CLINICAL TRIALS IN ORGAN TRANSPLANTATION IN CHILDREN

CTOTC-08

B Cell Targeted Induction to Improve Outcomes in Pediatric Lung Transplantation

Short Title: B Cell Induction in Pediatric Lung Transplantation

4.0 / July 16, 2018

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Protocol: CTOTC-08	**Version/Date:** 4.0/July 16, 2018
Title: B cell Targeted Induction to Improve Outcomes in Pediatric Lung Transplantation	
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:*

DAIT REGULATORY MANAGEMENT CENTER
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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	B cell Targeted Induction to Improve Outcomes in Pediatric Lung Transplantation
Clinical Phase	Phase 2
Number of Sites	7 US Sites
IND Sponsor/Number	NIAID/IND Number 121403
Primary Study Objective	To determine whether rituximab induction along with standard of care immunosuppression will improve outcomes following pediatric lung transplantation.
Secondary Study Objectives	1. To determine the effects of rituximab induction on post-transplant immunity in pediatric lung transplant recipients. 2. To assess the safety and tolerability of rituximab. 3. To assess the feasibility of a phone based intervention to decrease tacrolimus trough level variability.
Study Design/Treatment Description	Phase 2, prospective, multi-center, double-blind, randomized, placebo-controlled clinical trial in which 50 primary pediatric lung transplant recipients will be randomized (1:1) to receive either induction therapy with anti-CD20 mAb (375 mg/m^2) IV or placebo (IV day 0 and day 12 post-transplant) plus standard of care immunosuppression (thymoglobulin induction, tacrolimus or generic equivalent, MMF or generic equivalent, and steroids).
Primary Endpoint(s)	The primary endpoint will be the earliest time to any of the following events during the follow-up period: Chronic Allograft Dysfunction Listed for Retransplant Death
Secondary Clinical Endpoints	Post-transplant outcomes including: 1. Incidence of chronic allograft dysfunction, listing for retransplantation and death during the follow-up period, which will be a minimum of 12 months post-transplant; 2. Incidence of Primary Graft Dysfunction; 3. Incidence of Grade A Acute Rejection during the follow-up period, which will be a minimum of 12 months post-transplant; 4. Incidence of Antibody Mediated Rejection during the follow-up period, which will be a minimum of 12 months post-transplant; 5. Incidence of tacrolimus variability threshold during the follow-up period, which will be a minimum of 12 months post-transplant; 6. Percentage of participants meeting tacrolimus variability threshold who complete tacrolimus variability intervention; 7. Magnitude of change in standard deviation of tacrolimus levels following intervention. Post-transplant safety outcomes including: 1. Incidence and severity of infection episodes;

	2. Serious adverse events related to rituximab.
Secondary Mechanistic Endpoints	1. Incidence and kinetics of DSA and autoantibodies, specifically Collagen V (ColV) and k-alpha-1 tubulin (kα1T); 2. Frequency, kinetics, phenotype and function of peripheral B cells; 3. Frequency, kinetics and cytokine profiles of allo- and auto reactive T cells; 4. Incidence and quantity of B cells and B cell proximity to other cells in the graft tissue.
Accrual Objective	50 randomized pediatric lung transplant recipients
Study Duration	4.5 years (3.5 year accrual + 1-4.5 year follow-up period) All participants will be followed for a minimum of 1 year post-transplant. Participants will continue follow up visits (up to 4.5 years post-transplant) until the last participant completes 1 year of follow-up.
Enrollment Inclusion Criteria	1. Subject and/or parent/guardian must be able to understand and provide informed consent; 2. Less than or equal to 21 years of age; 3. Candidate for primary lung transplant (listed for lung transplant); 4. Female and male subjects with reproductive potential must agree to use FDA approved methods of control for 12-months after completion of treatment; 5. Adequate bone marrow function based on the following criteria: a. ANC > 1000mm^3 b. Platelets > 100,000/mm^3 c. Hemoglobin > 7 gm/dL d. AST or ALT <2x Upper Limit of Normal unless related to primary disease
Enrollment Exclusion Criteria	1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol; 2. Multi-organ transplant; 3. Previous treatment with rituximab (Rituxan®); 4. History of severe allergic anaphylactic reactions to humanized or murine monoclonal antibodies; 5. History of severe reaction to previous therapy with IVIG; 6. History of Burkholderia cenocepacia; 7. History of anti-CD20 therapy; 8. Persistent hypogammaglobulinemia (IgG < lower level of normal for age based on local laboratory ranges or 400 gm/dL for >2 months) and/or IVIG replacement therapy; 9. Positive blood culture, sepsis or other disease process with hemodynamic instability at time of enrollment; 10. Any history of serologic positivity to HIV, HBsAg, HBcAb and HCV Ab; 11. History of malignancy less than 2 years in remission of malignancy (any history

