactions].

REMICADE® should not be re-administered to participants who have experienced a severe hypersensitivity reaction to REMICADE®. Additionally, REMICADE® should not be administered to participants with known hypersensitivity to inactive components of the product or to any murine proteins.

Serious Infections

Patients treated with REMICADE® are at increased risk for developing serious infections involving various organ systems and sites that may lead to hospitalization or death.
Opportunistic infections due to bacterial, mycobacterial, invasive fungal, viral, or parasitic organisms including aspergillosis, blastomycosis, candidiasis, coccidioidomycosis, histoplasmosis, legionellosis, listeriosis, pneumocystosis and tuberculosis have been reported with TNF-blockers. Patients have frequently presented with disseminated rather than localized disease.

Treatment with REMICADE® should not be initiated in patients with an active infection, including clinically important localized infections. Patients greater than 65 years of age, patients with co-morbid conditions and/or patients taking concomitant immunosuppressive medications such as corticosteroids or methotrexate may be at greater risk of infection.

The risks and benefits of treatment should be considered prior to initiating therapy in patients:

- With chronic or recurrent infection
- Who have been exposed to tuberculosis
- With a history of an opportunistic infection
- Who have resided or traveled in areas of endemic tuberculosis or endemic mycoses, such as histoplasmosis, coccidioidomycosis, or blastomycosis
- With underlying conditions that may predispose them to infection.

Tuberculosis

Cases of reactivation of tuberculosis or new tuberculosis infections have been observed in patients receiving REMICADE®, including patients who have previously received treatment for latent or active tuberculosis. Patients should be evaluated for tuberculosis risk factors and tested for latent infection prior to initiating REMICADE® and periodically during therapy.

Treatment of latent tuberculosis infection prior to therapy with TNF blocking agents has been shown to reduce the risk of tuberculosis reactivation during therapy. Induration of 5 mm or greater with tuberculin skin testing should be considered a positive test result when assessing if treatment for latent tuberculosis is needed prior to initiating REMICADE®, even for patients previously vaccinated with Bacillus Calmette-Guerin (BCG).

Anti-tuberculosis therapy should also be considered prior to initiation of REMICADE® in patients with a past history of latent or active tuberculosis in whom an adequate course of treatment cannot be confirmed, and for patients with a negative test for latent tuberculosis but having risk factors for tuberculosis infection. Consultation with a physician with expertise in the treatment of tuberculosis is recommended to aid in the decision whether initiating anti-tuberculosis therapy is appropriate for an individual patient.

Tuberculosis should be strongly considered in patients who develop a new infection during REMICADE® treatment, especially in patients who have previously or recently traveled to countries with a high prevalence of tuberculosis, or who have had close contact with a person with active tuberculosis.

Monitoring

Patients should be closely monitored for the development of signs and symptoms of infection during and after treatment with REMICADE®, including the development of tuberculosis in patients who tested negative for latent tuberculosis infection prior to initiating therapy. Tests for latent tuberculosis infection may also be falsely negative while on therapy with REMICADE®.

REMICADE® should be discontinued if a patient develops a serious infection or sepsis. A patient who develops a new infection during treatment with REMICADE® should be closely monitored, undergo a prompt and complete diagnostic workup appropriate for an immunocompromised patient, and appropriate antimicrobial therapy should be initiated.

Invasive Fungal Infections

For patients who reside or travel in regions where mycoses are endemic, invasive fungal infection should be suspected if they develop a serious systemic illness. Appropriate empiric antifungal therapy should be considered while a diagnostic workup is being performed. Antigen and antibody testing for histoplasmosis may be negative in some patients with active infection.
When feasible, the decision to administer empiric antifungal therapy in these patients should be made in consultation with a physician with expertise in the diagnosis and treatment of invasive fungal infections and should take into account both the risk for severe fungal infection and the risks of antifungal therapy

Malignancies

Malignancies, some fatal, have been reported among children, adolescents and young adults who received treatment with TNF-blocking agents (initiation of therapy > 18 years of age), including REMICADE®. Approximately half of these cases were lymphomas, including Hodgkin's and non-Hodgkin's lymphoma. The other cases represented a variety of malignancies, including rare malignancies that are usually associated with immunosuppression and malignancies that are not usually observed in children and adolescents. The malignancies occurred after a median of 30 months (range 1 to 84 months) after the first dose of TNF blocker therapy. Most of the patients were receiving concomitant immunosuppressive medications. These cases were reported post-marketing and are derived from a variety of sources, including registries and spontaneous post-marketing reports.

Lymphomas

In the controlled portions of clinical trials of all the TNF-blocking agents, more cases of lymphoma have been observed among patients receiving a TNF blocker compared with control patients. In the controlled and open-label portions of REMICADE® clinical trials, 5 patients developed lymphomas among 5707 patients treated with REMICADE® (median duration of follow-up 1.0 years) vs. 0 lymphomas in 1600 control patients (median duration of follow-up 0.4 years). In rheumatoid arthritis patients, 2 lymphomas were observed for a rate of 0.08 cases per 10 patient-years of follow-up, which is approximately three-fold higher than expected in the general population. In the combined clinical trial population for rheumatoid arthritis, Crohn's disease, psoriatic arthritis, ankylosing spondylitis, ulcerative colitis, and plaque psoriasis, 5 lymphomas were observed for a rate of 0.10 cases per 100 patient-years of follow-up, which is approximately four-fold higher than expected in the general population. Patients with Crohn's disease, rheumatoid arthritis or plaque psoriasis, particularly patients with highly active disease and/or chronic exposure to immunosuppressant therapies, may be at a higher risk (up to several fold) than the general population for the development of lymphoma, even in the absence of TNF-blocking therapy. Cases of acute and chronic leukemia have been reported with post-marketing TNF-blocker use in rheumatoid arthritis and other indications. Even in the absence of TNF blocker therapy, patients with rheumatoid arthritis may be at a higher risk (approximately 2-fold) than the general population for the development of leukemia.

Hepatosplenic T-cell lymphoma (HSTCL)

Post marketing cases of hepatosplenic T-cell lymphoma (HSTCL), a rare type of T-cell lymphoma, have been reported in patients treated with TNF blockers including REMICADE®. These cases have

had a very aggressive disease course and have been fatal. All reported REMICADE® cases have occurred in patients with Crohn's disease or ulcerative colitis and the majority were in adolescent and young adult males. All of these patients had received treatment with the immunosuppressants azathioprine or 6-mercaptopurine concomitantly with REMICADE® at or prior to diagnosis. It is uncertain whether the occurrence of HSTCL is related to REMICADE® or REMICADE® in combination with these other immunosuppressive medications. When treating patients with inflammatory bowel disease, particularly in adolescents and young adults, consideration of whether to use REMICADE® alone or in combination with other immunosuppressive medications should take into account a possibility that there is a higher risk of HSTCL with combination therapy versus an observed increased risk of immunogenicity and hypersensitivity reactions with REMICADE® monotherapy from the clinical trial.

Skin cancer
Melanoma and Merkel cell carcinoma have been reported in patients treated with TNF blocker therapy, including REMICADE® [see Adverse Reactions (6.2)]. Periodic skin examination is recommended for all patients, particularly those with risk factors for skin cancer.
Other Malignancies

In the controlled portions of clinical trials of some TNF-blocking agents including REMICADE®, more malignancies (excluding lymphoma and non-melanoma skin cancer [NMSC]) have been observed in patients receiving those TNF-blockers compared with control patients. During the controlled portions of REMICADE® trials in patients with moderately to severely active rheumatoid arthritis, Crohn's disease, psoriatic arthritis, ankylosing spondylitis, ulcerative colitis, and plaque psoriasis, 14 patients were diagnosed with malignancies (excluding lymphoma and NMSC) among 4019 REMICADE®-treated patients vs. 1 among 1597 control patients (at a rate of 0.52/100 patient-years among REMICADE®-treated patients vs. a rate of 0.11/100 patient-years among control patients), with median duration of follow-up 0.5 years for REMICADE®-treated patients and 0.4 years for control patients. Of these, the most common malignancies were breast, colorectal, and melanoma. The rate of malignancies among REMICADE®-treated patients was similar to that expected in the general population whereas the rate in control patients was lower than expected.

In a clinical trial exploring the use of REMICADE® in patients with moderate to severe chronic obstructive pulmonary disease (COPD), more malignancies, the majority of lung or head and neck origin, were reported in REMICADE®-treated patients compared with control patients. All patients had a history of heavy smoking [see Adverse Reactions (6.1)]. Prescribers should exercise caution when considering the use of REMICADE® in patients with moderate to severe COPD.

Psoriasis patients should be monitored for non-melanoma skin cancers (NMSCs), particularly those patients who have had prior prolonged phototherapy treatment. In the maintenance portion of clinical trials for REMICADE®, NMSCs were more common in patients with previous phototherapy. The potential role of TNF-blocking therapy in the development of malignancies is not known [see Adverse Reactions (6.1)]. Rates in clinical trials for REMICADE® cannot be compared to rates in clinical trials of other TNF-blockers and may not predict rates observed in a broader patient population. Caution should be exercised in considering REMICADE® treatment in patients with a history of malignancy or in continuing treatment in patients who develop malignancy while receiving REMICADE®.

Hepatitis B Virus Reactivation

Use of TNF blockers, including REMICADE®, has been associated with reactivation of hepatitis B virus (HBV) in patients who are chronic carriers of this virus. In some instances, HBV reactivation occurring in conjunction with TNF blocker therapy has been fatal. The majority of these reports have occurred in patients concomitantly receiving other medications that suppress the immune system, which may also contribute to HBV reactivation. Patients should be tested for HBV infection before initiating TNF blocker therapy, including REMICADE®. For patients who test positive for hepatitis B surface antigen, consultation with a physician with expertise in the treatment of hepatitis B is recommended. Adequate data are not available on the safety or efficacy of treating patients who are carriers of HBV with anti-viral therapy in conjunction with TNF blocker therapy to prevent HBV reactivation. Patients who are carriers of HBV and require treatment with TNF blockers should be closely monitored for clinical and laboratory signs of active HBV infection throughout therapy and for several months following termination of therapy. In patients who develop HBV reactivation, TNF blockers should be stopped and antiviral therapy with appropriate supportive treatment should be initiated. The safety of resuming TNF blocker therapy after HBV reactivation is controlled is not known. Therefore, prescribers should exercise caution when considering resumption of TNF blocker therapy in this situation and monitor patients closely.

Hepatotoxicity

Severe hepatic reactions, including acute liver failure, jaundice, hepatitis and cholestasis, have been reported rarely in post-marketing data in patients receiving REMICADE®. Autoimmune hepatitis has been diagnosed in some of these cases. Severe hepatic reactions occurred between 2 weeks to more than 1 year after initiation of REMICADE®; elevations in hepatic aminotransferase levels were not noted prior to discovery of the liver injury in many of these cases. Some of these cases were fatal or necessitated liver transplantation. Patients with symptoms or signs of liver dysfunction should be evaluated for evidence of liver injury. If jaundice and/or marked liver enzyme elevations (e.g., ≥ 5 times the upper limit of normal) develop, REMICADE® should be discontinued, and a thorough investigation of the abnormality should be undertaken. In clinical trials, mild or moderate elevations of ALT and AST have been observed in patients receiving REMICADE® without progression to severe hepatic injury.

Patients with Heart Failure

REMICADE® has been associated with adverse outcomes in patients with heart failure, and should be used in patients with heart failure only after consideration of other treatment options. The results of a randomized study evaluating the use of REMICADE® in patients with heart failure (NYHA Functional Class III/IV) suggested higher mortality in patients who received 10 mg/kg REMICADE®, and higher rates of cardiovascular adverse events at doses of 5 mg/kg and 10 mg/kg. There have been post-marketing reports of worsening heart failure, with and without identifiable precipitating factors, in patients taking REMICADE®. There have also been rare post-marketing reports of new onset heart failure, including heart failure in patients without known pre-existing cardiovascular disease. Some of these patients have been under 50 years of age. If a decision is made to administer REMICADE® to patients with heart failure, they should be closely monitored during therapy, and REMICADE® should be discontinued if new or worsening symptoms of heart failure appear.

Hematologic Reactions

Cases of leukopenia, neutropenia, thrombocytopenia, and pancytopenia, some with a fatal outcome, have been reported in patients receiving REMICADE®. The causal relationship to REMICADE® therapy remains unclear. Although no high-risk group(s) has been identified, caution

should be exercised in patients being treated with REMICADE® who have ongoing or a history of significant hematologic abnormalities. All patients should be advised to seek immediate medical attention if they develop signs and symptoms suggestive of blood dyscrasias or infection (e.g., persistent fever) while on REMICADE®. Discontinuation of REMICADE® therapy should be considered in patients who develop significant hematologic abnormalities.

Hypersensitivity

REMICADE® has been associated with hypersensitivity reactions that vary in their time of onset and required hospitalization in some cases. Most hypersensitivity reactions, which include urticaria, dyspnea, and/or hypotension, have occurred during or within 2 hours of REMICADE® infusion. However, in some cases, serum sickness-like reactions have been observed in patients after initial REMICADE® therapy (i.e., as early as after the second dose), and when REMICADE® therapy was reinstituted following an extended period without REMICADE® treatment. Symptoms associated with these reactions include fever, rash, headache, sore throat, myalgias, polyarthralgias, hand and facial edema and/or dysphagia. These reactions were associated with a marked increase in antibodies to infliximab, loss of detectable serum concentrations of infliximab, and possible loss of drug efficacy.

REMICADE® should be discontinued for severe hypersensitivity reactions. Medications for the treatment of hypersensitivity reactions (e.g., acetaminophen, antihistamines, corticosteroids and/or epinephrine) should be available for immediate use in the event of a reaction.
In rheumatoid arthritis, Crohn's disease and psoriasis clinical trials, re-administration of REMICADE® after a period of no treatment resulted in a higher incidence of infusion reactions relative to regular maintenance treatment. In general, the benefit-risk of re-administration of REMICADE® after a period of no-treatment, especially as a re-induction regimen given at weeks 0, 2 and 6, should be carefully considered. In the case where REMICADE® maintenance therapy for psoriasis is interrupted, REMICADE® should be reinitiated as a single dose followed by maintenance therapy.

Neurologic Reactions

REMICADE® and other agents that inhibit TNF have been associated in rare cases with CNS manifestation of systemic vasculitis, seizure and new onset or exacerbation of clinical symptoms and/or radiographic evidence of central nervous system demyelinating disorders, including multiple sclerosis and optic neuritis, and peripheral demyelinating disorders, including Guillain-Barre syndrome. Prescribers should exercise caution in considering the use of REMICADE® in patients with these neurologic disorders and should consider discontinuation of REMICADE® if these disorders develop.

5.2. Risks of Investigational Product or Intervention cited in Medical Literature

Use of infliximab has been associated with impaired wound healing. There are also rare reports of autoimmune hepatitis.

5.3. Risks of Other Protocol Specified Medications

5.3.1. Risks of Thymoglobulin

Thymoglobulin is indicated for the treatment of renal transplant acute rejection in conjunction with concomitant immunosuppression.

Immune-mediated reactions

Serious immune-mediated reactions have been reported with the use of Thymoglobulin and consist of anaphylaxis or severe cytokine release syndrome (CRS). Fatal anaphylaxis has been reported. If an anaphylactic reaction occurs, the infusion should be terminated immediately. Medical personnel should be available to treat patients who experience anaphylaxis. Emergency treatment such as 0.3 mL to 0.5 mL aqueous epinephrine (1:1000 dilution) subcutaneously and other resuscitative measures including oxygen, intravenous fluids, antihistamines, corticosteroids, vasoactive amines, and airway management, as clinically indicated, should be provided. Any further administration of Thymoglobulin to a patient who has a history of anaphylaxis to Thymoglobulin is not recommended.

Severe, acute infusion-associated reactions (IARs) are consistent with CRS, which is attributed to the release of cytokines by activated monocytes and lymphocytes. Severe acute CRS can cause serious cardiorespiratory events and/or death.

Infection

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

Hematologic Effects

Thrombocytopenia and/or leukopenia (including lymphopenia and neutropenia) have been identified and are reversible following dose adjustments.

Malignancy

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

5.3.2. Risks of Maintenance Immunosuppression Medications

Administration of all immunosuppressive and immunomodulatory therapies used presently to prevent rejection of transplanted tissues carry general risks of opportunistic infection and malignancy, including lymphoma (~1%), and skin cancers. These agents are not recommended for nursing mothers, and it is recommended (and mandated in the current protocol) that women of childbearing potential (WOCBP) use effective contraception before, during and for at least 4 months following administration of these agents.

5.3.3. Risks of Methylprednisolone and Prednisone

Adverse effects of corticosteroid therapy associated with short-term therapy have included sodium retention-related weight gain and fluid accumulation, hyperglycemia and glucose intolerance, hypokalemia, gastrointestinal upset and ulceration, reversible depression of the hypothalamic-pituitary-adrenal (HPA) axis, and mood changes ranging from mild euphoria and insomnia to nervousness, restlessness, mania, catatonia, depression, delusions, hallucinations, and violent behavior. Long-term use of prednisone may be associated with hypertension, hyperlipidemia, weight gain with Cushingoid features, osteopenia, and skin changes including acne and easy bruisability.

5.3.4. Risks of Mycophenolic Acid Derivative (either Mycophenolate Mofetil/MMF or Enteric-coated Mycophenolic Acid/MPA)

Mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium are approved (in combination with cyclosporine and corticosteroids) as immunosuppressive agents for renal,

cardiac, and hepatic solid organ transplantation. Adverse events reported in > 30% of renal, cardiac or liver transplant patients receiving MMF were pain, fever, headache, asthenia, anemia, leukopenia, thrombocytopenia, leukocytosis, urinary tract infection, hypertension, hypotension, peripheral edema, hypercholesterolemia, hypokalemia, hyperglycemia, increased creatinine and BUN, cough, hypomagnesaemia, diarrhea, constipation, nausea, vomiting, respiratory infection, dyspnea, lung disorder, pleural effusion, tremor and insomnia. The side effect profile of enteric-coated mycophenolate sodium is similar to those of MMF.

There is an increased risk of developing lymphomas and other malignancies, particularly of the skin. Lymphoproliferative disease or lymphoma developed in 0.4% to 1% of patients receiving MMF 1 - 1.5 mg BID. Severe neutropenia developed in up to 2% of renal transplant recipients receiving MMF 1.5 mg BID. MMF can cause fetal harm (including spontaneous abortion in the first trimester and can cause congenital malformations in the offspring of women who are treated during pregnancy) when administered to a pregnant woman. Cases of progressive multifocal leukoencephalopathy (PML), sometimes fatal and pure red cell aplasia have been reported in patients treated with MMF. Gastrointestinal bleeding (requiring hospitalization) has been observed in approximately 3% of renal, in 1.7% of cardiac, and in 5.4% of hepatic transplant patients treated with MMF 1.5 g BID. Additional information about MMF can be found in the package insert.

5.3.5. Risks of Tacrolimus

Side effects of tacrolimus include hypertension, glucose intolerance, peripheral neuropathy, renal insufficiency, abnormal liver function studies, seizures, nausea, vomiting, confusion, hypomagnesaemia, tremulousness, neurotoxicity, posterior reversible encephalopathy syndrome (PRES), progressive multifocal leukoencephalopathy (PML), interstitial lung disease, BK nephropathy, and increased risk of secondary malignancies. Additional information about tacrolimus can be found in the package insert.

5.3.6. Risk of Prophylactic INH Therapy for Latent TB Infection

Subjects with a positive test for Latent TB Infection should receive prophylactic therapy.
Hepatotoxicity has been seen in 10-20% of patients that receive INH therapy. Liver function tests should be monitored to determine the need for reduction or cessation of the prescribed dose.
Allergic reactions such as skin rash can occur but are uncommon. Alcohol should be avoided during treatment with INH.

5.4. Risks of Study Procedures

5.4.1. Risks of Blood Draw (Venipuncture)

The amount of blood that may be drawn from adult subjects for research purposes will not exceed 10.5mL/kg or 550 mL; whichever is smaller, over an eight-week period. All blood samples for the mechanistic study will be obtained at the time of scheduled blood draws, so there will be minimal additional risk associated with obtaining the study samples.
The subject may experience some discomfort at the site of the needle entry, bruising, swelling, redness, fainting, or local infection. The additional amount of blood could contribute to the development of anemia. The subject's clinical condition will be taken into consideration to determine if research blood tests can be performed

5.4.2. Risk of Protocol-Directed Biopsy

A renal transplant biopsy will be performed for this protocol according to the following schedule:

Protocol-Directed Biopsies	
1. Pre-implantation biopsy	Day of Transplant/**Visit 01**
2. 3 month surveillance biopsy	Month 3 post-transplant/ **Visit 10**
3. 24 month biopsy	Month 24 post-transplant/**Visit 14**

There is a risk of bleeding associated with percutaneous transplant kidney biopsies. Transient hematuria occurs in 3 to 10% of patients and may prolong hospitalization, require bladder catheterization for clot drainage, or in approximately 1% of patients, require blood transfusion. Ureteral obstruction from blood clot may require percutaneous nephrostomy in < 1% of patients. Massive hemorrhage requiring surgical exploration, transplant nephrectomy, or arterial embolization occurs in ~0.1 % of patients. Death from massive hemorrhage is rare.

5.5. Potential Benefits

This study may show that infliximab, in combination with rATG, results in a decreased incidence and severity of ischemia reperfusion injury and delayed graft function, a decreased incidence of acute rejection, higher glomerular filtration rates, and increased graft survival rate compared to patients receiving placebo. The mechanistic studies may delineate the immune mechanisms underlying these potentially beneficial effects of infliximab, but will also add to a more general understanding of biomarkers and their role in predicting outcomes in kidney transplant recipients. The results of this study could influence future care of transplant patients. Participants in this study may benefit from the close follow-up dictated by the study protocol.

6. Investigational Agents:

The investigational agents for this trial are Remicade® (infliximab) and placebo (0.9% Sterile Normal Saline). Details describing the product labeling, supply, storage, monitoring and accountability are outlined in the Investigational Pharmacy Manual.

6.1. Investigational Agent: Remicade® (Infliximab)

The investigational agent that will be used in this study is Remicade® (Infliximab). Remicade® (infliximab) is manufactured by Janssen Biotech, Inc. Janssen Biotech, Inc. is part of the Janssen Pharmaceutical Companies of Johnson & Johnson. Remicade® is a drug approved by both FDA and Health Canada. Please refer to the product labeling for known and potential risks to human participants. The study therapy regimen for each arm is outlined in the table below.

Table1. Study Therapy Regimen

Experimental Arm	• **Remicade®** (Infliximab) • **Thymoglobulin®** (Anti-Thymocyte Globulin Rabbit) • **Tacrolimus** (Prograf®) or one of the following: o Generic equivalent o An equivalent once-daily formulation of Tacrolimus • **Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA** (or their generic equivalents) • **Methylprednisolone** or generic equivalent
Control Arm	• **Placebo** (Normal Saline for Injection) • **Thymoglobulin®** (Anti-Thymocyte Globulin Rabbit) • **Tacrolimus** (Prograf®) or one of the following: o Generic equivalent o An equivalent once-daily formulation of Tacrolimus • **Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA** (or their generic equivalents) • **Methylprednisolone** or generic equivalent

Please refer to section 8.0 Study Procedures for additional dosing and administration details.

6.1.1. Remicade® (Infliximab) Indications and Usage

Remicade® (infliximab) is a tumor necrosis factor (TNF) blocker indicated for treatment of Crohn's Disease, ulcerative colitis, rheumatoid arthritis in combination with methotrexate, ankylosing spondylitis, psoriatic arthritis, and plaque psoriasis. Use of Remicade® (infliximab) in the setting of solid organ transplantation as a TNF blocker has not been established, and thus is not approved by Health Authorities for use for this indication.

6.1.2. Remicade® (Infliximab) Formulation, Packaging, Labeling and Storage

Infliximab, the active ingredient in REMICADE®, is a chimeric IgG1κ monoclonal antibody (composed of human constant and murine variable regions) specific for human tumor necrosis factor-alpha (TNFα). It has a molecular weight of approximately 149.1 kilodaltons. Infliximab is produced by a recombinant cell line cultured by continuous perfusion and is purified by a series of steps that includes measures to inactivate and remove viruses.

Remicade® is supplied as a 100mg vial (100mg lyophilized infliximab in a 20mL vial for injection). Remicade® is supplied as a sterile, white, lyophilized powder for intravenous infusion. Following reconstitution with 10 mL of Sterile Water for Injection, USP, and the resulting pH is approximately 7.2. Each single-use vial contains 100 mg infliximab, 500 mg sucrose, 0.5 mg polysorbate 80, 2.2

mg monobasic sodium phosphate, monohydrate, and 6.1 mg dibasic sodium phosphate, dihydrate. No preservatives are present.

Each Remicade® 20mL vial is individually packaged in a carton. Remicade® is supplied in an accumulator carton containing 10 vials. Each single dose vial contains 100mg of infliximab for final concentration volume of 10mL.

Remicade® will be purchased using funds from the Investigator grant (U01-AI063594) and distributed by EMINENT Services Corporation (Frederick, MD).

Storage and Stability

REMICADE® must be refrigerated at 2° C to 8° C (36° F to 46° F). Do not use REMICADE® beyond the expiration date (Exp) located on the carton and the vial. This product contains no preservative.

6.1.3. Dosage, Preparation and Administration

Dosage and Administration

Remicade® (infliximab)/placebo will be given as a single 3mg/kg intravenous pre-perfusion dose, beginning after initiation of the corticosteroid infusion. Actual body weight will be used to calculate the dose. **Remicade®/placebo must be given through a dedicated intravenous line. Do not infuse Remicade® concomitantly with another IV solution or IV medication.**

- If the subject does not experience an adverse event during the first 30 minutes of the infusion, then Thymoglobulin® infusion will be initiated through a separate line from the Remicade® infusion.
- The Remicade®/placebo infusion should be administered over a period of greater than 2 hours and may continue beyond reperfusion (unclamping of the arterial anastomosis).
- Whenever possible, Remicade®/Placebo and rabbit ATG should be administered through two separate IV lines.
- **If only a single IV line with a single port is available**, the rabbit ATG infusion should begin only after the Remicade®/Placebo infusion has stopped and only after the line has been flushed with saline.
- **If the only available venous access is a multiport central venous catheter**, rabbit ATG can be started 30 minutes after initiation of Remicade®/Placebo through a separate port, but **only if that catheter has multiple exit ports**.

Pre-medications may attenuate infusion-related events. See the list of pre-medications in the table below. Please refer to the Investigational Pharmacy Manual for additional Remicade®/Placebo dosage, preparation, administration and storage details.

Participants will receive a standard of care dose of corticosteroids prior to transplantation.

Table 2. Pre-Medications-Experimental Arm

Pre-Medications-Experimental Arm
Acetaminophen
Antihistamines: Claritin (Loratadine) **or** Benadryl (Diphenhydramine)
Methylprednisolone or its Generic Equivalent

Please refer to Section 8 Study Procedures for additional dosing and administration details.

Preparation
Remicade® should be prepared by the site pharmacist using appropriate aseptic techniques. Discard any unused portion left in the vial. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

Remicade® is intended for use under the guidance and supervision of a physician. The reconstituted infusion solution should be prepared by a trained medical professional using aseptic technique by the following procedure:

1. Calculate the dose, total volume of reconstituted REMICADE® solution required and the number of REMICADE® vials needed. Each REMICADE® vial contains 100 mg of the infliximab antibody.
2. Reconstitute each REMICADE® vial with 10 mL of Sterile Water for Injection, USP, using a syringe equipped with a 21-gauge or smaller needle as follows: Remove the flip-top from the vial and wipe the top with an alcohol swab. Insert the syringe needle into the vial through the center of the rubber stopper and direct the stream of Sterile Water for Injection, USP, to the glass wall of the vial. Gently swirl the solution by rotating the vial to dissolve the lyophilized powder. Avoid prolonged or vigorous agitation. DO NOT SHAKE. Foaming of the solution on reconstitution is not unusual. Allow the reconstituted solution to stand for 5 minutes. The solution should be colorless to light yellow and opalescent, and the solution may develop a few translucent particles, as infliximab is a protein. Do not use if the lyophilized cake has not fully dissolved or if opaque particles, discoloration, or other foreign particles are present.
3. Dilute the total volume of the reconstituted REMICADE® solution dose to 250 mL with sterile 0.9% Sodium Chloride Injection, USP, by withdrawing a volume equal to the volume of reconstituted REMICADE® from the 0.9% Sodium Chloride Injection, USP, 250 mL bottle or bag. Slowly add the total volume of reconstituted REMICADE® solution to the 250 mL infusion bottle or bag. Gently mix. The resulting infusion concentration should range between 0.4 mg/mL and 4 mg/mL. NS. ***In order to maintain the blinding of the study treatment, amber bag/bottle covers will be used to administer both infliximab and placebo.*** *See the pharmacy manual for additional details.*
4. The REMICADE® infusion should begin within 3 hours of reconstitution and dilution. The infusion must be administered over a period of not less than 2 hours and must use an infusion set with an in-line, sterile, non-pyrogenic, low-protein-binding filter (pore size of 1.2 µm or less). The vials do not contain antibacterial preservatives. Therefore, any unused portion of the infusion solution should not be stored for reuse.
5. No physical biochemical compatibility studies have been conducted to evaluate the co-administration of REMICADE® with other agents. REMICADE® should not be infused concomitantly in the same intravenous line with other agents.
6. Parenteral drug products should be inspected visually before and after reconstitution for particulate matter and discoloration prior to administration, whenever solution and container permit. If visibly opaque particles, discoloration or other foreign particulates are observed, the solution should not be used.

6.1.4. Contraindications

REMICADE® at doses >5 mg/kg should not be administered to patients with moderate to severe heart failure. In a randomized study evaluating REMICADE® in patients with moderate to severe heart failure (New York Heart Association [NYHA] Functional Class III/IV), REMICADE® treatment at 10 mg/kg was associated with an increased incidence of death and hospitalization due to worsening heart failure [Refer to the product labeling].

REMICADE® should not be re-administered to patients who have experienced a severe hypersensitivity reaction to REMICADE®. Additionally, REMICADE® should not be administered to patients with known hypersensitivity to inactive components of the product or to any murine proteins.

6.1.5. Drug Interactions

Use with Anakinra or Abatacept

An increased risk of serious infections was seen in clinical studies of other TNFα-blocking agents used in combination with anakinra or abatacept, with no added clinical benefit. Because of the nature of the adverse reactions seen with these combinations with TNF-blocker therapy, similar toxicities may also result from the combination of anakinra or abatacept with other TNFα-blocking agents. Therefore, the combination of REMICADE® and anakinra or abatacept is not recommended [Refer to the product labeling].

Use with Tocilizumab

The use of tocilizumab in combination with biological DMARDs such as TNF antagonists, including REMICADE®, should be avoided because of the possibility of increased immunosuppression and increased risk of infection.

Use with Other Biological Therapeutics

The combination of REMICADE® with other biological therapeutics used to treat the same conditions as REMICADE® is not recommended [Refer to the product labeling].

Methotrexate (MTX) and Other Concomitant Medications

Specific drug interaction studies, including interactions with MTX, have not been conducted. The majority of patients in rheumatoid arthritis or Crohn's disease clinical studies received one or more concomitant medications. In rheumatoid arthritis, concomitant medications besides MTX were non-steroidal anti-inflammatory agents (NSAIDs), folic acid, corticosteroids and/or narcotics. Concomitant Crohn's disease medications were antibiotics, antivirals, corticosteroids, 6-MP/AZA and aminosalicylates. In psoriatic arthritis clinical trials, concomitant medications included MTX in approximately half of the patients as well as NSAIDs, folic acid and corticosteroids. Concomitant MTX use may decrease the incidence of anti-infliximab antibody production and increase infliximab concentrations.

Immunosuppressive Medications

Patients with Crohn's disease who received immunosuppressive medication tended to experience fewer infusion reactions compared to patients who were not. [Refer to the product labeling]. Serum infliximab concentrations appeared to be unaffected by baseline use of medications for the treatment of Crohn's disease including corticosteroids, antibiotics (metronidazole or ciprofloxacin) and aminosalicylates.

Cytochrome P450 Substrates

The formation of CYP450 enzymes may be suppressed by increased levels of cytokines (e.g., TNFα, IL-1, IL-6, IL-10, and IFN) during chronic inflammation. Therefore, it is expected that for a molecule that antagonizes cytokine activity, such as infliximab, the formation of CYP450 enzymes could be normalized. Upon initiation or discontinuation of REMICADE® in patients being treated with CYP450 substrates with a narrow therapeutic index, monitoring of the effect (e.g., warfarin) or drug concentration (e.g., cyclosporine or theophylline) is recommended and the individual dose of the drug product may be adjusted as needed.

Live Vaccines/ Therapeutic Infectious Agents
It is recommended that live vaccines not be given concurrently with REMICADE® [Refer to the product labeling]. It is recommended that therapeutic infectious agents not be given concurrently with REMICADE®.

6.1.6. Special Populations Use

Pregnancy
Pregnancy Category B- It is not known whether REMICADE® can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. REMICADE® should be given to a pregnant woman only if clearly needed. Because infliximab does not cross-react with TNFα in species other than humans and chimpanzees, animal reproduction studies have not been conducted with REMICADE®. No evidence of maternal toxicity, embryotoxicity or teratogenicity was observed in a developmental toxicity study conducted in mice using an analogous antibody that selectively inhibits the functional activity of mouse TNFα. Doses of 10 to 15 mg/kg in pharmacodynamic animal models with the anti-TNF analogous antibody produced maximal pharmacologic effectiveness. Doses up to 40 mg/kg were shown to produce no adverse effects in animal reproduction studies.
As with other IgG antibodies, REMICADE® crosses the placenta and has been detected up to 6 months in the serum of infants born to female patients treated with REMICADE® during pregnancy. Consequently, these infants may be at increased risk of infection, and caution is advised in the administration of live vaccines to these infants [Refer to the product labeling]

Nursing Mothers
It is not known whether REMICADE® is excreted in human milk or absorbed systemically after ingestion. Because many drugs and immunoglobulins are excreted in human milk, and because of the potential for adverse reactions in nursing infants from REMICADE®, women should not breast-feed their infants while taking REMICADE®. A decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

6.1.7. Infusion Supervision

Remicade® will be administered in a hospital environment where full resuscitation facilities are immediately available and under close supervision of the investigator or designated safety accessor. The intravenous infusion will be supervised by the clinical staff (i.e., clinical nurse, physician) at the participating institutions. A history of each infusion and any adverse side effects will be recorded and reported to the Clinical Research Organization (CRO) using the appropriate case report forms.

Vital signs (temperature, blood pressure, pulse, and respiratory rate) will be obtained prior to the start of **each** Remicade® infusion and every 15 minutes for the first hour of the infusion, every 30 minutes until the end of the infusion, and every hour thereafter for a total of 3 hours after the infusion. After the infusion, the IV line should remain in the participant for at least 1 hour to enable the administration of drugs, if necessary. Additional vital signs may then be obtained as clinically indicated.

6.2. Investigational Agent: Placebo

Placebo is considered an investigational agent in this study. The placebo is 0.9% sterile normal saline for injection (0.9% NS).

US sites will use only sterile normal saline that is produced by an FDA registered facility as designated by the presence of a National Drug Code (NDC) number on product labeling.

Canada sites will use only sterile normal saline that is associated with a valid DIN and is of compendial quality intended for human use.

Product information including manufacturer, lot number, and expiration date will be captured in the pharmacy log at each clinical center.

6.2.1. Dose, Preparation, Administration and Storage

Dosage and Administration

Placebo will be given as a single intravenous pre-perfusion dose, volume matched (250ml) to Remicade®, administered in the operating room. The infusion should be administered over a period of 2 hours or longer. Do not infuse placebo concomitantly with another IV solution or IV medication. **Placebo should be given through a dedicated line. Placebo can be administered at the same time as Thymoglobulin as long as placebo is given in a separate, dedicated intravenous line.** Pre-medications may attenuate infusion-related events. See Pre-Medications table below. Please refer to the Investigational Pharmacy Manual for additional Remicade®/Placebo dosage, preparation, administration and storage details. **Participants will receive a standard of care dose of corticosteroids prior to transplantation.**

Table 3. Pre-Medications-Control Arm

Pre-Medications-Control Arm
Acetaminophen
Antihistamines: Claritin (Loratadine) **or** Benadryl (Diphenhydramine)
Methylprednisolone or its Generic Equivalent

Please refer to section 8.0 Study Procedures for additional dosing and administration details.

Preparation, Storage and Stability

The Pharmacist of Record will prepare and label the placebo infusion bags (260mL bag) using site clinical stock 0.9% NS ***In order to maintain the blinding of the study treatment, amber bag/bottle cover will be used to administer both infliximab and placebo.*** *See the pharmacy manual for additional details.* Placebo will be prepared and stored using the standard procedures at each institution's Investigational Pharmacy. Placebo should be prepared using appropriate aseptic techniques.

6.2.2. Infusion Supervision

Remicade® and Placebo will be administered in a hospital environment where full resuscitation facilities are immediately available and under close supervision of the investigator or designated safety accessor. The intravenous infusion will be supervised by the clinical staff (i.e., clinical nurse, physician) at the participating institutions. A history of each infusion and any adverse side effects will be recorded and reported to the Clinical Research Organization (CRO) using the appropriate case report forms.

Vital signs (temperature, blood pressure, pulse, and respiratory rate) will be obtained prior to the start of each Placebo infusion and every 15 minutes for the first hour of the infusion, every 30 minutes until the end of the infusion, and every hour thereafter for a total of 3 hours after the infusion. After the infusion, the IV line should remain in the participant for at least 1 hour to enable

the administration of drugs, if necessary. Additional vital signs may then be obtained as clinically indicated.

6.3. Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) and Canadian regulations (C.05.012) the investigator will maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed.

The study site will maintain records for receipt, storage, use, and disposition. A drug-dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of drug dispensed.

All records regarding the disposition of the investigational product will be available for inspection. Any partially used, expired, or product remaining at the end of the study may be destroyed according to the center's standard operating center. If a center does not have an SOP for drug destruction, then the product will be returned to the CTOT drug distribution center for destruction.

6.4. Toxicity Prevention and Management

During infusion, mild to moderate infusion reactions may improve following slowing or suspension of the infusion, and upon resolution of the reaction, reinitiating at a lower infusion rate and/or therapeutic administration of antihistamines, acetaminophen, and/or corticosteroids. For participants that do not tolerate the investigational agent following these interventions, the infusion should be discontinued.

6.5. Premature Discontinuation of Investigational Agent

Study therapy may be prematurely discontinued for any participant if the participant has severe infusion-related hypersensitivity or an infusion related reaction. During or following infusion, participants who have severe infusion-related hypersensitivity reactions should be discontinued from further investigational agent administration. The management of severe infusion reactions should be dictated by the signs and symptoms of the reaction.

Study therapy may also be prematurely discontinued for any participant if the investigator believes that the study treatment is no longer in the best interest of the participant, if the subject is judged non-compliant, or due to other safety concerns. Participants that prematurely discontinue the study therapy regimen will remain in the study until study closure.

7. Other Medications

7.1. Concomitant Medications

7.1.1. Protocol-Mandated Medications

Study Therapy Regimen

The study therapy regimen is outlined in the table below. Please refer to section 8.0 Study Procedures for additional dosing and administration details.

Study Therapy Regimen	
Experimental Arm	• **Remicade®** (Infliximab) • **Thymoglobulin®** (Anti-Thymocyte Globulin, Rabbit) • **Tacrolimus** (Prograf®) or one of the following: o Generic equivalent o An equivalent once-daily formulation of Tacrolimus • **Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA** (or their generic equivalents) • **Methylprednisolone** or generic equivalent
Control Arm	• **Placebo** (Normal Saline for Injection) • **Thymoglobulin®** (Anti-Thymocyte Globulin, Rabbit) • **Tacrolimus** (Prograf®) or one of the following: o Generic equivalent o An equivalent once-daily formulation of Tacrolimus • **Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA** (or their generic equivalents) • **Methylprednisolone** or generic equivalent

7.1.2. Other permitted concomitant medications

Concomitant medications are not defined by the CTOT-19 study; this is defined by each centers standard of care. Information regarding Immunosuppressant use will be collected during study visits. *Please refer to Appendix 1 (Schedule of Events) for additional details*.

7.2. Prophylactic Medications

7.2.1. Infection Prophylaxis

CMV Prophylaxis

- *Sero-Negative Donor and Recipient*
  These subjects will not receive CMV prophylaxis mandated by the protocol. Treatment will be dictated by the standard of care at each site.
- *Sero-Positive Donor and Negative Recipient*
  When tolerating PO or at discharge, subjects will receive Valganciclovir (Valcyte®) PO, 450 to 900 mg QD. Dose adjusted according to renal function, for 6 months post- transplant as per local site standard of care.
- *Sero-Positive Recipient with donor that is either sero-positive or sero-negative*
  When tolerating PO or at discharge, subjects will receive Valganciclovir (Valcyte®) PO, 450 to 900 mg QD. Dose adjusted according to renal function, for 3 months post- transplant as per local site standard of care.