	of adequately treated in-situ cervical carcinoma, or adequately treated basal or squamous cell carcinoma of the skin will be permitted); 12. Any condition, including psychiatric disorders, that in the opinion of the investigator would interfere with the subject's ability to comply with study requirements; 13. Participation in another investigational trial within 4 weeks of enrollment; 14. Currently lactating or plans to become pregnant during the timeframe of the study follow-up period; 15. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.
Randomization Inclusion Criteria	1. Serum IgG immunoglobulin level greater than lower level of normal for age based on local laboratory ranges or 400 mg/dL within 90 days prior to randomization; 2. Female subjects of childbearing potential must have a negative pregnancy test within 48 hours of transplant; 3. Negative for Hepatitis B infection (if at time of transplant, subject does not exhibit effective immunization, the subject should be re-tested).
Randomization Exclusion Criteria	1. Use of an induction agent other than Thymoglobulin®; 2. Renal insufficiency requiring hemodialysis or ultrafiltration; 3. Inability to obtain intravenous access; 4. Positive blood culture, sepsis or other disease process with hemodynamic instability at time of transplant; 5. Use of investigational agent(s) within 5 half-lives of the investigational drug or 4 weeks, whichever is longer; 6. Receipt of a MMR vaccine within 30 days prior to randomization; 7. Any condition that, in the opinion of the investigator, would interfere with the subject's ability to comply with study requirements.
Study Stopping Rules	Satisfaction of any of the following **stopping rules** in study subjects at any time of follow-up in the treatment arms will trigger an *ad hoc* DSMB Safety Review: • Any occurrence of confirmed PML. • Incidence of death of 30% or more subjects. • Incidence of at least mild acute rejection of 35% or more. • Incidence of humoral rejection of 25% or more. • Incidence of primary graft dysfunction of 50% or more. • Incidence of PTLD of 5% or more. • Incidence of infections of any type requiring hospitalization of 40% or more.
Individual Subject Stopping Rules	Individuals who meet any of the criteria listed below will not receive the second dose of rituximab. 1. Serious adverse event casually related to the rituximab infusion;

	2. Acute pulmonary infectious process with evidence of graft dysfunction; 3. Positive blood culture, sepsis or other disease process with hemodynamic instability; 4. Renal insufficiency requiring hemodialysis or ultrafiltration; 5. Inability to obtain intravenous access; 6. Use of an investigational drug after the first dose of placebo or rituximab; 7. Any other event which in the opinion of the principal investigator may pose additional risk to the participant.

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APPENDICES

Glossary of Abbreviations

Abs	Antibodies
Ags	Antigens
ACR	Acute Cellular Rejection
AE	Adverse Event
AEC	Airway Epithelial Cells
AIB	Autoimmune B cells
ALT	Alanine Aminotransferase
AMR	Antibody Mediated Rejection
ANC	Absolute Neutrophil Count
APC	Antigen Presenting Cell
AST	Aspartate Aminotransferase
BAL	Bronchoalveolar Lavage
BOS	Bronchiolitis Obliterans Syndrome
cc	Cubic Centimeters
CBC	Complete Blood Count
CDC	Center for Disease Control
cDNA	Complementary DNA
CF	Cystic Fibrosis
CFR	Code of Federal Regulations
CMV	Cytomegalovirus
CNI	Calcineurin Inhibitor
CoIV	Type V Collagen
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTOT	Clinical Trials in Organ Transplantation
CTOT-02	B-Cell Depletion by Anti-CD20 (Rituximab) in Renal Allograft Recipients Who Develop Early de Novo Anti-HLA Alloantibodies Will Result in Inhibition of Alloantiody Production and Attenuation of Chronic Humoral Rejection (NCT00307125)
CTOT-11	Prevention of Cardiac Allograft Vasculopathy Using Rituximab (Rituxan®) Therapy in Cardiac Tranpslantation (NCT01278745)