Table 4 below summarizes the protocol mandated CMV prophylaxis based on the CMV status of the donor and recipient prior to transplant.

Table 4. Donor/Recipient CMV Status and Prophylaxis

Donor CMV	Recipient CMV	CMV Prophylaxis
+	-	• When tolerating PO or at discharge, subjects will receive Valganciclovir (Valcyte*) PO, 450 to 900 mg QD. • Dose adjusted according to renal function, for **6 months post- transplant** as per local site standard of care.
-	+	• When tolerating PO or at discharge, subjects will receive Valganciclovir (Valcyte*) PO, 450 to 900 mg QD. • Dose adjusted according to renal function, for **3 months post- transplant** as per local site standard of care.
+	+	• When tolerating PO or at discharge, subjects will receive Valganciclovir (Valcyte*) PO, 450 to 900 mg QD. • Dose adjusted according to renal function, for **3 months post- transplant** as per local site standard of care.
-	-	• These subjects will **not** receive CMV prophylaxis mandated by the protocol. Treatment will be dictated by the standard of care at each site.

All sites will use PCR based assays to diagnose CMV. Treatment of symptomatic CMV disease will be treated per the local center standard of care.

7.3. Prohibited Medications

The use of any other Investigational Drugs is prohibited. Participants should not use an investigational agent within 4-weeks prior to study entry and for the duration of the protocol.

7.4. Rescue Medications

Subjects suspected of having a rejection episode on the basis of clinical signs, symptoms or on the basis of laboratory tests will have a renal ultrasound and will undergo a renal transplant biopsy. The local pathologist at the participating clinical site will make rejection diagnoses. A sample of blood, urine and a portion of the biopsy will be collected and sent to a CTOT-19 Core laboratory for mechanistic studies.

Acute cellular rejection or acute humoral rejection (C4d+) detected on any biopsy will be treated per local practice.

8. Study Procedures

8.1. Screening/Enrollment

The research study will be explained in lay terms to each potential research participant. As part of the informed consent process outlined in CFR Title 21 Part 50 and ICH E6 GCP Guidance, the investigator or physician listed on the FDA 1572 or Investigator of Record Form will conduct a meeting with the study candidate to review all of the required elements of informed consent. The potential participant will sign an informed consent form before undergoing any study procedures. After the informed consent has been signed and the participant meets entry criteria, the participant will be enrolled in the study. Once enrolled, the participant will be assigned a unique participant number. Participants enrolled in the study will be followed for approximately 24 months after their transplant surgery.

8.1.1. Testing and Prophylaxis for Latent TB Infection

All enrolled subjects must have a negative test result for latent TB infection. Subjects who have a positive test result for latent TB infection at any time prior to enrollment are not eligible for enrollment, even if they completed a full course of treatment. Any subject with a history of active TB disease are not eligible for enrollment.

All enrolled subjects should be evaluated for new risks of exposure to latent TB infection since their last test. If there are new risks of exposure to latent TB infection since the last test, these subjects should not be enrolled without sufficient time to get a latent TB infection test result prior to enrollment.

Once it has been determined that there are no new risks of exposure to latent TB infection since the last test for latent TB infection, subjects who have a negative test result for latent TB infection within 1 year of transplant date are eligible for enrollment and no further action is required.

Subjects who have a negative test for latent TB infection that is greater than 1 year old are eligible for enrollment but will need to repeat the test. Samples for testing latent TB infection can be obtained during the hospital admission for the transplant but must be collected prior to the initiation of immunosuppression and prior to transplant. The results of this repeat test will determine the next step.

- If the test is negative, no further action is required
- If the test is positive, lost, indeterminant or unavailable **the subject must be treated for latent TB infection** (if the subject is enrolled, received study drug/placebo and transplanted). Treatment is required for all in this group.
    - The treatment regimen (including the dose and duration of treatment) will be determined by the local Infectious Disease team.
        - Rifampin-based prophylaxis is discouraged due to a drug-drug interaction with tacrolimus and other drugs metabolized by the cytochrome P450 system.
- If the subject has no evidence of latent TB testing available, then the subject cannot be enrolled.

8.2. Baseline Visit

Subjects will have their baseline visit prior to transplant. During this visit blood, urine and tissue will be collected from participants.

Randomization

Randomization will occur after enrollment, prior to transplant surgery. Participants will be randomized to either the Treatment group (receive Remicade®) or the Control Group (receive placebo). The site personnel will enter the information in the web-based system. This action will generate an automatic email and/or faxed communication to the site staff indicating successful randomization.

This is a blinded study so participants and the study team will not know the treatment assignment of the participant. Only the research pharmacist will receive a communication identifying the treatment assignment. Randomization treatment assignments will be generated by the SACCC statisticians and stored on the SACCC server. The SACCC will maintain a central web-based randomization system.

8.3. Study Therapy Regimen

Table 5 below summarizes the study therapy regimen defined by this protocol. Please refer to the Appendices for additional information.

Table 5. Study Therapy Regimen Summary

Study Therapy Regimen Summary
Pre-Meds *(Acetaminophen, Antihistamines & Methylprednisolone)* • **All pre-meds must be administered** up to 4 hours prior to transplant and prior to start of Remicade® infusion   ○ **Acetaminophen**   ▪ 600mg to 1000mg given by mouth, suppository or IV   ○ **Antihistamines** (either can be used)   ▪ ***Claritin/Loratadine*** 10mg given by mouth or IV   ▪ ***Benadryl/Diphenhydramine*** 25 to 50mg given by mouth or IV   ○ **Methylprednisolone**   ▪ 500mg given by IV
Remicade® (Infliximab) or **Placebo** • **Initiated** prior to reperfusion. • **Administered** over a period greater than 2 hours   ○ Remicade®-single dose, 3mg/kg infusion   ○ Placebo- single dose, volume matched to Remicade® (250mL) infusion
Thymoglobulin® (Rabbit ATG) • **Initiated** on the day of transplant • **Administered** daily with the intention of achieving a total dose of 4.5 to 6.0 mg/kg, as tolerated
Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA (or their generic equivalent) • **Initiated** no later than 48 hours post-transplant • **Administered** at a target dose of 2000mg daily, as tolerated, until study closure
Tacrolimus (or its generic equivalent) • **Initiated** no later than 48 hours post-transplant • **Administered** at a target dose of 0.1mg/kg per day divided into BID dosing (0.05 mg/kg BID), or an equivalent starting dose of a once-daily formulation of tacrolimus post-op   ○ Then adjusted to target trough levels of 8-12ng/ml during 1st 3-months post-op   ○ Finally adjusted to target trough levels of 5-8ng/ml until study closure
Prednisone (or its generic equivalent) • **Prednisone administered** perioperatively according to center standard of practice • Prednisone should be gradually tapered to no less than 5 mg QD or 10 mg QOD by 3 months post-transplant.

8.3.1. Pre-Medications

Participants will receive all pre-medications (antihistamines acetaminophen and methylprednisolone) up to 4 hours prior to transplant followed by either the investigational drug or placebo based on randomization assignment. When necessary, intravenous formulations may be used for pre-medications. Methylprednisolone 500 mg will be administered by intravenous infusion beginning up to 4 hours prior to or at the initiation of the transplant operation, and prior to initiating the infusions of Remicade®/placebo or thymoglobulin.

8.3.2. Remicade®/Placebo

Remicade® is administered as a single 3mg/kg intravenous pre-perfusion dose. Placebo is administered as a single, intravenous, pre-perfusion, volume matched to Remicade® (250mL) dose.

- Actual body weight will be used in the calculation.
- Do not infuse Remicade® or placebo concomitantly with another IV solution or IV medication.
- Remicade® and Placebo infusions should be administered through a separate, dedicated intravenous line.
- The Remicade® and Placebo infusions should be administered in the operating room, over a period greater than 2 hours.

Please refer to protocol Section 6.1 Investigational Agent: Remicade® and Section 6.2 Investigational Agent: Placebo *for additional details regarding Remicade®/Placebo dosing, administration, preparation and storage.*

8.3.3. Thymoglobulin

Thymoglobulin will be initiated on the day of transplant. It will be administered at a dose of 1.5 mg/kg daily (as tolerated) with the intention of achieving a total dose of 4.5 to 6.0 mg/kg total dose. Subjects who receive less than 4.5mg/kg total dose will continue in the study until study closure. Receiving less than 4.5 mg/kg will not be considered a deviation from the protocol. Thymoglobulin can be administered at the same time as Remicade® or Placebo as long as Remicade® or Placebo is given through a separate, dedicated intravenous line.

8.3.4. Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA

Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA (or its generic equivalent) will be administered at a target dose of 2000 mg daily until study closure. MMF/MPA will be adjusted based on clinical complications. If participants cannot tolerate MMF/MPA (or its generic equivalent) or is outside the target dose range, this will not be considered a protocol deviation.

8.3.5. Tacrolimus (Prograf®)

Tacrolimus (or its generic equivalent) will be initiated no later than 48 hours post-transplant and administered at a dose of 0.1 mg/kg per day divided into BID dosing (0.05 mg/kg BID) or an equivalent dose of a once-daily tacrolimus postoperatively, then adjusted to target trough levels of 8-12 ng/ml during the first 3 months post-transplant, and then adjusted to target trough levels of 5-8 ng/ml thereafter until study closure.

8.3.6. Prednisone

Prednisone or its generic equivalent should be administered perioperatively according to center practice. Methylprednisolone (SOLU-MEDROL) can be administered perioperatively according to center practice. Prednisone should be gradually tapered to no less than 5 mg/day or 10 mg QOD by 3 months post-transplant thereafter until study closure.

8.4. Follow-up Study Visits

Subjects will have study visit everyday up to 7 days while they are in the hospital after their transplant surgery. Blood and/or urine samples will be collected during scheduled visits. Protocol mandated biopsies will be performed on the day of transplant, month 3 and month 24 post-transplant.

8.5. MEMS®

Prior to discharge, subjects will be given a MEMS® cap and bottle to store their prednisone medication. Subjects will utilize the MEMS® from the time they are discharged from the hospital after transplant surgery until 3 months post-transplant. Subjects will return MEMS® cap and bottle to the research team once they have reached the 3-month post-transplant visit.

Please refer to Appendix 1 for detailed information regarding assessments.

8.6. Additional Clinical Data Collection

In addition to data collected during study visits, Tacrolimus trough levels will be collected every month from 1 month post-transplantation until the end of the study period.

8.7. Modification of Immunosuppression Protocol

At the discretion of the site investigator, immunosuppression protocols may be modified for clinical reasons. This would not be considered a protocol deviation.

8.8. Unscheduled Visits

If disease activity increases or other concerns arise between regularly scheduled visits, participants should be instructed to contact study personnel and may be asked to return to the study site for an "unscheduled" visit.

8.9. Unscheduled Study Visits after Month 24 Post-Transplant

Once sibjects complete the last planned study visit at month 24 after your kidney transplant the subject may continue to have study visits every 6 months up to 5 years after your transplant. This portion of the research study is optional. During these visits, a review of the medical record to determine graft status and a sample of blood will be collected for research.

8.10. Reduced Follow-Up

Subjects that withdraw or terminate from the study early or experience graft loss will follow a reduced follow-up schedule.

Subjects that experience graft loss can remain in the study until study closure. These subjects will be followed on a reduced schedule. Please refer to Appendix 3 for details.

For subjects who withdraw or terminate from the study early, the last study visit should be considered the final study visit. Please refer to Appendix 4 for details regarding data collection.

8.11. COVID-19 Pandemic-Study Follow-up

During the COVID-19 pandemic sites should use the **COVID-19 Study Visit Windows** outlined below. The COVID-19 Pandemic Study Visit Windows will remain in effect until each center reinstitutes standard protocols and allows participants to come into their center for their standard clinic and/or research visits. If there is a re-emergence of the COVID-19 pandemic, these changes may be reinstituted to accommodate site restrictions.

8.12. Standard Study Visit Windows

STUDY VISIT WINDOWS		
VISIT Post-Transplant	**VISIT NO.**	**VISIT WINDOW** Study visits should take place within the time limits specified below
Day 0	00	• Blood and urine samples should be collected prior to perfusion ○ The specimens (urine and blood) may be collected up to 4 months prior to transplant, while the subject is on the wait list for transplant. If the transplant occurs greater than 4 months after screening sample collection, then the site should collect the specimens prior to transplant/perfusion. Missed screening samples (visit 00) will not be considered a protocol deviation. • Latent TB infection test must occur within 1 year prior to transplant ○ If latent TB infection test results are not available within 1 year of transplant, sites must collect sample for latent TB test prior to the initiation of immunosuppression and prior to transplant with the understanding that results may not be available until after transplant surgery. ○ Section **8.1.1 Testing and Prophylaxis for Latent TB Infection**, provides instructions on how to proceed based on test results.
Days 1 to 7	01 02 03 04 05 06 07	• ± 12 hours • After transplant, **urine samples** (for Urinary Chemokines and Necroptosis Markers and mRNA Profiling, Gene Expression) will be collected daily until subject is discharge from hospital. • Subjects will have study visits daily while they are hospitalized post-transplant up to Day 7. If a subject is discharged prior to Day 7 the day of discharge will be the last study visit until Day 14. • **There are no study visits from Day 8 to Day 13.** ○ Note: If discharge occurs prior to Day 7, then complete the discharge visit/core labs (using Kit #3) at the time of the discharge. Any remaining core labs due between discharge and Day 7 are not required. ▪ *Example: If discharge occurs at Day 4, use the Kit #3 to complete the core labs at Day 4. The Day 4, 5, 6, and 7 core labs (Kit#2) are not required.* ○ Note: If discharge occurs after Day 7, then complete the Day 7 core labs (Kit#3) on Day 7. No further core labs should be collected until Month 1. ▪ *Example: If discharge occurs at Day 9, use the Kit #3 to complete the core labs at Day 7. There are no scheduled visits between Day 8 to 14. The next time a protocol visit is due is Day 14, and the next time core labs are due is Month 1 (Kit #4).*
Day of Discharge	07	• Day 3 to Day 7
Day 14	08	• ± 7 Days
Month 1	09	• -7 Days/+ 21 days
Month 3	10	• ± 14 days
Month 6	11	• ± 21 days

VISIT Post-Transplant	VISIT NO.	VISIT WINDOW Study visits should take place within the time limits specified below
Month 12	12	• ± 21 days
Month 18	13	• ± 21 days
Month 24	14	• ± 1 month
Tacrolimus Trough levels	07, 09-14, UV1	• ± 2 weeks • ***Collected monthly from Month 1 to Month 24***
Unscheduled Visit 1	UV1	• Day of unscheduled biopsy
Unscheduled Visit 2	UV2	• Between 2 to 6 weeks after UV1 visit
Unscheduled Visit 3	UV3	• ± 2 months
Final Study Visit	FV	• Day of the visit

8.13. COVID-19 Pandemic Study Visit Window

COVID-19 PANDEMIC STUDY VISIT WINDOWS		
VISIT Post-Transplant	**VISIT NO.**	**VISIT WINDOW** Study visits should take place within the time limits specified below
Month 12	12	• - 21 days/+ 4 months
Month 18	13	• - 21 days/+ 5 months
Month 24	14	• - 1 month/+ 6 months
Tacrolimus Trough levels	07, 09-14, UV1	• +/- 2 weeks • ***Collected monthly from Month 1 to Month 24***
Unscheduled Visit 1	UV1	• Day of unscheduled biopsy
Unscheduled Visit 2	UV2	• Between 2 to 6 weeks after UV1 visit
Unscheduled Visit 3	UV3	• +/- 2 months
Final Study Visit	FV	• Day of the visit

9. Mechanistic Assays

9.1. Overview

Previous work by our group and others has identified multiple biomarkers that strongly correlate with, or predict, episodes of acute rejection and/or poor late graft function in low risk recipients of living donor kidneys. We will validate the utility of these markers in larger populations of deceased donor graft recipients and in the context of a clinically applicable testing frequency. The biomarker analyses will be supplemented by studies to address mechanisms of anti-TNFa mAb effects on recipient inflammation and alloimmunity. Our hypothesis predicts that neutralization of TNFa at the time of renal transplantation will dampen IR-induced inflammation and result in weaker anti-donor T cell immunity, preservation of Treg number/function, limiting production of DSA and endothelial injury and preventing induction of fibrogenesis, together resulting in better graft outcomes.

9.2. Cellular Immunology Core

The Cellular Immunology Core laboratory is located at Icahn School of Medicine at Mount in New York, NY (PI: Dr. Peter Heeger, MD). Selective validations will be done at the Cleveland Clinic (PI: Dr. Robert Fairchild)

9.2.1. T and B Cell Phenotyping by Flow Cytometry

We hypothesize that addition of anti-TNFa to rATG as induction therapy will dampen post-transplant inflammation thereby improving ATG-induced depletion, limiting re-expansion and function of donor reactive memory T cells, and preventing induction of new donor reactive T cells, which together will result in decreased acute and chronic allograft injury and improved graft outcome. We will test the hypothesis first, by performing comprehensive, serial, multicolor flow cytometric phenotyping on PBMC from each patient using standardized flow cytometric approaches. Patterns of depletion and reconstitution will be compared between the 2 study arms and correlated with clinical outcomes. We will assess frequencies of CD4 and CD8, naïve and memory T cells as well as Treg and B cell subsets as each time point. Blood samples will be collected pre and serially post-transplant, as outlined in the Schedule of Events (appendix 1). Samples will be stained using antibody panels shown in **Table 6** and analyzed by flow cytometry. Depending upon available cells we will also stain for NKG2D and chemokine receptors CXCR3 and CCR6 on the T cell subsets.

Table 6. Antibody panels for Flow Cytometric Analyses

fluorochrome	TruCount	T Cell	Treg	B Cell
FITC	CD16	CD45RA	CD45RA	IgG
PE	CD19	CCR7	CD25	CD24
PerCP6Cy565	CD45	CD3	CD3	IgD
PE6Cy7	CD3	CD28	CD127	CD38
APC	CD56	CD27	CCR4	CD27
APC6Cy7	CD8	CD8		CD19
Pacific Blue	CD4	CD4	CD4	IgM
Krome Orange	CD14	CD14/CD19	CD14/CD19	CD14/CD3

Because intracellular expression of pSTAT3 has been reported by others to be a useful marker of effector T cell function in patients with multiple sclerosis (among other diseases) while Treg pSTAT5 expression correlates with Treg function (46-49), we will include these 2 markers in our analyses as indirect measures of function.

9.2.2. T Cell Functional Assays (ELISPOT) and Archived PBMC for CyTOF Analysis

Evidence suggests that effector and/or memory T cells reactive to donor antigens and or to a panel of alloantigens as measured by IFNy ELISPOT assays are detrimental to graft survival. In previous studies we showed that pre- (and post-) transplant frequency of anti-donor memory T

cells in peripheral blood, as determined by an IFNy ELISPOT assay directly correlates with the risk of developing AR (within 6 mo. Post-transplant) and decreased 12 mo. GFR (50-55). These findings have been subsequently validated by our group and several others using independent patient cohorts (56-59). Completion of ELISPOT assays requires ~36 hours, a time frame which essentially precludes using anti-donor ELISPOT testing to make clinical decisions regarding use of, or therapy for, deceased donor transplants. In an effort to circumvent this practical issue and to develop a screening test for alloreactive T cell memory (analogous to the panel of reactive antibody, PRA, screening test for humoral immunity), we developed and validated the "panel of reactive T cells," or PRT, assay (60-62). In the PRT, PBMC from transplant candidates (e.g. dialysis patients on the transplant waiting list) are tested in IFNy ELISPOT assays against a panel of HLA disparate B cell stimulator lines. Our goals here are to a) prospectively validate the pre-transplant PRT as an independent risk assessment tool in deceased donor transplant recipients and b) to compare the results of the PRT to anti-donor responses as predictors of outcomes in study population. Using serially collected post-transplant blood samples we will assess the impact of anti-TNFa induction therapy on the strength of the post-transplant anti-donor T cell immune responses over time. Based on new data published by the Fairchild group that CD28neg effector T cells are important mediators of allograft injury and that this population of cells only produce IFNy in ELISPOT assays if stimulated with IL-15 (63) we will perform side by side PRT and anti-donor ELISPOT assays with and without recombinant IL-15 compare the results and determine how well the results of each assay correlate with outcomes. Readouts will be both IFNy and Granzyme B ELISPOTs.

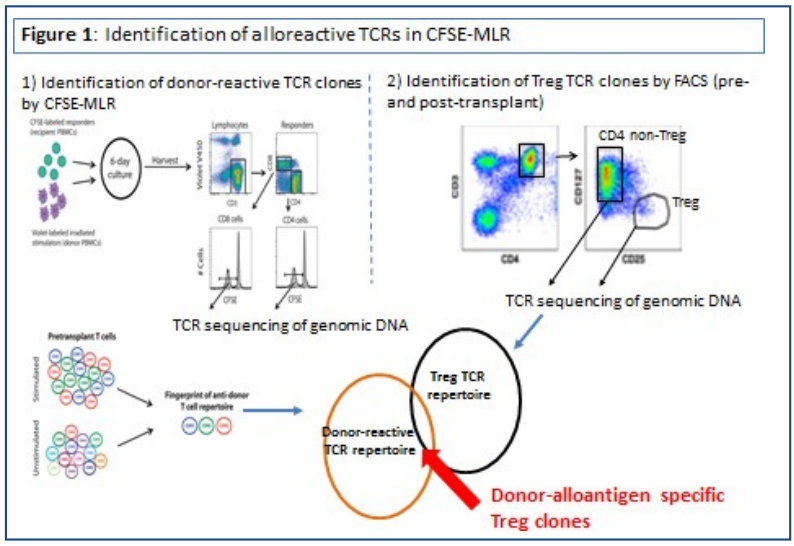
We will obtain blood samples in heparinized green top tubes pre-transplant (pre-induction) and serially post-transplant and will perform PRT and anti-donor ELISPOT assays with and without IL-15 as published. In vitro expanded B cell lines will be used as stimulator cells for these assays. HLA typed B cells will be expanded from donor and or recipients within the study population. PRT assays will be done with the same panel of up to 8 stimulator lines for each patient at each time point. As it is anticipated that donor tissue will only be obtainable from a subset of the deceased donors in this study, anti-donor immunity will only be assessed in a subset of recipients (anticipated to be ~30% of the total enrollees).

In exploratory studies, we will use 1-2 million PBMCs obtained from these cells or from the cells obtained in 9.3 below to evaluate expression >30 concurrent markers by CyTOF analysis and to assess B cell reactivity in vitro to allo-, auto- and infectious antigens.

9.2.3. TCR Sequencing Substudy

We have recently demonstrated that biologically-relevant donor-reactive T cell clones can be identified by high-throughput sequencing of recipient T cells proliferating in an anti-donor MLR and expanded compared to unstimulated T cell populations[100]. In patients achieving tolerance by combined kidney and bone marrow transplantation, the number of circulating donor-reactive CD4 T cell clones declined over time, whereas conventional transplant patients showed an increase in these clones in the circulation. We hypothesize, therefore, that a decline in these donor-specific CD4 clones at one year compared to pre-transplant blood may identify patients who are operationally "tolerant" and whose graft outcomes at two years will be excellent. We hypothesize that there may be more frequent reductions in these clones in the infliximab-treated group than the controls, though this hypothesis cannot be addressed until the study is completed, due to the double-blind study design. We also hypothesize that a high level of expansion of donor-specific Tconv and lack of expansion of donor-specific Tregs will be associated with the presence of rejection episodes. Conversely, high levels of expansion of donor-specific Tregs and/or conversion of donor-reactive CD4 Tconv to Tregs post-transplant may be associated with the absence of rejection. We have been able to detect CFSE-low CD4

cells in the anti-donor MLR that appear in the sorted Treg fraction of transplant patients (Figs. 1,2) as well as donor-reactive T cells that appear in the sorted Tconv fraction pre-transplant and in the Treg fraction post-transplant (Fig.2). Thus, donor-specific Tregs may be identified and tracked with this method.



Furthermore, in pilot studies we have detected donor-specific TCR DNA in urine of kidney transplant patients prior to and during rejection episodes but have not detected such DNA in urine of patients without rejection episodes. We hypothesize that the presence of donor-reactive TCR DNA in the urine may be a diagnostic marker and/or predictor of rejection. We propose a substudy to obtain preliminary data relevant to these hypotheses in 10 subjects. This work will be performed at Columbia University (Megan Sykes, MD).

1) The degree of expansion or reduction of donor-reactive CD4 TCRs in the circulating T cell pool at 6 and/or 12 months correlates with graft outcomes at 2 years.
2) Expansion of donor-specific Tregs or conversion of donor-reactive Tconv to Tregs post-transplant correlates with excellent graft function and absence of rejection.

The presence of donor-reactive TCR DNA in the urine predicts and/or correlates with the presence of rejection.

9.2.4. T Regulatory Cells Substudy

The flow cytometric analyses will determine the effect of anti-TNFa induction on Treg frequencies. To assess impact of anti-TNFa on Treg function, in a subset of patients in each group (n=10 per group without anemia) we will isolate 50-100 cc of blood to perform functional analyses of Treg suppressive capacity to verify the correlations between phenotype and function. We will flow sort $CD4^{+}CD25^{hi}CD127^{lo}$ T cells and test them in in vitro suppression assays (64), using effector T cells from normal controls as the responders. We will quantify DNA methylation of the Foxp3 gene (65) and phosphorylation (66) using commercial assays in selected samples as an indirect measure of Foxp3 stability of the Treg. Only participants from the Cleveland Clinic, University Hospitals of Cleveland/Case Western, and Mount Sinai will be included in this Substudy.

9.2.5. Urinary Chemokine Protein and Necroptosis Markers

Urinary chemokine assays will be performed at Icahn School of Medicine at Mount Sinai in New York, NY (PI: Dr. Peter Heeger, MD).

One goal of these studies is to determine the effectiveness of the anti-TNFa mAb in attenuating TNFa-mediated inflammation and its associated downstream sequelae. Urinary chemokines, including CXCL9, CXCL10 are elevated immediately post-transplant as a reflection of ischemia reperfusion injury. We hypothesize that anti-TNFa induction therapy will limit post-transplant inflammation and ischemia reperfusion injury. To test this we will serially collect urine samples over the first month post-transplant and test supernatants for CXCL9 and CXCL10 (among other

potential markers), perform time dependent analyses and compare the results between the experimental and the control groups.

A second goal of these studies is to validate the utility of urinary CXCL9 as a biomarker for ongoing or incipient acute rejection in a high-risk study population. We previously showed that in low risk kidney allograft recipients urinary CXCL9measurements are an accurate noninvasive approach to diagnosing acute cellular (but not humoral rejection) and can detect subclinical allograft injury (with a stable creatinine) that will develop over time into clinically evident rejection (67). Our goal herein is to determine the utility of serial urinary CXCL9 measurements to: a) diagnose and or predict incipient, clinically evident, biopsy proven acute rejection, b) to assess whether the type (cellular and or antibody) and/or grade of rejection impacts diagnostic/predictive utility, and c) to define causes of false positive results (predicted to be infection and resolving IR injury). Urine samples will be obtained from each patient at each site at predefined time points between transplantation and 6 months post-transplant because the majority of rejections occur during the first 6 months. We will also obtain urine samples at the time of a biopsy performed for evaluation of acute kidney dysfunction.

Urine supernatants will be sent to a core lab and frozen in aliquots. Samples will be tested by ELISA for urinary CXCL9, CXCL10 and additional markers. Pilot data indicate that values > 200 mg/dl represents a positive test indicative of ongoing or incipient rejection. Results will be correlated with clinical evidence of rejection (we will not direct therapy based on the results of the assays). As we have previously shown that urinary infections including BK virus infections lead to increases in urinary CXCL9, we will correlate the urinary CXCL9 results with tests for BK and UTI.

9.3. Genomics Analysis Core Laboratory

The Genomics Core Lab is located at the Icahn School of Medicine at Mount Sinai, NY, NY (PI: Dr. Barbara Murphy).

9.3.1. mRNA Profiling, Gene Expression in Blood

We will isolate RNA from PBLs and perform RT-PCR (or nanostring). There is some evidence that these markers may be useful for predicting AR in renal allograft recipients. We will include these studies to confirm published findings and to test whether recurrent detection of these markers is associated with the development of the late endpoints. Blood samples will be collected in PAXgene RNA tubes. Results will be correlated with pathological interpretation of the 3-month. biopsy (core lab) and with incipient biopsy proven rejection.

9.3.2. mRNA Profiling, Gene Expression Profiles in Tissue

We also hypothesize that chronic allograft injury manifested pathologically as progressive interstitial fibrosis and tubular atrophy (IF/TA) is initiated early post-transplant by a pro-inflammatory environment, compounded by T cell and antibody mediated injury. Our hypothesis is that anti-TNFa induction will dampen early post-transplant inflammation and prevent or attenuate induction of a fibrogenic gene profile in the allograft that ultimately leads to chronic allograft injury. This hypothesis predicts that differences in molecular signatures indicating fibrosis are likely to be present prior to the histological development of the injury and that they will be less prevalent (and or occur later) in patients treated with anti-TNFa induction. Data in animal models of liver and renal fibrosis and clinical studies clearly implicate a role for TNFa and for TNF receptor 1 signaling in the instigation of this fibrogenesis (154-159). We will test this hypothesis by evaluating gene expression profiles in urine between the treatment and control arms, and comparing the results to the pathological analysis of IF/TA progression between implantation and 24 mo. (as well as with eGFR which is likely to correlate with the IF/TA progression).

Molecular analyses of the graft tissue will be performed for 2 purposes: a) to determine the effects of anti-TNFa induction therapy on intragraft inflammation at 3 months and b) to validate a molecular biomarker that predicts incipient rejection.

a) Effects of anti-TNFa induction on intragraft inflammation. To supplement the urinary gene expression profiling described above and to directly test the effects of anti-TNFa on graft tissue, we will make RNA from a portion of the 3-month protocol biopsy from each patient and perform qPCR (or nanostring) to quantify gene expression of inflammatory and profibrotic genes (same as those described in section 8.1.4). For each gene, we will compare the results between the experimental and control groups and will also determine the strength of the correlations between urinary gene expression patterns and intragraft gene expression patterns.

b) In unpublished work from co-I B Murphy, analysis of RNA obtained prospectively from 3 mo. surveillance transplant biopsies in patients with stable renal function at 3 mos. showed that molecular profiles in the graft at 3 mo. predict subsequent 12 mo. chronic injury, independent of clinical and histopathological parameters. This genomic biomarker of 20 differentially regulated genes (some up-regulated, others down-regulated) strongly correlate with chronic allograft damage at 1 year, but importantly not with fibrosis at 3 mo. Statistical analysis of this preliminary panel reveals an average AUC score of multiple iterations of ROC curves of 0.947 within 95% confidence interval 0.942-0.952. Our goal here is to independently validate and refine the gene set as a biomarker for incipient rejection. We will prospectively test the predictive value of the gene set expressed in the 3-month biopsy RNA using the new cohort from this deceased donor kidney transplant trial and will test the impact of anti-TNFa therapy on the utility of the biomarker. We will isolate RNA from surveillance/protocol biopsies obtained at 3 mos. from our study cohort (control and anti-TNFa treatment arms). We will perform qRT-PCR on each RNA sample to detect expression levels of the minimal gene set (10-20 genes) as a biomarker. We will test the strength of the relationship of this intragraft gene expression profile biomarker with: a) 12m eGFR, b) 24m eGFR and c) change in histology scores between implantation and 24 months accounting for confounders (see statistical analysis below).

9.3.3. Genomics Analysis

In unpublished work, Dr. Murphy (Co-Investigator) and colleagues, using a set of 147 kidney transplant recipients identified a 100-gene set from peripheral blood obtained at 3 months post-transplant that strongly correlated with subclinical acute cellular rejection (ACR) of 3 mo. surveillance biopsies. Our goal here is to test the diagnostic utility of this peripheral blood derived RNA biomarker for ongoing and incipient acute cellular rejection in this new deceased donor cohort, and to test whether the therapy with anti-TNFa impacts the diagnostic/predictive utility.

9.3.4. Cell Free DNA Analysis

Blood samples will be obtained in Streck Cell-Free DNA BCT tubes to perform cell-free plasma DNA analysis. Streck Cell-Free DNA BCT tubes contain a formaldehyde-free preservative in Cell-Free DNA BCT that stabilizes white blood cell, preventing the release of genomic DNA, allowing isolation of high-quality cell free DNA. Samples will be collected and shipped to the Genomics Core Laboratory for processing. Samples will be provided to CareDx, Inc. for final analysis. CareDx, Inc. (Brisbane, CA) is a commercial stage company that develops, markets and delivers a diagnostic surveillance solution for transplant recipients to help clinicians make personalized treatment decisions throughout a transplant patient's lifetime.

9.4. Molecular Immunology Core Lab

The Molecular Core laboratory is located at the Cleveland Clinic, Cleveland OH (PI: Dr. Robert Fairchild).

9.4.1. Nanostring Analysis

We will isolate RNA from PBLs and perform RT-PCR and Nanostring analysis. Blood samples will be collected in PAXgene RNA tubes, and shipped to the Genomics Core Lab (PI: Barbara Murphy). The samples will be processed and shared with the Molecular Immunology Core lab.

9.4.2. mRNA Profiling, Gene Expression Profiles in Urine Pellet

To further determine the effectiveness of the anti-TNFa mAb in attenuating TNFa-mediated inflammation and its associated downstream sequelae, we will study and compare urinary RNA expression of a panel of selected molecules relevant to IR injury and are known to be up-regulated by TNFa (**Table 7**).

Table 7. Genes to be studied in Graft tissue and urine

Cytokines: TNFa, IL-1, -6, -8, -15, -18
Chemokines: CCL2, CCL3, CCL5, CXCL-9, -10 CCR2
Complement components: C3, fB, C5, C5aR, C3aR, DAF
Other: ICAM-1, TLR4, HMGB1
Control: succinyl dehydrogenase (SDHA)

We will examine and compare: urinary cellular RNA expression profiles and urinary proteins peri-transplant, in the control and treatment arms. For cost containment purposes, we will use quantitative real time PCR (qPCR) or nanostring technology as the primary methodology. Urine samples from each patient obtained at the predefined time points *(Schedule of Events, Appendix 1)* will be pelleted and the cellular sediment homogenized in RNA Lysis Solution. We will not differentiate or separate immune cells from intrinsic renal cells in this process. RNA will be isolated from biopsy samples or urine cells and reversed transcribed using random hexamers in our published and validated SOPs. qPCR will be done as published (68) using primers for the mouse BAK gene to establish a standard curve and normalized to 18S. The expression levels of each gene will be expressed as copy number per µg of the control BAK standard. Additional inflammatory and fibrosis genes will be assessed by nanostring once the procedures have been optimized and validated (exploratory analyses).

We will compare the results between treatment groups and all laboratory results will be correlated with graft outcomes, specifically endpoints related to early graft function (DGF, immediate graft function, slow graft function, *see Endpoints in Sections 3.2 and 3.3*), testing the hypothesis that dampening ischemia IR-inflammation improves outcome (e.g. prevents DGF).

We will isolate urine cell RNA obtained serially post-transplantation to measure RNA for genes encoding proteins involved in the progression of interstitial graft fibrosis including TGFb, n-cadherin, a-smooth muscle actin, connective tissue growth factor, vimentin, fibronectin, collagen I, collagen III, MMP-2, BMP7 (among others) in urine cells using qPCR as described above and published (109). The findings will be correlated with the degree of chronic injury and will be compared between the groups of patients in the 2 study arms.

9.5. Pathology Core Lab

The Pathology Core laboratory is located at the University of Manitoba in Winnipeg, Manitoba, Canada (PI: Dr. Ian Gibson, MD).

9.5.1. Histology

Our hypothesis is that the anti-TNFa induction therapy will lower the incidence of acute T cell and antibody mediated rejection and result in improved 2-year graft histology (lower chronicity scores).

Graft tissue will be obtained pre-transplant (prior to implantation), at 3 months post-transplant and at 2 years post-transplant. Histologic examination will be done at the University of Manitoba at Winnipeg site. Biopsy results will be reported using the Banff '07 schema for acute rejection and chronic allograft injury. The centers will send renal tissue (1-1.5 wedge or core tissue s in a pre-filled formalin jar). If tissue cannot be sent to the Histology Core Lab, then the site should send or stained slides (3 PAS, 1 Trichrome or unstained and 1 H&E).

Morphological evaluations will be recorded in a standardized format, according to Banff '07 criteria. Primary data will be sent to the Statistical and Clinical Coordinating Center for final analysis. High-resolution images of entire sections of all stains from each biopsy will be archived in an Aperio Digital database system. Slide images will be stored at the University of Manitoba at Winnipeg (PI: Dr. Ian Gibson) for additional analysis, if needed.

9.5.2. TNFR1 Expression Patterns in Renal Allografts

We will test the hypothesis that the efficacy of anti-TNF therapy will depend upon the state of the kidney at the time of transplantation. Specifically, the prediction is that transplanted kidneys expressing high levels of TNFR1 but low levels of TNFR2 and DR3 have less pre-existing injury and are less likely to manifest DGR. Some patients still develop DGF and this is the group of patients who would benefit from anti TNF therapy. In contrast, those transplanted kidneys expressing low levels of TNFR1 but high levels of DR3 and TNFR2 (i.e., those with ischemia-reperfusion injury) are more likely to develop DGF and less likely to show benefits of anti-TNF therapy. We plan to assess the expression patterns of TNFR1, TNFR2 and DR3 by multicolor immunofluorescence staining on pre-implantation FFPE biopsies and correlate the findings with clinical variables. A portion of renal allograft tissue collected at implantation will be shared with Yale School of Medicine (Jordan Prober, MD).

9.5.3. Immunofluorescence/IHC

Peritubular capillary (PTC) loss induced by IR correlates with interstitial inflammation and subsequent fibrosis in animal models of renal disease (75), in native kidneys with progressive dysfunction (76) and in kidney transplants (77). PTC loss as quantified by endothelial cell immunostaining for CD31 and CD34, has been reported to increase between baseline and 3 months post-transplant more often in recipients of deceased donor kidneys (more so in recipients of brain dead donors than in recipients of donors after cardiac death) than in living donor kidneys, in recipients of older donors, and in recipients with early immunological events (77). Furthermore, PTC loss occurs more commonly in recipients who develop DGF, in whom serum levels of the soluble VEGF-R1 levels are elevated (78). As we hypothesize that these processes will be dampened by perioperative anti-TNFa, our working model predicts that PTC loss will be prevented in patients in the treatment group. We will compare the change in PTC density of biopsy tissue by immunofluorescence or IHC between baseline and 3 mos. in a subset of patients from each arm of the study that have developed DGF (or SGF) or have had good function as controls. As PTC loss correlates with subsequent fibrosis (77), we will determine whether PTC density at 3 months correlates with the prevalence and/or intensity of interstitial fibrosis at 2 years. Tissue obtained for Histology (described above) will be shared to complete the Immunofluorescence/IHC work. If tissue is not received from the local center, they will send the core pathology 3 unstained slides mounted on positively-charged glass slides (e.g. Superfrost Plus treated slides), 1 C4d stained slide

and 1 SV-40 stained slide. If the C4d and SV-40 stained slides were not prepared at the local center, then a total of 5 unstained slides will be sent to the core pathologist.

Primary data will be sent to the CTOT Statistical and Clinical Coordinating Center for final analysis. High-resolution images of entire sections of all stains from each biopsy will be archived in an Aperio Digital database system. Slide images will be stored at the University of Manitoba at Winnipeg (PI: Dr. Ian Gibson) for additional analysis, if needed.

9.6. Humoral Core Lab

The Humoral core lab is located at the University of Manitoba at Winnipeg, (PI: Dr. Peter Nickerson).

9.6.1. Anti-HLA Antibodies and Donor Specific Antibodies

Preexisting and de novo post-transplant donor specific antibodies (DSA) can be pathogenic and contribute to acute and chronic allograft injury. We hypothesize that blocking early inflammatory events post-transplant with anti-TNFa induction therapy will prevent de novo alloantibody formation in human transplant recipients.

Serum samples will be collected from recipients at predefined time point, divided into aliquots and store at -80 C. For these analyses, serum samples will be obtained prior to transplantation, every 3 months post transplantation, as well as at the time of indication biopsies. We will use standardized methods (79) to define kinetics, specificities and quantity (median fluorescence intensity, MFI) of DSA in each patient. The HLA specificity, class I vs. II, and approximate titer of the anti-HLA IgG will be determined using Luminex single HLA class I and class II antigen microbeads in combination with phycoerythrin (PE) secondary antibody conjugates for the detection of human IgG. To minimize any prozone effect (interfering factors which mask the antibody strength (MFI) serum samples will be treated with or without EDTA as part of the evaluation to assess if there is an MFI increment with EDTA treatment. The Luminex beads will be analyzed using the LABScan™ 100 flow analyzer and an analysis program will assist in the assignment of the reaction strength (MFI). We will compare the incidence of de novo DSA between the control and experimental arms and assess the correlations de novo DSA and outcomes.

We will also collect serum samples in the early post-transplant time period (days 1-5) and measure TNFa levels by ELISA as another approach to test the hypothesis that anti-TNFa mAb induction therapy limits post-transplant inflammation.