CTOT-C	Clinical Trials in Organ Transplantation in Children
CTOTC-03	Viral Triggers of Alloimmunity and Autoimmunity in Pediatric Lung Transplantation (NCT00891865)
CXR	Chest X-Ray
DAIT	Division of Allergy, Immunology, and Transplantation
dL	Deciliters
DLBCL	Diffuse Large B cell Lymphoma
DSA	Donor Specific Antibody
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Form
EBV	Epstein Barr Virus
eCRF	Electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
GCP	Good Clinical Practice
gm	Gram
H&E	Hematoxylin and Eosin Stain
HACA	Human Anti-Chimeric Antibody
HBcAb	Hepatitis B Antibody
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
hCOV	Human Coronavirus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
hMPV	Human Metapneumovirus
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IFNg	Interferon Gamma
IL-1B	Interleukin-1 beta
IL-10	Interleukin-10
IL-17	Interleukin-17
IND	Investigational New Drug

IPLTC	International Pediatric Lung Transplant Collaborative
IRB	Institutional Review Board
ISHLT	The International Society for Heart & Lung Transplantation
ISMMS	Icahn School of Medicine at Mount Sinai
ITN	Immune Tolerance Network
ITT	Intent to Treat
IVIG	Intraveneous Immunoglobulin
JC	John Cunningham (Virus)
Kα1T	K-A1-Tubulin
kD	Kilo Dalton
Kg	Kilogram
LTx	Lung Transplanation
LTxR	Lung Transplant Recipients
mg	Milligram
mH	Minor Antigenic
µL	Microliter
mm	Millimeter
MMF	Mycophenolate Mofetil
MMR	Measles Mumps and Rubella
MOP	Manual of Procedures
ng	Nanogram
NG	Nasogastric
NHL	Non-Hodgkin Lymphoma
NIAID	National Institute of Allergy and Infectious Diseases
nM	Nanomolar
NP	Nasopharyngeal
OAD	Obliterative Airway Disease
OB	Obliterative Bronchilitis
PCR	Polymerase Chain Reaction
PDT	Protocol Development Team
PFT	Pulmonary Function Test
PGD	Primary Graft Dysfunction
pg	picogram

PI	[Site] Principal Investigator
PML	Progressive Multifocal Leukoencephalopathy
PO	Per Oral
PP	Per Patient
PRA	Panel Reactive Antibody
PTLD	Post-Transplant Lymphoproliferative Disorder
RA	Rheumatoid Arthritis
rATG	rabbit Anti-thymocyte Globulin
RESTARRT	Research Study of ATG and Rituximab in Renal Transplantation (NCT01318915)
RSV	Respiratory Syncytial Virus
RVI	Respiratory Viral Infection
SAE	Serious Adverse Event
SACCC	Statistical and Clinical Coordinating Center
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SD	Standard Deviation
SLE	Systemic Lupus Erythematosis
SOP	Standard Operating Procedure
SUSAR	Serious Unexpected Suspected Adverse Reaction
TBBx	Transbronchial Biopsy
TLR	Toll-like Receptors
Treg	Regulatory T-Cells
TVI	Tacrolimus Variability Intervention
TVT	Tacrolimus Variability Threshold
UNOS	United Network for Organ Sharing