9.7. Glomerular Filtration Rate (GFR) Core Lab

All creatinine values will be performed at the core lab at the Cleveland Clinic foundation (PI: Dr. Emilio Poggio, MD) for standardization.

GFR analysis will be calculated by the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI). This method uses a serum sample for creatinine at month 6 post-transplant, then months 6 and 18 post-randomization. To accomplish this in a uniform manner, serum samples will be collected as part of the protocol at these time points and creatinine values will be determined at a single site (CCF). Demographic characteristics, gender, and race are required for calculation.

9.8. Mechanistic Studies Data Analysis Plan

We will test, validate and expand our biomarker panel to predict and diagnose incipient ACR and to determine the accuracy of early post-transplant markers to define individuals most at risk for late graft dysfunction. This evaluation will be based primarily on examining the Receiver Operating Characteristic (ROC) Curves generated for each candidate biomarker and the associated classification accuracy in terms of sensitivity and specificity (as well as positive and negative predictive value). We will also explore combining the candidate biomarkers to determine whether there are distinct combinations of biomarkers that may improve performance relative to individual biomarkers. The ROC curve puts different biomarkers on a common relevant scale and shows the entire range of possible performance. The information in these curves, describing the overall potential performance of each biomarker, will be quantified by the area under the curve (AUC-ROC) and its associated 95% confidence interval using the nonparametric method of Hanley and McNeil (80). Cut points, based on optimizing sensitivity and specificity, will be determined, and based on the ROC curves. The sensitivity and specificity so obtained (and also the positive and negative predictive values) will serve as the primary summaries of the predictive ability of each biomarker.

Logic regression (81-83) will be used to construct Boolean combinations of biomarkers, dichotomized into indicator variables using the optimized cut points referred to above. Logic regression is an adaptive regression methodology that attempts to construct predictors as Boolean combinations of binary covariates. This method determines a classification algorithm that identifies predictive rules for combining biomarkers by searching the space of possible rules for an optimal rule. The optimal rule is one that minimizes a specified objective function; in this case, misclassification of progression. Cross-validation will be used to select the optimal number of trees and leaves, and the resulting score used to construct an ROC curve. The AUC for this curve will be computed with the associated confidence interval determined using a bootstrap percentile method. This “combination” AUC-ROC will be qualitatively compared to those obtained from the individual biomarkers. Multiple logistic regression models will quantify the association of the biomarkers to risk, and assess the potential confounding effects of key characteristics such as age, sex, race, treatments and comorbidities.

Our hypothesis that anti-TNFa limits activation of donor reactive immunity and prevents initiation and progression of graft fibrosis by dampening early inflammation within the allograft will be assessed by a series of linear mixed models. Models will be fit sequentially, with specific models fit to address specific elements of the hypothesized mechanism. Initially we will use regression models to quantify the extent to which infliximab, when added to therapy with ATG, reduces “early” inflammation at the time of transplantation, measured both by cellular reactivity in blood and from post-implantation biopsies. Corresponding linear regression models will assess differences between treatment groups with respect to inflammation measures at 24 mos., and mixed linear models will be used to assess the inflammation measures over the entire time course subsequent to the “early” evaluation. Additionally, proportional odds models will be used to assess treatment differences in ordinal grade of graft fibrosis at 24 mos. and, through a generalized estimating equation (GEE) approach, over time. A finding of reduced inflammation with combination therapy would be consistent with our mechanistic hypothesis. Further confirmation would be a finding that the estimated benefit for added infliximab therapy at 24 hours and times subsequent the “early” assessment were diminished after adjusting for the “early” measures of inflammation. The adjusted estimate will be based on models that include the “early” measures as covariates in the assessment of treatment on 24 hour measures and on the entire time course. A formal statistical analysis plan is being prepared separately

Sample Size: Our sample size will allow precise estimation of sensitivity and specificity, especially for those biomarkers that perform well. For sensitivities and specificities in the range of 0.80 to 0.90, this sample size (300 subjects) ensures that the associated standard error is in the range of 0.02 to 0.03.

9.9. Sample Collection

The mechanistic studies schedule of events is located in **Appendix 1 & 2-Schedule of Events** of this protocol.

10. Biospecimen Storage

Biological specimens (whole blood, plasma, serum, urine supernatant, urine pellet) 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, or assays performed by other CTOT consortium members for cross-validation studies. 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 subject ID number that could be directly linked to the subject and the subject's medical record. When the funding period is over, samples will be anonymized, which means a sample that was previously identifiable, has had all identifiers removed and can no longer be linked back to the subject or the subject's medical record by any means.

11. Criteria for Participant and Study Completion and Premature Study Termination

11.1. Participant Completion

11.2. Participant Withdrawal Criteria

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

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. The participant dies.
4. The Investigator no longer believes participation is in the best interest of the participant.

11.3. Participant Replacement

Participants that are randomized but did not receive the investigational agent will be replaced. Participants who received the investigational agent and subsequently withdrew consent or are withdrawn will *not* be replaced.

11.4. Follow-up after Early Study Withdrawal

If a participant is withdrawn from the study for any reason, the participant may be asked to complete a final visit and/or final assessments. Please refer to Appendix 4 for details regarding final visit assessments.

11.5. Stopping Rules

Study enrollment at all participating clinical sites will be temporarily suspended 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) upon satisfaction of any of the following stopping rules at any time during the post-transplant (treatment) follow-up period:

- Any single occurrence of a life-threatening or fatal AE that is possibly, probably, or definitely related to either the investigational agent (infliximab/infliximab placebo) or a study mandated procedure.

<u>Across both treatment arms</u>

- Incidence of PTLD of 1% or more subjects
- Incidence of Adverse Events for tuberculosis active disease of 1% or more subjects
- Incidence of invasive fungal infection of 3% or more subjects
- Incidence of coccidioidomycosis of 1% or more subjects
- Incidence of histoplasmosis of 1% or more subjects
- Incidence of death of 10% or more subjects

<u>Within a given treatment arm</u>

- Incidence of infection of any type requiring hospitalization of 40% or more subjects
- Incidence of graft loss of 20% or more subjects
- Incidence of BPAR (Banff Grade 1 or higher) or AMR based on local read of 25% or more subjects

These thresholds of concern will be continuously monitored by the SACCC throughout the study to determine if any of their observed subject-based incidence rates exceed the pre-specified incidence rate of concern for each particular event. They will be implemented by comparing the exact lower 95% confidence limit on the currently observed subject-level incidence rate to the threshold rate described for that event in the stopping rule. If the lower confidence limit is greater than the threshold, the stopping rule will be considered to have been met and the Medical Monitor, DSMB and study PI will be notified.

The following are tables illustrating, for each stopping rule, the minimum numbers of subjects with the respective event that would meet the stopping rule for selected numbers of subjects.

Table 8a: Minimum Numbers of Subjects with the Event in All Randomized Subjects that meet the Stopping Rule for PTLD, Adverse Events for Tuberculosis Active Disease, Coccidioidomycosis & Histoplasmosis disease with a Threshold of Concern of 1%

Number of Subjects with Event	Number of Randomized Subjects Total	Cumulative Incidence Rate (%)	Lower 95% Exact Confidence Limit (%)
1	5	20.00	1.02
2	30	6.67	1.20
3	80	3.75	1.03
4	130	3.08	1.06
5	190	2.63	1.04
6	260	2.31	1.01

Table 8b- Minimum Numbers of Subjects with the Event in All Randomized Subjects that meet the Stopping Rule for Incidence of Invasive Fungal Infections of 3% or More

Number of Subjects with Event	Number of Randomized Subjects Total	Cumulative Incidence Rate (%)	Lower 95% Exact Confidence Limit (%)
7	110	6.36	3.02
9	150	6.00	3.17
11	190	5.79	3.28
12	230	5.22	3.04
14	270	5.19	3.16

Table 9: Minimum Numbers of Subjects with the Event in All Randomized Subjects That meet the Stopping Rule for Death with a Threshold of Concern of 10%

Number of Subjects with Event	Number of Randomized Subjects Total	Cumulative Incidence Rate (%)	Lower 95% Exact Confidence Limit (%)
4	10	40.00	15.00
7	30	23.33	11.50
10	50	20.00	11.27
16	100	16.00	10.30
22	150	14.67	10.14
28	200	14.00	10.14
34	250	13.60	10.17

Table 10: Minimum Numbers of Subjects with the Event in Either Randomized Treatment Arm That meet the Stopping Rule for Infection of any type requiring Hospitalization With a Threshold of Concern of 40%

Number of Subjects with Event	Number of Randomized Subjects in Either Treatment Arm	Cumulative Incidence Rate (%)	Lower 95% Exact Confidence Limit (%)
8	10	80.00	49.31
17	30	56.67	40.16
27	50	54.00	41.48
36	70	51.43	40.99
45	90	50.00	40.88
54	110	49.09	40.89
62	130	47.69	40.20

Table 11: Minimum Numbers of Subjects with the Event in Either Randomized Treatment Arm That meet the Stopping Rule for Graft Loss with a Threshold of Concern of 20%

Number of Subjects with Event	Number of Randomized Subjects in Either Treatment Arm	Cumulative Incidence Rate (%)	Lower 95% Exact Confidence Limit (%)
5	10	50.00	22.24
11	30	36.67	22.11
16	50	32.00	21.21
21	70	30.00	21.06
25	90	27.78	20.11
30	110	27.27	20.36
35	130	26.92	20.59

Table 12: Minimum Numbers of Subjects with the Event in Either Randomized Treatment Arm That meets the Stopping Rule for BPAR (Banff Grade 1 or higher) or AMR
Based on local read **with a Threshold of Concern of 25%**

Number of Subjects with Event	Number of Randomized Subjects in Either Treatment Arm	Cumulative Incidence Rate (%)	Lower 95% Exact Confidence Limit (%)
6	10	60.00	30.35
13	30	43.33	27.87
19	50	38.00	26.51
25	70	35.71	26.19
30	90	33.33	25.12
36	110	32.73	25.34
42	130	32.31	25.54

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

12. Safety Monitoring and Reporting

12.1. 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. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly (per Section 12.5, *Reporting of Serious Adverse Events and Adverse Events*) to the sponsor, DAIT/NIAID. Appropriate notifications will also be made to site principal investigators, Institutional Review Boards (IRBs)/Institutional Ethics Committees (IECs) and health authorities.

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, and Division 5 of the Canadian Food and Drug Regulations applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version *4.0*: (http://ctep.cancer.gov/reporting/ctc.html).

12.2. Definitions

12.2.1. Adverse Event (AE)

Any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject'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/policy/advevntguid.html#Q2).

12.2.2. Suspected Adverse Reaction (SAR)

Any adverse event for which there is a reasonable possibility that the investigational drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)/ICHE2A).

Suspected adverse reactions associated with the study therapy regimen or study procedures are collected and reported to the sponsor. The sponsor will relay any suspected adverse reactions to the DSMB, as appropriate.

12.2.3. Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the package insert or is not listed at the specificity, severity or rate of occurrence that has been observed; or is not consistent with the risk information described in the general investigational plan or elsewhere in the IND.

"Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the 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)*] Division 5 of the Canadian Food and Drug Regulations and ICH E2A}*

For events assessed in association with kidney biopsy, an AE or suspected adverse reaction is considered "unexpected" if it is not listed in the protocol or is not listed at the specificity, severity or rate of occurrence that has been observed.

12.2.4. Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or Sponsor DAIT/NIAID, it results in any of the following outcomes (21 CFR 312.32(a), Division 5 of the Canadian Food and Drug Regulations and ICH E2A):

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 [add DAIT/NIAID or other Sponsor, *if applicable],* its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
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 subject 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.

12.3. Grading and Attribution of Adverse Events

12.3.1. Grading Criteria

The study site will grade the severity of adverse events experienced by the study subjects according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE). This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. The NCI-CTCAE has been reviewed by the *Protocol Chair(s), Principal Investigator, etc.* and has been deemed appropriate for the subject population to be studied in this protocol.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Table 13. NCI-CTCAE Adverse Event Grading Scale

Grade 1	Mild Adverse Event
Grade 2	Moderate Adverse Event
Grade 3	Severe and Undesirable Adverse Event
Grade 4	Life-Threatening or Disabling Adverse Event
Grade 5	Death

For this trial, only events that are ≥ 3 will be recorded as adverse events. Additionally, all events that meet serious criteria will be recorded as AEs/SAEs, regardless of severity grade.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent adverse event is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to baseline will also be recorded as adverse events, but are not treatment-emergent. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an adverse event if changes in therapy or monitoring are implemented as a result of the event/result.

12.3.2. Attribution Definitions

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE electronic case report form (AE/SAE eCRF). Final determination of attribution for safety reporting will be determined by DAIT/NIAID. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided 12.2.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: http://ctep.cancer.gov/reporting/ctc.html.

Table 14. Attribution of Adverse Events

Code	Descriptor	**Relationship** (to primary investigational product and/or other concurrent mandated study therapy or study procedure)
UNRELATED CATEGORY		
1	**Unrelated**	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.
RELATED CATEGORIES		
2	**Possible**	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	**Definite**	The adverse event is clearly related.

For this study, attribution assessment for the study therapy regimen and study mandated procedures would be made when an adverse event/Serious Adverse Event is recorded.

Table 15. Study therapy regimen

Experimental Arm	• **Investigational Agent** o Remicade® (Infliximab) • **Standard of Care Immunosuppression** o Thymoglobulin® (Anti-Thymocyte Globulin-Rabbit) o Tacrolimus (Prograf®) or equivalent, or an equivalent starting dose of a once-daily formulation of tacrolimus o Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA (or their generic equivalents) o Prednisone or generic equivalent
Control Arm	• **Standard of Care Immunosuppression** o Placebo o Thymoglobulin® (Anti-Thymocyte Globulin-Rabbit) o Tacrolimus (Prograf®) or equivalent, or an equivalent starting dose of a once-daily formulation of tacrolimus o Mycophenolate Mofetil/MMF or Mycophenolate Acid/MPA (or their generic equivalents) o Prednisone or generic equivalent

Table 16. Study mandated procedures

Procedures	• Blood Draw • Biopsy

12.4. Collection and Recording of Adverse Events

12.4.1. Collection Period

Adverse events will be collected from the initiation of the investigational treatment until one of the following time points: a subject completes study participation or 45 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

<u>After Month 24/Visit 14</u>

After Month 24/Visit 14 sites will only report grade 3 or higher AEs and any SAE regardless of severity that occur within 24 hours after a protocol mandated procedure of blood draw.

<u>Collecting Adverse Events</u>

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

- Observing the subject
- Interviewing the subject [e.g., using a checklist, structured questioning, diary, etc.]
- Receiving an unsolicited complaint from the subject

In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in Section 12.3, *Grading and Attribution of Adverse Events.*

12.4.2. Recording Adverse Events

Throughout the study, the investigator will record adverse events and serious adverse events as described previously (Section 12.2, *Definitions)* on the appropriate AE/SAE eCRF regardless of the relationship to study therapy regimen or study procedure.

- COVID-19 infections of any grade

Certain adverse events occur commonly in this study population and will not be recorded as an adverse event, unless it meets the definition of a serious adverse event.

- Upper respiratory infection, nasopharyngitis, bronchitis, diarrhea, constipation, nausea, vomiting, abdominal pain, hypocalcaemia, hypercalcemia, hypercholesterolemia, hypomagnesemia, hyperuricemia, edema, pyrexia, hematuria, proteinuria, dysuria, cough, dyspnea, arthralgia, back pain, hip pain, fracture, headache, dizziness, tremor, acne, insomnia, anxiety, depression, stomatitis/aphthous ulcers, wound complications, AVF thrombosis, neutropenia, renal impairment, renal artery stenosis, incontinence, hydronephrosis, hematoma, lymphocele, musculoskeletal pain, alopecia, hyperhidrosis, atrial fibrillation, anemia, lymphocytopenia
- An elective hospitalization or hospital admission for the purpose of conducting protocol-mandated procedures is not to be reported as an SAE unless the hospitalization is prolonged due to complications.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 45 days after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

12.5. Reporting of Serious Adverse Events and Adverse Events

12.5.1. Reporting of Serious Adverse Events to Sponsor

This section describes the responsibilities of the site investigator to report serious adverse events to the sponsor via the SACCC eCRF. Timely reporting of adverse events is required by 21 CFR, Division 5 of the Canadian Food and Drug Regulations and ICH E6 guidelines.

Site investigators will report all serious adverse events (see Section 12.2.3, *Serious Adverse Event)*, regardless of relationship or expectedness **within 24 hours of discovering the event**.

For serious adverse events, all requested information on the AE/SAE *eCRF* will be provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE *eCRF* will be updated and submitted.

12.5.2. Reporting to Health Authority

After an adverse event requiring 24-hour reporting (per Section 12.5.1, *Reporting of Serious Adverse Events to Sponsor*) is submitted by the site investigator and assessed by *DAIT/NIAID*, there are two options for *DAIT/NIAID* to report the adverse event to the appropriate health authorities (Annual Reporting and Expedited Reporting).

12.5.2.1 Annual Reporting

DAIT/NIAID will include in the annual study report to health authorities all adverse events classified as:

- Serious, expected, suspected adverse reactions (see Section 12.2.1.1, Suspected Adverse Reaction, and Section 12.2.2, Unexpected Adverse Event)
- Serious and not a suspected adverse reaction (see Section 12.2.2, Suspected Adverse Reaction)
- Pregnancies

Note that all adverse events (not just those requiring 24-hour reporting) will be reported in the Annual IND Report.

12.5.2.2 Expedited Safety Reporting

This option, with 2 possible categories, applies if the adverse event is classified as one of the following:

Category 1: **Serious and unexpected suspected adverse reaction** (**SUSAR)**
(See Section 12.2.1.1, *Suspected Adverse Reaction* and Section 12.2, *Unexpected Adverse Event* and 21 CFR 312.32(c) (1)i, Division 5 of the Canadian Food and Drug Regulations and ICH E2A). The sponsor shall report any suspected adverse reaction that is both serious and unexpected. The sponsor shall report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the adverse event, such as:

1. A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, or Stevens-Johnson Syndrome);
2. One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
3. An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

Category 2: **Any findings from studies that suggests a significant human risk**
The sponsor shall report any findings from other epidemiological studies, analyses of adverse events within the current study or pooled analysis across clinical studies or animal or *in vitro* testing (e.g. mutagenicity, teratogenicity, carcinogenicity) that suggest a significant risk in humans exposed to the drug that would result in a safety-related change in the protocol, informed consent, investigator brochure or package insert or other aspects of the overall conduct of the study.

DAIT/NIAID shall notify the appropriate health authorities (*FDA and Health Canada)* and all participating investigators of *Expedited Safety Reports* within 15 calendar days; unexpected fatal or immediately life-threatening suspected adverse reaction(s) shall be reported as soon as possible or within 7 calendar days.

12.5.3. 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 and Health Canada shall be distributed by DAIT/NIAID or designee to all participating institutions for site IRB/IEC submission.

12.6. Pregnancy Reporting

The investigator shall be informed immediately of any pregnancy in a study subject or a partner of a study subject. *A* pregnant subject will not be infused. The investigator shall counsel the subject per site standard of care.

The investigator shall report to the *SACCC and DAIT/NIAID* all pregnancies within 1 business day of becoming aware of the event using the Pregnancy *eCRF*. All pregnancies identified during the study shall be followed to conclusion and the outcome of each must be reported. The Pregnancy *eCRF* shall be updated and submitted to *SACCC and DAIT/NIAID* when details about the outcome are available. When possible, similar information shall be obtained for a pregnancy occurring in a partner of a study subject.

Information requested about the delivery shall include:

- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

All pregnancy complications that result in a congenital abnormality, birth defect, miscarriage, and medically indicated abortion will be considered an SAE. Pregnancies reported as SAEs will be submitted to the SACCC, DAIT/NIAID, the FDA and Health Canada using the SAE reporting procedures described above.

12.7. COVID-19 Reporting

COVID-19 is an event of medical importance. Centers must report any instance of COVID-19 infection as a Serious Adverse Event. If none of the standard criteria for a serious event are met, the event should still be reported as an SAE due to its medical importance. This event should be reported (entered on the AE/SAE form) within 24 hours of awareness even if the event does not meet serious criteria.

12.8. Reporting of Other Safety Information

An investigator shall promptly notify the site *IRB/ IEC* as well as the *SACCC using the AE/SAE form when an "unanticipated problem involving risks to subjects or others" is identified, which is not otherwise reportable as an AE.*

12.9. Review of Safety Information

The PI, the DAIT/NIAID Medical Monitor, and the NIAID/DAIT Transplant DSMB will review safety data on an ongoing basis. Enrollment and initiation of study treatment may be suspended at any time if any of these reviews conclude there are significant safety concerns.

12.9.1. Medical Monitor Review

DAIT/NIAID Medical Monitor shall receive monthly reports from the *SACCC* compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study sites on appropriate eCRFs.

In addition, the Medical Monitor shall review and make decisions on the disposition of the SAE and pregnancy reports received by the *SACCC* (See Sections 12.5.1, *Reporting of Serious Adverse Events to Sponsor*, and 12.6, *Pregnancy Reporting*).

12.9.2. DSMB Review

12.8.2.1 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. The DSMB will be informed of an Expedited Safety Report in a timely manner.

12.8.2.2 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 study principal investigator or DAIT/NIAID. Additionally, satisfaction of any of the stopping rules listed in Section 11.5 will trigger an *ad hoc* DSMB Safety Review.

12.8.2.3 Temporary Suspension of Enrollment/Drug Dosing for Ad Hoc DSMB Safety Review

A temporary halt in enrollment, randomization and administration of Remicade®/infliximab or Remicade®/infliximab placebo will be implemented if an ad hoc DSMB safety review is required.

13. Statistical Considerations and Analytical Plan

13.1. Overview

CTOT-19 is a Phase 2, randomized, double blind, controlled clinical trial in which 300-deceased donor kidney transplant recipients will be randomized 1:1 to the experimental or control arms. Each group will receive rATG induction in combination with either infliximab or placebo pre-reperfusion followed by maintenance therapy consisting of tacrolimus, either MMF or enteric coated MPA, and prednisone. The objective of the study is to determine the efficacy of intravenous infliximab administered at the time of transplantation on 2-year kidney transplant survival and function.

13.2. Endpoints

As described in section 2, the study endpoints are outlined in the table below.

Endpoints
Primary Endpoint The difference between the mean 24-month eGFR (modified MDRD) in the experimental vs. control arms.
Secondary Endpoints (Efficacy, Safety and Mechanistic Endpoints) **<u>Efficacy Endpoints</u>** **1.** Proportion of subjects with biopsy proven acute cellular rejection (BPAR) within **a)** 6 month and **b)** 2 years of transplant **2.** BANFF grades of first Acute Cellular Rejections (ACR) within 6 month of transplant **3.** Proportion of subjects with biopsy proven acute cellular rejection (BPAR) or borderline rejection within **a)** 6 month and **b)** 2 years of transplant **4.** Proportion of subjects with biopsy proven acute antibody mediated rejection (AMR) within 6 months and 2 years of transplant **5.** Proportion of subjects with biopsy proven acute antibody mediated rejection AMR or suspicious for AMR within **a)** 6 months and **b)** 2 years of transplant **6.** BANFF grades of first AMR within 6 months of transplant **7.** Proportion of subjects with BANFF chronicity scores >or equal 2 on 24-month biopsy **8.** Change in BANFF chronicity scores between implantation and 24 month biopsies **9.** eGFR (as measured by both MDRD and CKD-EPI) **a)** Change in eGFR between 3 months and 24 months **b)** Change in eGFR between post-transplant nadir (lowest eGFR in first 6 months) and 24 months **c)** eGFR on days 7, 30, 90, and 180 post-transplant **10.** Proportion of subjects with: **a)** Death or graft failure within 2 years **b)** Only graft failure within 2 years

11. Each of the following:
- **a)** Proportion of subjects that required at least one dialysis treatment within the first week after transplantation
- **b)** Number of dialysis sessions in the first 8 weeks post-transplantation.
- **c)** Duration of DGF defined as time from transplantation to the last required dialysis treatment
- **d)** The incidence of primary non-function (PNF), defined as for dialysis-dependency for more than 3 months or an eGFR 20 ml/min or less at 90 days post-transplant
- **e)** Change from baseline (immediately after surgery) in serum creatinine and serum creatinine concentration at 24, 48, and 72 hours.

12. Days from transplantation until event (ACR, AMR, or hospitalization for infection and or malignancy)

13. Rate of Slow Graft Function (SGF):
- **a)** The proportion of patients with a serum creatinine of more than 3 mg/dL at day 5 post-transplant,
- **b)** Creatinine reduction ratio (CRR) on day 2
- **c)** Creatinine reduction ratio (CRR) on day 5
- **d)** The proportion of patients whose day 5 serum CRR was less than 70%
- **e)** The proportion of patients whose day 2 serum CRR was less than 30%
- **f)** Proportion of subjects who need dialysis after 1 week.

Safety/Complication Endpoints

1. Proportion of subjects with:
- **a)** Any infection requiring hospitalization or resulting in death
- **b)** With mycobacterial or fungal infections

2. Proportion of subjects with CMV viremia that require a change in immunosuppression or anti-viral treatment as per standard of care at the site

3. Proportion of subjects with BK viremia that require a change in immunosuppression or anti-viral treatment as per standard of care at the site

4. Proportion of subjects with malignancy

5. Proportion of subjects with impaired wound healing manifested by wound dehiscence, wound infection, or hernia at the site of the transplant incision.

Mechanistic Endpoints

1. Sensitivity, specificity, PPV, and NPV of biomarkers, including PRT, urinary CXCL9, blood genomic profile, and 3-month allograft genomic profile (alone and/or in combination) to predict:
- **a)** Incident biopsy-proven acute rejection.
- **b)** Graft loss
- **c)** Chronic graft injury, as measured by 2-year eGFR

2. Each of the following:
- **a)** Inflammatory gene expression profiles
- **b)** Frequency of donor reactive T-cells
- **c)** Frequency and function of Treg
- **d)** Proportion of subjects with de novo DSA within 24 months
- **e)** Fibrogenic gene expression profiles
- **f)** Amount of peritubular capillary loss by histology

Ancillary Endpoints

1. Percentage of predicted prednisone bottle openings as measured by a medication event monitoring system (MEMS®) in the first 3 months post-transplantation
2. Standard deviation of the monthly tacrolimus trough levels from 6 months post-transplantation to 2 years post-transplantation

13.3. Measures to Minimize Bias

Randomization will be carried out with fixed block sizes and stratification by enrolling center to balance the intervention assignments. In order to avoid center-specific measurement error, mechanistic assays will be performed in central laboratories where the lab personnel will be blinded to the randomization status of the study subject. All endpoints are predefined. We will also attempt to enroll all consecutive patients fulfilling inclusion criteria.

13.4. Analysis Plan

All analyses will be detailed in a statistical analysis plan. All analyses will be performed using the SAS System version 9.3 (or higher).

13.4.1. Analysis Populations.

- **Intent-to-Treat (ITT) Sample** – All transplanted and randomized subjects who receive the infliximab/placebo infusion. This sample will be used for efficacy and some safety analyses. Transplanted subjects who receive an infusion, but have no additional follow up will be used in this population
- **Per Protocol (PP) Sample** – All randomized subjects who receive the infliximab/placebo infusion who are compliant with their medications and do not have any major protocol deviations that preclude analysis of the primary endpoint.
- **Safety Sample** - All subjects who receive any infliximab/placebo infusion. This sample will be used for some safety summaries/analyses.
- **Delayed Graft Function (DGF) Sample** –All subjects with delayed graft function. This is defined as any dialysis in the first 7 days. This sample will be used for all DGF related analyses.
- **Screening Sample** – All subjects who are enrolled in the study. This sample will be used for disposition summaries.

Subjects will be analyzed in the group to which they were randomized, regardless of the treatment received.

13.4.2. Primary Analysis of Primary Endpoint(s)/Outcome(s)

All subjects in the ITT sample will be used for the primary analysis. Mean eGFR of each of the two treatment arms will be compared within a restricted maximum likelihood repeated measures model (MMRM) framework, wherein repeated measures of eGFR at months 1, 3, 6 12, 18, and 24 will be modeled using fixed effects of treatment group, collection time point, and the interaction between time point and treatment group. A random subject effect will also be included in the model to appropriately adjust for the correlation of having multiple eGFR values for each subject. The following material presents the notation and the model.

Let

t index the two treatment arms, $t = 1$ for placebo control, i.e., rATG induction in combination with placebo; $t = 2$ for experimental treatment arm, i.e., rATG induction in combination with infliximab;

s index subjects within treatment arms, $s = 1, 2, \ldots, N_t$= number of subjects in treatment arm t whose data are in the ITT Analysis Set of Data (the data from the subjects in the ITT Analysis Set of Subjects or "ITT Analysis Population");

m index the post-randomization measurement or evaluation occasions, with $m = 1 \leftrightarrow$ month 1 evaluation, $m = 2 \leftrightarrow$ month 3 evaluation, $m = 3 \leftrightarrow$ month 6 evaluation, $m = 4 \leftrightarrow$ month 12 evaluation, $m = 5 \leftrightarrow$ month 18 evaluation, and $m = 6 \leftrightarrow$ month 24 evaluation.

Y_{tssm} = eGFR value from subject number s (s=1, 2, …, N_t) within treatment arm t (t = 1 for placebo control, i.e., rATG induction in combination with placebo; t = 2 for experimental treatment arm, i.e., rATG induction in combination with infliximab), $m = 1 \leftrightarrow$ month 1 evaluation, $m = 2 \leftrightarrow$ month 3 evaluation, $m = 3 \leftrightarrow$ month 6 evaluation, $m = 4 \leftrightarrow$ month 12 evaluation, $m = 5 \leftrightarrow$ month 18 evaluation, and $m = 6 \leftrightarrow$ month 24 evaluation.

The MMRM for Y_{tssm} is, for the values of t, s, and m given above:

$$Y_{tsm} = \mu_{tm} + \delta_{ts} + \varepsilon_{tsm}$$

where

$\mu_{tm} = E[Y_{tsm}]$ is the population mean eGFR for subjects treated with treatment t and followed for the time indicated by the index m;

δ_{ts} is the random effect on eGFR associated with subject s in treatment arm t;

$\varepsilon_{tsm} = Y_{tssm} - (\mu_{tm} + \delta_{ts})$ is the random component in the eGFR measurement from subject s in treatment arm t at evaluation occasion m.

Assumptions:

- Each μ_{tm} is an unknown, fixed constant parameter; an MMRM will be used to estimate the values of the μ_{tm}.
- Each δ_{ts} is a random variable that is stochastically independent of each other $\delta_{t's'}$, where $(t,s) \neq (t',s')$; $E[\delta_{ts}]=0$, $\sigma^2_\delta = Cov(\delta_{ts}, \delta_{ts})$ for each combination of t and s
- Each ε_{tsm} is a random variable that is stochastically independent of each other $\varepsilon_{t's'm'}$, where $(t,s,m) \neq (t',s',m')$; $E[\varepsilon_{tsm}]=0$, $\sigma^2_\varepsilon = Cov(\varepsilon_{tsm}, \varepsilon_{tsm})$ for each combination of t, s, and m.

It follows that $Cov[Y_{tsm}, Y_{tsm'}] = \sigma^2_\delta + \sigma^2_\varepsilon$ and $Cov[Y_{tsm}, Y_{tsm'}] = \sigma^2_\delta$ for $m \neq m'$. That is, the expected-value component of the MMRM is a cell means model and the covariance component is a compound symmetric model.

Let τ denote the "treatment effect at 24 months", defined as $\tau = \mu_{t6} - \mu_{c6}$, i.e., the difference in 24-month population mean eGFR for the experimental treatment minus the corresponding placebo mean. τ is a parameter of primary interest; the MMRM will be used to estimate τ.

The primary null and alternative hypotheses are: $H_0: \tau = 0$ vs. $H_1: \tau \neq 0$. The MMRM will be used to test these hypotheses at the $\alpha = 0.05$ Type I error rate.

SAS PROC MIXED (ver. 9.3 or higher) will be used to fit the MMRM to the data, estimate the parameters, and test the primary hypotheses along with others.

The mean eGFR at 24 months will also be summarized and the difference will be presented with a 95% confidence interval. Additional graphical summaries of the eGFR over time will be used to further illustrate any differences between the two treatment groups. MMRM assumptions will be evaluated graphically via standard residual analyses. If the assumptions fail, a suitable transformation (e.g., natural logarithms) will be utilized and the data will be reanalyzed on the transformed scale. Results associated with the F-test for the contrast and the corresponding mean treatment difference with two-sided 95% confidence intervals will be reported. Missing data will not be imputed for the primary analysis; however, a sensitivity analysis will be employed to assess the impact of missing data due to subjects who terminate the study early.

13.4.3. Supportive Analyses of the Primary Endpoint(s)/Outcome(s)

Because subject drop-outs represent an important area to carefully examine, (86) we propose to compare 1) the proportion of subjects who drop out prior to the 24-month visit (i.e., premature withdrawals), 2) the timing of premature withdrawals, and 3) reasons for premature withdrawals. These comparisons will look for differences between treatment groups that could indicate that the missing at random (MAR) assumption in the primary analysis model does not hold. Additionally, we will utilize the methods of Ma et al (87) to provide an objective index of the level of non-ignorable missingness.

If the MAR assumption fails, we will perform a sensitivity analysis to examine the impact of the missing data on our inference. The first sensitivity analysis will use a pattern mixture model (PMM) fitted via multiple imputation (88), and is an example of a “controlled imputation” method (85, 89) and will implement a jump to control (89) approach. For subjects who drop out of the study, this method imputes missing values for both treatment groups from the placebo population, which reduces any bias away from the null hypothesis of no difference between treatment groups.

Additionally, we will perform a second sensitivity analysis that imputes a single point for subjects (in either treatment group) that have had a graft loss or have died. Specifically, subjects who have a graft loss prior to 24 months would have an eGFR of 10 imputed for their remaining eGFR collection time points. Subjects who die with a functioning kidney would have a “last observation carried forward” imputation, which means that we would impute their last known eGFR prior to death for all remaining eGFR collection time points. This final eGFR would be based on the central evaluation of eGFR if the collection time was within 1 month of death and on a local assessment of eGFR if the last collection for the central labs was more than 1 month from death.

A third sensitivity analysis will compare the eGFR at 24 months between the two treatment arms using a Wilcoxon test. Subjects who experience death or graft loss will be given the worst ranks. Subjects who are missing their 24-month eGFR due to other causes will receive a rank based on an imputed eGFR from the model used in the primary analysis.

13.4.4. Analyses of Secondary and Other Endpoint(s)/Outcome(s)

The secondary endpoints are to be computed and summarized by treatment and, where appropriate, by day/month and treatment. Continuous variables will be summarized using means and standard deviations or, if appropriate, medians and inter-quartile ranges, and categorical variables will be summarized using frequencies and percentages. 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 (Table 17) lists each endpoint and its measurement scale (continuous, dichotomous, and ordinal). Secondary analyses are considered descriptive.

Longitudinal continuous variables will be modeled using a MMRM similar to the one used to analyze the primary endpoint. Non-longitudinal continuous variables will be analyzed using an ANOVA (e.g. change from baseline in eGFR). Dichotomous measures of prevalence and incidence will be analyzed using a chi-square test. Time to event variables will be analyzed by fitting Cox regression models to the data (90). The change in BANFF cell-mediated AR score will be analyzed using a generalized McNemar's test.

Table 17. Summary of Proposed Analyses for CTOT-19 Secondary Clinical Endpoints

Response Type (Sample)	Response	Measurement Scale	Summary Statistics	Models to test for treatment effects
Incidence and Severity of Rejection Endpoints (ITT, PP)	Proportion of subjects with biopsy proven acute cellular rejection (BPAR) within a) 6 month and b) 2 y of transplant	dichotomous	proportion + 95% CI	Chi-square test
	BANFF grades of first Acute Cellular Rejections (ACR) within 6 month of transplant	categorical	Table	Chi-square test
	Proportion of subjects with biopsy proven acute antibody mediated rejection (AMR) within 6 months and 2 y of transplant	dichotomous	proportion + 95% CI	Chi-square test
	BANFF grades of first AMR within 6 months of transplant	categorical	Table	Chi-square test
	Proportion of subjects with BANFF chronicity scores >or equal 2 on 24 month biopsy	dichotomous	proportion + 95% CI	Chi-square test
	Change in BANFF chronicity scores between implantation and 24 month biopsies	Categorical	Shift table	Generalized McNemar's test
	Days from transplantation until event (ACR, AMR, or hospitalization for infection and or malignancy)	Time to event	Kaplan-Meier estimates	Cox model
Renal Function Endpoints (ITT, PP)	Change in eGFR (as measured by both MDRD and CKD-EPI) between 3 months and 24 months	continuous	Mean + 95% CI	ANOVA
	Change in eGFR (as measured by both MDRD and CKD-EPI) between post-transplant nadir (lowest eGFR in first 6 months) and 24 months	continuous	mean + 95% CI	ANOVA

	Estimated GFR (as measured by both MDRD and CKD-EPI) on days 7, 30, 90, and 180 post-transplant	continuous	mean + 95% CI	MMRM, (repeated time effect; fixed effects of treatment, time and treatment by time interaction)
Graft Failure Endpoints	Proportion of subjects with death or graft failure within 2 years	dichotomous	proportion + 95% CI	Chi-square test
	Proportion of subjects with only graft failure within 2 years	dichotomous	proportion + 95% CI	Chi-square test
Delayed Graft Function Endpoints (DGF Sample)	Proportion of subjects that required at least one dialysis treatment within the first week after transplantation	dichotomous	proportion + 95% CI	Chi-square test
	Number of dialysis sessions in the first 8 weeks post-transplantation	continuous	mean and/or geometric mean + 95% CI	Poisson regression
	Duration of DGF defined as time from transplantation to the last required dialysis treatment	continuous	mean and/or geometric mean + 95% CI	ANOVA
	The incidence of primary non-function (PNF), defined as dialysis-dependency for more than 3 months or an eGFR 20 ml/min or less at 90 days post-transplant	dichotomous	proportion + 95% CI	Chi-square test
	Change from baseline (immediately after surgery) in serum creatinine and serum creatinine concentration at 24, 48, and 72 hours	continuous	mean + 95% CI	ANOVA
	Proportion of patients with total 24-hour urine output of more t00 ml on days 2, 3	dichotomous	proportion + 95% CI	Chi-square test
Slow Graft Function (ITT, PP)	The proportion of patients with a serum creatinine of more than 3 mg/dL at day 5 post-transplant	dichotomous	proportion + 95% CI	Chi-square test
	Creatinine reduction ratio (CRR) on day 2	ratio	mean + 95% CI	ANOVA
	Creatinine reduction ratio (CRR) on day 5	ratio	mean + 95% CI	ANOVA
	The proportion of patients whose day 5 serum CRR was less than 70%	dichotomous	proportion + 95% CI	Chi-square test

	The proportion of patients whose day 2 serum CRR was less than 30%	dichotomous	proportion + 95% CI	Chi-square test
	Proportion of subjects who need dialysis after 1 week	dichotomous	proportion + 95% CI	Chi-square test

We will test a biomarker panel to predict and diagnose incipient ACR and to determine the accuracy of early post-transplant markers to define individuals most at risk for late graft dysfunction and chronic graft injury. This panel will include reactive T cell (PRT) assays, urinary chemokine, urinary PCR assays, gene expression profiles in peripheral blood, and gene expression profiles in graft tissue. If the comparison of the biomarkers between the two treatment groups (see previous paragraph) yields no differences, we will utilize the entire study population. If, however, we find an influence of the infliximab on the biomarkers, we will limit this investigation to the control subjects. This evaluation will be based primarily on examining the Receiver Operating Characteristic (ROC) Curves generated for each candidate biomarker and the associated classification accuracy in terms of sensitivity and specificity as well as positive and negative predictive value. We will also explore combining the candidate biomarkers to determine whether there are distinct combinations of biomarkers that may improve performance relative to individual biomarkers.

Our hypothesis that anti-TNFa limits activation of donor reactive immunity and prevents initiation and progression of graft fibrosis by dampening early inflammation within the allograft will be assessed by a series of linear mixed models. The assays to assess these effects will include T cell (PRT) assays, urinary chemokine, urinary PCR assays, gene expression profiles in peripheral blood, gene-expression profiles in graft tissue, donor-reactive effector T cells, markers of inflammation, regulatory T cells, donor specific antibody, fibrogenic gene profiles, and peritubular capillary loss. Models will be fit sequentially, with specific models fit to address specific elements of the hypothesized mechanism. Initially we will use regression models to quantify the extent to which infliximab, when added to therapy with ATG, reduces “early” inflammation at the time of transplantation, measured both by cellular reactivity in blood and from post-implantation biopsies. Corresponding linear regression models will assess differences between treatment groups with respect to inflammation measures at 24 mos., and mixed linear models will be used to assess the inflammation measures over the entire time course subsequent to the “early” evaluation. Additionally, proportional odds models will be used to assess treatment differences in ordinal grade of graft fibrosis at 24 mos. and, through a generalized estimating equation (GEE) approach, over time.

Should chance imbalances occur despite randomization, estimates of treatment effects on primary and some secondary outcomes will be obtained from multivariable models that include the treatment factor, the potential confounders and statistically significant interactions between the covariates and the treatment factor. In particular, the two ancillary adherence measures, the standard deviation of tacrolimus trough levels and the MEMS® percentage of predicted medication use will be included in the analysis of potential confounding variables.

13.4.5. Analyses of Safety Endpoints

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 NCI-CTCAE 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.

Specific AEs of interest include infections, hospitalizations, malignancies, and wound healing. The accompanying table (Table X) lists each of these AE endpoints and its measurement scale (continuous, dichotomous, and ordinal) as well as the summary statistics and the statistical model that will be used to compare the endpoints between treatment groups.

Table 18. Summary of Proposed Safety Endpoints and Analyses for CTOT-19

Response Type (Sample)	Response	Measurement Scale	Summary Statistics	Models to test for treatment effects
Safety Endpoints (ITT)	Proportion of subjects with **a)** any infection requiring hospitalization or resulting in death **b)** mycobacterial or fungal infections	dichotomous	proportion + 95% CI	Chi-square test
	Proportion of subjects with CMV viremia that require a change in immunosuppression or anti-viral treatment as per standard of care at the site	dichotomous	proportion + 95% CI	Chi-square test
	Proportion of subjects with BK viremia that require a change in immunosuppression or anti-viral treatment as per standard of care at the site	dichotomous	proportion + 95% CI	Chi-square test
	Proportion of subjects with malignancy	dichotomous	proportion + 95% CI	Chi-square test
	Proportion of subjects with impaired wound healing manifested by wound dehiscence, wound infection, or hernia at the site of the transplant incision	dichotomous	proportion + 95% CI	Chi-square test

13.4.6. Descriptive Analyses

Disposition of subjects will be summarized by treatment group (including the number of subjects who complete the study, number lost to follow-up, number withdrawn from study, times to lost to follow-up, and reasons for discontinuation). Baseline and demographic subject characteristics will also be summarized by treatment group. Other clinical characteristics will be summarized by treatment group and study visit as needed.

13.5. Interim Analysis

There are no planned interim analyses for this study.

13.6. Sample Size Calculations

Primary endpoint of eGFR (MDRD) at 24 months: Data from published studies employing similar control arms with deceased donor allograft recipients induced with ATG on CNI, MMF and prednisone (BENEFIT trial among others) indicate 24 mo. GFRs range from 55-70 ml/min with s.d. ~23-27 ml/min. Conservatively assuming a s.d. of 25 ml/min, 130 patients per arm will provide 90% power to detect a difference of 10 ml/min in eGFR between groups using a two-sided two-sample t-test at an alpha level of 0.05. We will be able to detect smaller differences in eGFR should the size of the s.d. be less than 25 mL/min. Note that in our preliminary assessment of eGFRs in CTOT-01, the mean ± s.d. 6 mo. values were 57.7± 17.1, a lower s.d. that would permit detection of eGFR differences of < 8 ml/min between groups with 90% power.

Assuming a 15% dropout rate (10% due to death or graft loss, 5% for other reasons) we require 150 patients per arm. Based on a sample size of 300 (150 patients/arm), power calculations for selected secondary endpoints are given in **Table 18**.

Table 19. Power for secondary endpoint analyses

Secondary Endpoint	Expected Outcome in Control Group	Expected outcome in treatment group (80%/90% power)	Detectable Risk-Reduction (80%/90% Power)
BPAR at 12 months	15%	5.3%/4.1%	65%/73%
Graft loss at 12 months	10%	2.3%/1.4%	77%/86%
SGF	23%	10.9%/9.3%	53%/60%
DGF	40%	24.9%/22.7%	38%/43%

(**BPAR**: Biopsy Proven Acute Rejection, **SGF**: Slow Graft Function, **DGF**: Delayed Graft Function)

14. Identification and Access to Source Data

14.1. Source Data

Source documents and source data are considered to be the original documentation where subject information, visits consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a clinical trial.

14.2. Access to Source Data

The site investigators and site staff will make all source data available to the DAIT/NIAID, as well as to relevant health authorities. Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

15. Quality Assurance and Quality Control

15.1. Quality Assurance

The sponsor will review site processes for quality management of the protocol prior to enrollment at each clinical center, to include processes for data and biological specimen collection. Expectations will be communicated to each site regarding study conduct. In addition, all study staff are required to have GCP and ICH training.

15.2. Quality Control

A quality control plan for electronic data capture and data management will be created by the data center and will be reviewed by the sponsor prior to study onset. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

The Sponsor will develop a risk-based monitoring plan to direct study monitoring. Sponsor monitors will follow written Standard Operating Procedures (SOPs) to verify that the clinical trial is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

16. Protocol Deviations

16.1. Protocol Deviation Definitions

Protocol Deviation – The investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

Major Protocol Deviation - A Major Protocol Deviation is a deviation from the IRB approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.

Non-Major Protocol Deviation - A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data. Non-Major Protocol Deviations will not be collected in this study.

16.2. Reporting and Managing Protocol Deviations

The study site principal investigator has the responsibility to identify, document and report protocol deviations as directed by the study Sponsor. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

17. Ethical Considerations and Compliance with Good Clinical Practice

17.1. 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 *IRB or Ethics Committee*. The IRB, Ethics Committee or relevant health authority (FDA or Health Canada) will also approve any amendments to the protocol or to the consent materials before they are implemented.

17.2. 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 site investigator or a study physician designee listed on the 1572, must conduct a meeting with the potential study participant to review all of the required elements of informed consent, review the consent form and answer questions. The conversation between investigator or their physician designee and the potential study participant must be documented in participant's medical record. The study physician designee listed on the 1572 must be a medical doctor licensed to practice medicine in the United States for US sites and possess appropriate licenses to practice medicine in Canada for the Canadian sites. Coordinators, nurses, physician assistants, nurse practitioners, non-physicians listed on the 1572 and other support staff may provide additional trainings and discussions to potential study participants, but **cannot** serve as the physician designee listed above.

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.

17.3. 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.

18. Publication Policy

The CTOT policy on the publication of study results will apply to this trial. The CTOT Publications Policy is located on the CTOT website at www.ctotstudies.org.

19. References

1. Pascual M, Theruvath T, Kawai T, Tolkoff-Rubin N, Cosimi AB. Strategies to improve long-term outcomes after renal transplantation. *N Engl J Med* 2002;346:580-590.
2. Bostom AD, Brown RS, Jr., Chavers BM, Coffman TM, Cosio FG, Culver K et al. Prevention of post-transplant cardiovascular disease--report and recommendations of an ad hoc group. *Am J Transplant* 2002;2:491-500.
3. Meier-Kriesche HU, Baliga R, Kaplan B. Decreased renal function is a strong risk factor for cardiovascular death after renal transplantation. *Transplantation* 2003;75:1291-1295.
4. Padiyar A, Augustine JJ, Hricik DE. Induction antibody therapy in kidney transplantation. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2009;54:935-944.
5. Brennan DC, Flavin K, Lowell JA, Howard TK, Shenoy S, Burgess S et al. A randomized, double-blinded comparison of Thymoglobulin versus Atgam for induction immunosuppressive therapy in adult renal transplant recipients. *Transplantation* 1999;67:1011-1018.
6. Brennan DC, Daller JA, Lake KD, Cibrik D, Del Castillo D, Thymoglobulin Induction Study G. Rabbit antithymocyte globulin versus basiliximab in renal transplantation. *N Engl J Med* 2006;355:1967-1977.
7. Noel C, Abramowicz D, Durand D, Mourad G, Lang P, Kessler M et al. Daclizumab versus antithymocyte globulin in high-immunological-risk renal transplant recipients. *J Am Soc Nephrol* 2009;20:1385-1392.
8. Goggins WC, Pascual MA, Powelson JA, Magee C, Tolkoff-Rubin N, Farrell ML et al. A prospective, randomized, clinical trial of intraoperative versus postoperative Thymoglobulin in adult cadaveric renal transplant recipients. *Transplantation* 2003;76:798-802.
9. Perico N, Cattaneo D, Sayegh MH, Remuzzi G. Delayed graft function in kidney transplantation. *Lancet* 2004;364:1814-1827.
10. Siedlecki A, Irish W, Brennan DC. Delayed graft function in the kidney transplant. *Am J Transplant* 2011;11:2279-2296.
11. Shoskes DA, Cecka JM. Deleterious effects of delayed graft function in cadaveric renal transplant recipients independent of acute rejection. *Transplantation* 1998;66:1697-1701.
12. Yokoyama I, Uchida K, Kobayashi T, Tominaga Y, Orihara A, Takagi H. Effect of prolonged delayed graft function on long-term graft outcome in cadaveric kidney transplantation. *Clinical transplantation* 1994;8:101-106.
13. Yarlagadda SG, Coca SG, Formica RN, Jr., Poggio ED, Parikh CR. Association between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 2009;24:1039-1047.
14. Rosenthal JT, Danovitch GM, Wilkinson A, Ettenger RB. The high cost of delayed graft function in cadaveric renal transplantation. *Transplantation* 1991;51:1115-1118.
15. Koning OH, Ploeg RJ, van Bockel JH, Groenewegen M, van der Woude FJ, Persijn GG et al. Risk factors for delayed graft function in cadaveric kidney transplantation: a prospective study of renal function and graft survival after preservation with University of Wisconsin solution in multi-organ donors. European Multicenter Study Group. *Transplantation* 1997;63:1620-1628.

16. Giblin L, O'Kelly P, Little D, Hickey D, Donohue J, Walshe JJ et al. A comparison of long-term graft survival rates between the first and second donor kidney transplanted--the effect of a longer cold ischaemic time for the second kidney. *Am J Transplant* 2005;5:1071-1075.

17. Salahudeen AK, Haider N, May W. Cold ischemia and the reduced long-term survival of cadaveric renal allografts. *Kidney Int* 2004;65:713-718.

18. Bonventre JV. Pathophysiology of acute kidney injury: roles of potential inhibitors of inflammation. *Contributions to nephrology* 2007;156:39-46.

19. Farrar CA, Zhou W, Lin T, Sacks SH. Local extravascular pool of C3 is a determinant of postischemic acute renal failure. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2006;20:217-226.

20. Zhou W, Farrar CA, Abe K, Pratt JR, Marsh JE, Wang Y et al. Predominant role for C5b-9 in renal ischemia/reperfusion injury. *J Clin Invest* 2000;105:1363-1371.

21. Zhou W, Medof ME, Heeger PS, Sacks S. Graft-derived complement as a mediator of transplant injury. *Curr Opin Immunol* 2007;19:569-576.

22. Irish WD, Ilsley JN, Schnitzler MA, Feng S, Brennan DC. A risk prediction model for delayed graft function in the current era of deceased donor renal transplantation. *Am J Transplant* 2010;10:2279-2286.

23. Sacks SH, Zhou W. The role of complement in the early immune response to transplantation. *Nat Rev Immunol* 2012;12:431-442.

24. Ishii D, Schenk AD, Baba S, Fairchild RL. Role of TNFalpha in early chemokine production and leukocyte infiltration into heart allografts. *Am J Transplant* 2010;10:59-68.

25. Kruger B, Krick S, Dhillon N, Lerner SM, Ames S, Bromberg JS et al. Donor Toll-like receptor 4 contributes to ischemia and reperfusion injury following human kidney transplantation. *Proc Natl Acad Sci U S A* 2009;106:3390-3395.

26. Matsumoto CS, Zasloff MA, Fishbein TM. Chronic mucosal inflammation/inflammatory bowel disease-like inflammation after intestinal transplantation: where are we now? *Curr Opin Organ Transplant* 2014;19:276-280.

27. Muro M, Lopez-Hernandez R, Mrowiec A. Immunogenetic biomarkers in inflammatory bowel diseases: Role of the region. *World journal of gastroenterology : WJG* 2014;20:15037-15048.

28. Geiler J, Buch M, McDermott MF. Anti-TNF treatment in rheumatoid arthritis. *Current pharmaceutical design* 2011;17:3141-3154.

29. Taylor PC. Pharmacology of TNF blockade in rheumatoid arthritis and other chronic inflammatory diseases. *Current opinion in pharmacology* 2010;10:308-315.

30. Cacciapaglia F, Navarini L, Menna P, Salvatorelli E, Minotti G, Afeltra A. Cardiovascular safety of anti-TNF-alpha therapies: facts and unsettled issues. *Autoimmunity reviews* 2011;10:631-635.

31. Chang RJ, Lee SH. Effects of interferon-gamma and tumor necrosis factor-alpha on the expression of an Ia antigen on a murine macrophage cell line. *J Immunol* 1986;137:2853-2856.

32. Enk AH, Katz SI. Early molecular events in the induction phase of contact sensitivity. *Proc Natl Acad Sci U S A* 1992;89:1398-1402.

33. Wei RQ, Schwartz CF, Lin H, Chen GH, Bolling SF. Anti-TNF antibody modulates cytokine and MHC expression in cardiac allografts. *The Journal of surgical research* 1999;81:123-128.

34. Bolling SF, Kunkel SL, Lin H. Prolongation of cardiac allograft survival in rats by anti-TNF and cyclosporine combination therapy. *Transplantation* 1992;53:283-286.

35. Coito AJ, Binder J, Brown LF, de Sousa M, Van de Water L, Kupiec-Weglinski JW. Anti-TNF-alpha treatment down-regulates the expression of fibronectin and decreases cellular infiltration of cardiac allografts in rats. *J Immunol* 1995;154:2949-2958.

36. Coito AJ, Binder J, Brown LF, de Sousa M, Van de Water L, Kupiec-Weglinski JW. TNF-alpha upregulates the expression of fibronectin in acutely rejecting rat cardiac allografts. *Transplantation proceedings* 1995;27:463-465.

37. Langrehr JM, Gube K, Hammer MH, Lehmann M, Polenz D, Pascher A et al. Short-term anti-CD4 plus anti-TNF-alpha receptor treatment in allogeneic small bowel transplantation results in long-term survival. *Transplantation* 2007;84:639-646.

38. FrancoSalinas G, Mai HL, Jovanovic V, Moizant F, Vanhove B, Boeffard F et al. TNF blockade abrogates the induction of T cell-dependent humoral responses in an allotransplantation model. *J Leukoc Biol* 2011;90:367-375.

39. Eason JD, Wee S, Kawai T, Hong HZ, Powelson JA, Widmer MB et al. Inhibition of the effects of TNF in renal allograft recipients using recombinant human dimeric tumor necrosis factor receptors. *Transplantation* 1995;59:300-305.

40. Azzawi M, Grant SD, Hasleton PS, Yonan N, Campbell CS, Deiraniya AK et al. TNF alpha mRNA and protein in cardiac transplant biopsies: comparison with serum TNF alpha levels. *Cardiovascular research* 1996;32:551-556.

41. Kirk AD, Hale DA, Hoffmann SC, Kampen RL, Kleiner DE, Mannon RB et al. Results from a Human Tolerance Trial Using Campath-1H With and Without Infliximab. *Am J Transplant* 2002;2:S379.

42. Torre-Amione G, Wallace CK, Young JB, Koerner MM, Thohan V, McRee S et al. The effect of etanercept on cardiac transplant recipients: a study of TNFalpha antagonism and cardiac allograft hypertrophy. *Transplantation* 2007;84:480-483.

43. Bellin MD, Kandaswamy R, Parkey J, Zhang HJ, Liu B, Ihm SH et al. Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. *Am J Transplant* 2008;8:2463-2470.

44. Gangemi A, Salehi P, Hatipoglu B, Martellotto J, Barbaro B, Kuechle JB et al. Islet transplantation for brittle type 1 diabetes: the UIC protocol. *Am J Transplant* 2008;8:1250-1261.

45. Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA* 2005;293:830-835.

46. Li J, Andreyev O, Chen M, Marco M, Iwase H, Long C et al. Human T cells upregulate CD69 after coculture with xenogeneic genetically-modified pig mesenchymal stromal cells. *Cell Immunol* 2013;285:23-30.

47. Betts BC, Abdel-Wahab O, Curran SA, St Angelo ET, Koppikar P, Heller G et al. Janus kinase-2 inhibition induces durable tolerance to alloantigen by human dendritic cell-stimulated T cells yet preserves immunity to recall antigen. *Blood* 2011;118:5330-5339.

48. Yu GP, Chiang D, Song SJ, Hoyte EG, Huang J, Vanishsarn C et al. Regulatory T cell dysfunction in subjects with common variable immunodeficiency complicated by autoimmune disease. *Clin Immunol* 2009;131:240-253.

49. Schneider A, Long SA, Cerosaletti K, Ni CT, Samuels P, Kita M et al. In active relapsing-remitting multiple sclerosis, effector T cell resistance to adaptive T(regs) involves IL-6-mediated signaling. *Sci Transl Med* 2013;5:170ra115.

50. Augustine JJ, Poggio ED, Clemente M, Aeder MI, Bodziak KA, Schulak JA et al. Hemodialysis vintage, black ethnicity, and pretransplantation antidonor cellular immunity in kidney transplant recipients. *J Am Soc Nephrol* 2007;18:1602-1606.

51. Augustine JJ, Siu DS, Clemente MJ, Schulak JA, Heeger PS, Hricik DE. Pre-transplant IFN-gamma ELISPOTs are associated with post-transplant renal function in African American renal transplant recipients. *Am J Transplant* 2005;5:1971-1975.

52. Gebauer BS, Hricik DE, Atallah A, Bryan K, Riley J, Tary-Lehmann M et al. Evolution of the enzyme-linked immunosorbent spot assay for post-transplant alloreactivity as a potentially useful immune monitoring tool. *Am J Transplant* 2002;2:857-866.

53. Heeger PS, Greenspan NS, Kuhlenschmidt S, Dejelo C, Hricik DE, Schulak JA et al. Pretransplant frequency of donor-specific, IFN-gamma-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. *J Immunol* 1999;163:2267-2275.

54. Hricik DE, Poggio ED, Woodside KJ, Sarabu N, Sanchez EQ, Schulak JA et al. Effects of cellular sensitization and donor age on acute rejection and graft function after deceased-donor kidney transplantation. *Transplantation* 2013;95:1254-1258.

55. Hricik DE, Rodriguez V, Riley J, Bryan K, Tary-Lehmann M, Greenspan N et al. Enzyme linked immunosorbent spot (ELISPOT) assay for interferon-gamma independently predicts renal function in kidney transplant recipients. *Am J Transplant* 2003;3:878-884.

56. Nather BJ, Nickel P, Bold G, Presber F, Schonemann C, Pratschke J et al. Modified ELISPOT technique--highly significant inverse correlation of post-Tx donor-reactive IFNgamma-producing cell frequencies with 6 and 12 months graft function in kidney transplant recipients. *Transplant immunology* 2006;16:232-237.

57. Nickel P, Presber F, Bold G, Biti D, Schonemann C, Tullius SG et al. Enzyme-linked immunosorbent spot assay for donor-reactive interferon-gamma-producing cells identifies T-cell presensitization and correlates with graft function at 6 and 12 months in renal-transplant recipients. *Transplantation* 2004;78:1640-1646.

58. Bestard O, Nickel P, Cruzado JM, Schoenemann C, Boenisch O, Sefrin A et al. Circulating alloreactive T cells correlate with graft function in longstanding renal transplant recipients. *J Am Soc Nephrol* 2008;19:1419-1429.

59. Kim SH, Oh EJ, Kim MJ, Park YJ, Han K, Yang HJ et al. Pretransplant donor-specific interferon-gamma ELISPOT assay predicts acute rejection episodes in renal transplant recipients. *Transplantation proceedings* 2007;39:3057-3060.

60. Poggio ED, Augustine JJ, Clemente M, Danzig JM, Volokh N, Zand MS et al. Pretransplant cellular alloimmunity as assessed by a panel of reactive T cells assay correlates with acute renal graft rejection. *Transplantation* 2007;83:847-852.

61. Poggio ED, Clemente M, Hricik DE, Heeger PS. Panel of reactive T cells as a measurement of primed cellular alloimmunity in kidney transplant candidates. *J Am Soc Nephrol* 2006;17:564-572.

62. Sawinski D, Uribarri J, Peace D, Yao T, Wauhop P, Trzcinka P et al. 25-OH-vitamin D deficiency and cellular alloimmunity as measured by panel of reactive T cell testing in dialysis patients. *Am J Transplant* 2010;10:2287-2295.

63. Traitanon O, Gorbachev A, Bechtel JJ, Keslar KS, Baldwin WM, 3rd, Poggio ED et al. IL-15 induces alloreactive CD28(-) memory CD8 T cell proliferation and CTLA4-Ig resistant memory CD8 T cell activation. *Am J Transplant* 2014;14:1277-1289.

64. Akimova T, Kamath BM, Goebel JW, Meyers KE, Rand EB, Hawkins A et al. Differing effects of rapamycin or calcineurin inhibitor on T-regulatory cells in pediatric liver and kidney transplant recipients. *Am J Transplant* 2012;12:3449-3461.

65. van der Touw W, Cravedi P, Kwan WH, Paz-Artal E, Merad M, Heeger PS. Cutting edge: Receptors for C3a and C5a modulate stability of alloantigen-reactive induced regulatory T cells. *J Immunol* 2013;190:5921-5925.

66. Nie H, Zheng Y, Li R, Guo TB, He D, Fang L et al. Phosphorylation of FOXP3 controls regulatory T cell function and is inhibited by TNF-alpha in rheumatoid arthritis. *Nat Med* 2013;19:322-328.

67. Hricik DE, Nickerson P, Formica RN, Poggio ED, Rush D, Newell KA et al. Multicenter validation of urinary CXCL9 as a risk-stratifying biomarker for kidney transplant injury. *Am J Transplant* 2013;13:2634-2644.

68. Keslar KS, Lin M, Zmijewska AA, Sigdel TK, Tran TQ, Ma L et al. Multicenter evaluation of a standardized protocol for noninvasive gene expression profiling. *Am J Transplant* 2013;13:1891-1897.

69. Duerrschmid C, Crawford JR, Reineke E, Taffet GE, Trial J, Entman ML et al. TNF receptor 1 signaling is critically involved in mediating angiotensin-II-induced cardiac fibrosis. *J Mol Cell Cardiol* 2013;57:59-67.

70. Therrien FJ, Agharazii M, Lebel M, Lariviere R. Neutralization of tumor necrosis factor-alpha reduces renal fibrosis and hypertension in rats with renal failure. *Am J Nephrol* 2012;36:151-161.

71. Osawa Y, Hoshi M, Yasuda I, Saibara T, Moriwaki H, Kozawa O. Tumor necrosis factor-alpha promotes cholestasis-induced liver fibrosis in the mouse through tissue inhibitor of metalloproteinase-1 production in hepatic stellate cells. *PLoS One* 2013;8:e65251.

72. Liew R, Khairunnisa K, Gu Y, Tee N, Yin NO, Naylynn TM et al. Role of tumor necrosis factor-alpha in the pathogenesis of atrial fibrosis and development of an arrhythmogenic substrate. *Circ J* 2013;77:1171-1179.

73. Verjee LS, Verhoekx JS, Chan JK, Krausgruber T, Nicolaidou V, Izadi D et al. Unraveling the signaling pathways promoting fibrosis in Dupuytren's disease reveals TNF as a therapeutic target. *Proc Natl Acad Sci U S A* 2013;110:E928-937.

74. Borthwick LA, Corris PA, Mahida R, Walker A, Gardner A, Suwara M et al. TNFalpha from classically activated macrophages accentuates epithelial to mesenchymal transition in obliterative bronchiolitis. *Am J Transplant* 2013;13:621-633.

75. Basile DP. The endothelial cell in ischemic acute kidney injury: implications for acute and chronic function. *Kidney Int* 2007;72:151-156.

76. Kaukinen A, Lautenschlager I, Helin H, Karikoski R, Jalanko H. Peritubular capillaries are rarefied in congenital nephrotic syndrome of the Finnish type. *Kidney Int* 2009;75:1099-1108.

77. Steegh FM, Gelens MA, Nieman FH, van Hooff JP, Cleutjens JP, van Suylen RJ et al. Early loss of peritubular capillaries after kidney transplantation. *J Am Soc Nephrol* 2011;22:1024-1029.

78. Chapal M, Neel M, Le Borgne F, Meffray E, Carceles O, Hourmant M et al. Increased soluble flt-1 correlates with delayed graft function and early loss of peritubular capillaries in the kidney graft. *Transplantation* 2013;96:739-744.

79. Reed EF, Rao P, Zhang Z, Gebel H, Bray RA, Guleria I et al. Comprehensive assessment and standardization of solid phase multiplex-bead arrays for the detection of antibodies to HLA. *Am J Transplant* 2013;13:1859-1870.

80. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982;143:29-36.

81. Schwender H, Ruczinski I. Logic regression and its extensions. *Adv Genet* 2010;72:25-45.

82. Kooperberg C, Ruczinski I, LeBlanc ML, Hsu L. Sequence analysis using logic regression. *Genet Epidemiol* 2001;21 Suppl 1:S626-631.

83. Ruczinski I, Kooperberg C, LeBlanc ML. Logic Regression. *Journal of Computational and Graphical Statistics* 2003;12:475-511.

84. Carpenter JR, Roger JH, Kenward MG. Analysis of longitudinal trials with protocol deviation: a framework for relevant, accessible assumptions, and inference via multiple imputation. *Journal of biopharmaceutical statistics* 2013;23:1352-1371.

85. Little R, Yau L. Intent-to-treat analysis for longitudinal studies with drop-outs. *Biometrics* 1996;52:1324-1333.

86. The Prevention and Treatment of Missing Data in Clinical Trials Panel on Handling Missing Data in Clinical Trials; National Research Council ISBN: 0-309-15815-X, 2010

87. Ma G, Troxel AB, Heitjan DF. An index of local sensitivity to non-ignorable drop-out in longitudinal modelling. Stat Med. 2005 Jul 30;24(14):2129-50.

88. Kenward M, Multiple Imputation and its Application James Carpenter, ISBN: 978-0-470-74052-1 February 2013.

89. Carpenter, J. R., and Kenward, M. G. , Multiple Imputation and its Application (Statistics in Practice), Wiley. 2010

90. DR Cox and D Oakes, Analysis of Survival Data ISBN: 041224490 X. 1984

APPENDIX 1. CTOT-19 SCHEDULE OF EVENTS

ASSESSMENTS		Days Post-transplant									Months Post-transplant						Clinically Indicated Biopsy	Clinically Indicated Biopsy Follow-Up	Every 6 months to study end[4]
Days/Months Post-transplant		**0**[9]	**1**	**2**	**3**	**4**	**5**	**6**	**7/ Discharge**[8]	**14**	**1**	**3**	**6**	**12**	**18**	**24**	**UV1**	**UV2**	**UV3**
Visit Number		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**			
GENERAL ASSESSMENTS																			
Informed Consent, Screening	Study Enrollment- Inclusion/Exclusion Criteria	X																	
Randomization	Assignment received	X																	
Medical History	All Body Systems	X										X	X	X		X			
Medical Record Review	Graft Status																		X
Physical Exam/ Vital Signs	Height, Weight, Temperature, Blood Pressure, Pulse	X										X	X	X		X			
Concomitant Medications	Drug Name	X									X	X	X	X	X	X			
Warm Ischemia Time	**Warm Ischemia Time** (Recipient) **Warm Ischemia Time** (DCD Donors only)	X																	
Cold Ischemia Time	(Defined by site SOC practices)	X																	
Delayed Graft Function/Dialysis	**Hemodialysis** (# of days) **Peritoneal Dialysis** (# of days)											X							

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ASSESSMENTS		Days Post-transplant									Months Post-transplant						Clinically Indicated Biopsy	Clinically Indicated Biopsy Follow-Up	Every 6 months to study end[4]
Days/Months Post-transplant		**0**[9]	**1**	**2**	**3**	**4**	**5**	**6**	**7/ Discharge**[8]	**14**	**1**	**3**	**6**	**12**	**18**	**24**	**UV1**	**UV2**	**UV3**
Visit Number		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**			
Assessment of Events	AE/SAE, Infections, Rejections, Hospitalizations, Graft Loss	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
STUDY MEDICATIONS																			
Investigational Agent/Placebo	Total Dose	X																	
Thymoglobulin	Drug Name, Total Dose Administered								X										
Mycophenolate Mofetil /Mycophenolic Acid (or generic equivalents)	Drug Name, Total Daily Dose								X			X	X	X		X	X		
Tacrolimus (or generic equivalent)	Drug Name, Total Daily Dose								X			X	X	X		X	X		
Tacrolimus (or generic equivalent)[5] (Only Trough levels)	Drug Name, Trough Levels *(Collected monthly from 1-24 months post-transplant)*								X		X	X	X	X	X	X	X		
Prednisone (or generic equivalent)	Drug Name, Total Daily Dose	X							X			X	X	X		X	X		
Pneumocystis Infection Prophylaxis	Drug Name, Total Daily Dose								X			X	X	X	X	X	X		
CMV Infection Prophylaxis	Drug Name, Total Daily Dose								X			X	X	X	X	X	X		

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ASSESSMENTS		Days Post-transplant									Months Post-transplant						Clinically Indicated Biopsy	Clinically Indicated Biopsy Follow-Up	Every 6 months to study end[4]
Days/Months Post-transplant		0[9]	1	2	3	4	5	6	7/ Discharge[8]	14	1	3	6	12	18	24	UV1	UV2	UV3
Visit Number		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14			
Medication Event Monitoring System (MEMS®)-Prednisone (Given to Subject *before* discharge from the hospital)	Assess accurate use of MEMS® Cap by Subject										X	X							
LOCAL LABORATORY ASSESSMENTS																			
Blood Type	A, B, O, Rh	X																	
HLA Typing	I (A, B, C), II (DR, DP, DQ)	X																	
PRA	Peak and Current (Calculated) *(Flow Based, Cellular, ELISA, Luminex)*	X																	
Crossmatch	T-Cell and B-Cell *(Flow Cytometry)*	X																	
Pregnancy Test	Urine or Serum	X																	
Hematology Panel [8]	CBC, Differential	X	X	X	X		X		X			X	X	X		X			
Chemistry Panel [8]	Creatinine, BUN, glucose, sodium, AST, ALT, ALP, bilirubin, albumin, calcium, phosphate, magnesium	X	X	X	X		X		X	X	X	X	X	X		X			
Urine Sample	Urine Protein, Urine Creatinine, Urine Protein to Creatinine Ratio (Calculated from above)										X	X	X	X		X			

APPENDIX 1. CTOT-19 SCHEDULE OF EVENTS

ASSESSMENTS		Days Post-transplant									Months Post-transplant						Clinically Indicated Biopsy	Clinically Indicated Biopsy Follow-Up	Every 6 months to study end[4]
Days/Months Post-transplant		**0**[9]	**1**	**2**	**3**	**4**	**5**	**6**	**7/ Discharge**[8]	**14**	**1**	**3**	**6**	**12**	**18**	**24**	**UV1**	**UV2**	**UV3**
Visit Number		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**			
CMV Matching	Donor and Recipient Status prior to transplant	X																	
Viral Monitoring	BKV by PCR in blood, Viral load										X	X	X	X					
Tuberculosis Testing	QuantiFERON, PPD, ELISPOT	X[10]																	
Local Histology Read												X[7]				X[7]	X		
Standard of Care Biopsies [11]	Biopsy Results, Treatment provided (if applicable)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
CORE MECHANISTIC ASSESSMENT																			
CELLULAR IMMUNOLOGY CORE –ICAHN SCHOOL OF MEDICINE AT MSSM (PI: PETER HEEGER)																			
T and B Cell Phenotyping by Flow Cytometry (FLO)	**10 mL Blood** Cyto-Chex ® Tubes 2 x 5mL tubes	X							X		X	X	X	X		X			
T Cell Functional Assays (ELISPOT) / CyTOF Assay (ELI)	**30 mL Blood** Green-Top – Sodium Heparin 3x 10mL tubes	X									X		X	X					
T Regulatory Cells Sub-study *(CCF, UHC/Case Western, MSSM)*[1] (REG)	**26 mL Blood** Green-Top – Sodium Heparin, 2x 10mL tubes; 1x 6mL tube												X						

APPENDIX 1. CTOT-19 SCHEDULE OF EVENTS

ASSESSMENTS		Days Post-transplant									Months Post-transplant						Clinically Indicated Biopsy	Clinically Indicated Biopsy Follow-Up	Every 6 months to study end[4]
Days/Months Post-transplant		**0**[9]	**1**	**2**	**3**	**4**	**5**	**6**	**7/ Discharge**[8]	**14**	**1**	**3**	**6**	**12**	**18**	**24**	**UV1**	**UV2**	**UV3**
Visit Number		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**			
Urinary Chemokines and Necroptosis Markers[6, 8] (USC)	**50-100ml Urine**	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	
Plasma for Necroptosis Markers, donor and mitochondrial DNA, and other Inflammatory Markers (PIM) (PTA)	**2 mL Blood** Lavender-top (EDTA) Tube Remaining EDTA Plasma Tube Archived	X							X		X	X		X		X			
GENOMICS ANALYSIS CORE – ICAHN SCHOOL OF MEDICINE AT MSSM (PI: BARBARA MURPHY)																			
mRNA Profiling, Gene Expression (Blood) (BGE)	**2.5 mL Blood** PAXgene RNA 1 x 2.5mL tube	X							X		X	X	X	X		X	X	X	
mRNA Profiling, Gene Expression (Tissue) (TGE)	1 Core/Wedge Tissue	X[2]										X				X	X		
Genomics (SNP analysis, epigenetics, CDR3 TCR sequencing) Archived (GEN)	**4mL Blood** Lavender-top (EDTA) Tube 1 x 4mL tube	X							X		X	X		X		X	X	X	
Cell Free DNA Analysis (CFD)	**10 mL Blood** Streck Cell Free DNA BCT 1x10mL tube								X		X	X	X	X	X	X	X	X	

APPENDIX 1. CTOT-19 SCHEDULE OF EVENTS

ASSESSMENTS		Days Post-transplant									Months Post-transplant						Clinically Indicated Biopsy	Clinically Indicated Biopsy Follow-Up	Every 6 months to study end[4]
Days/Months Post-transplant		**0**[9]	**1**	**2**	**3**	**4**	**5**	**6**	**7/ Discharge**[8]	**14**	**1**	**3**	**6**	**12**	**18**	**24**	**UV1**	**UV2**	**UV3**
Visit Number		**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**			
MOLECULAR IMMUNOLOGY CORE – CLEVELAND CLINIC (PI: ROBERT FAIRCHILD)																			
Nanostring Analysis **(sample shared from the mRNA Profiling- blood)** (NSA)	**2.5mL Blood** PAXgene RNA 1x2.5mL tube	X							X		X	X	X	X		X	X	X	
mRNA Profiling, Gene Expression (Urine Pellet)[6, 8] (UPR)	**50-100ml Urine**	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	
HISTOLOGY CORE – UNIVERSITY OF MANITOBA (PI: IAN GIBSON)																			
Histology **(1 Core/Wedge Tissue in Formalin Jar- Core to be shared with IHC)** (HIS)	*** **If tissue core cannot be sent** to the Core Pathology Lab, then the site should send (1) H&E, (3) PAS, and (1) Trichrome or unstained slide[3]	X[2]										X				X	X		
Immunohistochemistry **(1 Core/Wedge Tissue in Formalin Jar- Core to be shared with HIS)** (IHC)	*** **If tissue core cannot be sent** to the Core Pathology Lab, then the site should send (3) unstained slides, (1) C4d stained, (1) SV-40 stained **If C4d and/or SV-40 are not available**, then the site will send an unstained slide in its place (5 slides total for Immunohistochemistry) [3]	X[2]										X				X	X		

APPENDIX 1. CTOT-19 SCHEDULE OF EVENTS

ASSESSMENTS		Days Post-transplant									Months Post-transplant						Clinically Indicated Biopsy	Clinically Indicated Biopsy Follow-Up	Every 6 months to study end[4]
Days/Months Post-transplant		0[9]	1	2	3	4	5	6	7/ Discharge[8]	14	1	3	6	12	18	24	UV1	UV2	UV3
Visit Number		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14			
HUMORAL ANTIBODY CORE – UNIVERSITY OF MANITOBA (PI: PETER NICKERSON)																			
Anti-HLA Antibodies, Donor Specific Antibodies (DSA) (ALO)	**5 mL Blood** Red-Top (No Additive) Tubes 1 x 5mL tubes	X									X	X		X		X	X		
HLA Typing for Epitope Analysis (EPI-R)	**4mL Blood**	X																	
GLOMERULAR FILTRATION RATE (GFR) CORE –CLEVELAND CLINIC (PI: EMILIO POGGIO)																			
eGFR by MDRD (GFR)	**5ml Blood** Red-Top (No Additive) Tubes 1 x 5mL tubes	X							X		X	X	X	X	X	X			X

1. T Regulatory Cells Sub-study will be performed at a subset of centers (Cleveland Clinic, Case Western/UHC, and Mount Sinai SOM). These centers will receive a separate Sub-study kit for this blood collection.
2. Day 0 (implantation biopsy) can be a wedge biopsy or 2 cores.
3. If unstained slides are shipped, these should be prepared and mounted on positively-charged glass slides (e.g. Superfrost Plus treated slides).
4. The UV3 visit will occur in subjects whom have reached the month 24 visit, however the study is still actively enrolling or following subjects. Subjects will be seen every 6 months until the last subject has completed their last visit (LSLV).
5. Trough levels will be collected monthly from Month 1 to Month 24.
6. After transplant, urine will be collected daily until subject is discharge from hospital. If subject is unable to produce urine it will not constitute a deviation.
7. Collected if available. Would not constitute a deviation if not available.
8. Subjects will have study visits every day while they are hospitalized post-transplant up to Day 7/Visit 07. If a subject is discharged prior to Day 7/Visit 07 the day of discharge will be the last study visit until Day 14. There are no study visits Day 8 to Day 13.
9. Day 0 is defined as the day when the initial Thymoglobulin administration ends. Day 1 is at least 12 hours after Day 0
10. TB exposure test sample should be drawn pre-transplant.
11. Collect Standard of Care/Surveillance biopsy results when performed post-transplant, throughout the study follow-up period. Additionally, collect if subject was treated and treatment provided.

Appendix 2. Donor Schedule of Events

Assessments		Day of Transplant Visit 00
Donor Demographics	Age, Ethnicity, Gender, CMV status, Cause of Death, DCD/Non-DCD, Cold Ischemia Time, HLA Typing, KDPI Score (including components), Kidney Pumped/Not Pumped, Renal Resistive Index	X
Mechanistic Sample Collection		
Donor Sample Collection for HLA Typing for Epitope Analysis	***4mL Blood*** in a Lavender-top EDTA Tube **or** ***20µL of DNA*** at a concentration of 20ng/µL. • The purity should be between 1.6 to 1.9	X
Donor Sample Collection for Genomics	***4mL Blood*** in a Lavender-top EDTA Tube **or** ***20µL of DNA*** at a concentration of 20ng/µL. • The purity should be between 1.6 to 1.9	X
Donor Sample Collection for T Cell Functional Assays	Spleen, Lymph node or Blood[1] • **1st Priority:** Spleen Tissue • **2nd Priority:** 30ml Blood in Green Top Sodium Heparin Tubes • **3rd Priority:** Lymph Nodes	X

1. The donor samples will be collected when the transplant center is able to obtain the samples. At a minimum, the transplant center is required to collect the sample for the HLA Typing for Epitope Analysis (EPI-D), as this is required for the planned study analysis. The transplant center may obtain this sample from the HLA Histocompatibility Laboratory at their center.

APPENDIX 3. CTOT-19 REDUCED FOLLOW-UP SCHEDULE OF EVENTS

ASSESSMENTS		Days Post-transplant									Months Post-transplant						Clinically Indicated Biopsy	Clinically Indicated Biopsy Follow-Up	Every 6 months to study end[4]
Days/Months Post-transplant		0[9]	1	2	3	4	5	6	7/ Discharge[8]	14	1	3	6	12	18	24	UV1	UV2	UV3
Visit Number		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14			
ASSESSMENTS																			
Assessment of Events	AE/SAE, Infections, Death, Malignancies													X	X	X			
Physical Exam	Height, Weight, Temperature, Blood Pressure, Heart Rate													X	X	X			
Immunosuppressive Medications	Drug Name, Dose Total Daily Dose													X	X	X			
Anti HLA Antibodies	Results of test that are done as part of clinical care (If not done, not a deviation)													X	X	X			

APPENDIX 4. REDUCED FOLLOW-UP SCHEDULE OF EVENTS (Withdrawn/Terminated Subjects)		
ASSESSMENTS		**FINAL VISIT**
	Visit Number	**FV**
Assessment of Events	AE/SAE, Infections, Rejections, Death, Malignancies, Graft Loss	**X**
Physical Exam	Height, Weight, Temperature, Blood Pressure, Heart Rate	**X**
Immunosuppressive Medications	Drug Name, Dose Total Daily Dose	**X**
Anti HLA Antibodies	Results of test that are done as part of clinical care (if not done, not a deviation)	**X**