Study Definitions Page

Abnormal Histology	Abnormal histology will be defined as one or more of the following: • Neutrophilic capillaritis or septal margination; • high grade (≥A3) or persistent /recurrent acute rejection; • acute lung injury/diffuse alveolar damage; • high grade (B2R) or persistent low grade (B1R) lymphocytic bronchitis obliterative bronchiolitis; • arteritis in the absence of acute rejection or other finding not explained by clinical circumstances (i.e. infectious causes thoroughly excluded).
Acute Cellular Rejection	Acute rejection, Grade A, will be defined based on pathology specimens obtained from transbronchial biopsy according to the working formulation for the revision of the classification of pulmonary allograft rejection in 2007.[103]
Antibody Mediated Rejection	Antibody Mediated Rejection (AMR) will be defined based on the Revision of the 1996 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Lung Rejection and the Pathology of pulmonary antibody mediated rejection and the 2012 update from the Pathology Council of the ISHLT.[103,104] AMR will be diagnosed based on the presence of one or more of the following: • Donor Specific Antibody (DSA) or autoantibody; • Abnormal Histology; • Graft Dysfunction.
Anonymized	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.
Chronic Allograft Dysfunction	Chronic allograft dysfunction will be diagnosed locally based on ISHLT criteria for BOS or histologic evidence of oblitereans bronchilitis.[103, 105] Local sites will rule out acute rejection, acute infection and airway stenosis or narrowing prior to diagnosing chronic allograft dysfunction.
Donor Specific Antibodies or Autoantibodies	Circulating antibody to HLA or other antigens expressed on donor endothelial cells.
Inadequate Weight Gain	Drop between 5-10 percentile points
Infectious Events	Infectious events will be defined using published criteria from the ISHLT.[106] The 2010 guidelines on defining infections in cardiothoracic transplantation provide classification of bacterial, fungal and viral pulmonary infections in lung transplant recipients.
Lost to Follow-Up	The CTOTC-08 subject may be considered "lost to follow up" after the subject misses a minimum of 3 consecutive study visits, and the site personnel has made a number of unsuccessful phone contacts. The decision to early terminate the subject will be the decision of the site PI, all attempts to establish contact with the subject will be documented in the study files.

Tacrolimus Variability Intervention	A series of calls with the participant, parent(s)/guardian(s) and specifically trained call center personnel to address barriers to adherence, using a manualized intervention.
Tacrolimus Variabililty Threshold	SD of 2.0 or more (outpatient tacrolimus trough levels) or an undetectable tacrolimus trough level.
Primary Graft Dysfunction	PGD is defined according to the recent summary statement from the ISHLT and will be graded from 0 to 3 based on radiographic changes and the ratio of PaO2/FiO2.[107]
Retransplantation	Listed for a second lung 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 (inclusion and exclusion); 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, and signed the informed consent document.
Study Termination	The subject will no longer be seen for any study related procedure; including clinical assessments, local laboratory assessments, study therapy, core mechanistic studies. No data will be submitted on any subject as of the date of termination.
Study Therapy	The investigational agent and all protocol required therapies include the following; Rituximab (Rituxan®) or Rituximab Placebo, Standard of Care Immunosuppression (Thymoglobulin® (induction), Tacrolimus, Mycophenolate Mofetil and corticosteroids).

1. Study Hypothesis/Objectives

1.1. Hypothesis

Rituximab induction along with standard of care immunosuppression will improve outcomes following pediatric lung transplantation by reducing the development of antibodies reactive to donor mismatched HLA (DSA) and to lung expressed self-antigens and by limiting T-cell autoimmunity and alloimmunity without compromising patient safety.

1.2. Primary Objective

To determine whether rituximab induction along with standard of care immunosuppression will improve outcomes following pediatric lung transplantation.

1.3. Secondary Objectives

1. To determine the effects of rituximab induction on post-transplant immunity in pediatric lung transplant recipients.
2. To assess the safety and tolerability of rituximab.
3. To assess the feasibility of a phone based intervention to decrease tacrolimus trough level variability.

2. Background and Rationale

2.1. Background and Scientific Rationale

Outcomes after pediatric lung

Pediatric lung transplantation is an accepted treatment for end-stage lung disease. Despite advancements in recipient selection, surgical techniques, prophylaxis against infection and development of new immunosuppressive regimens, annual reports from the International Society for Heart and Lung Transplantation (ISHLT) indicate no statistical improvement in long term outcome or survival for pediatric lung transplant recipients[1]. Further, data from the United Network for Organ Sharing (UNOS) supports that while survival has improved marginally, improvements in short-term survival account for this difference[2]. In addition, outcomes in both pediatric and adult lung transplantation are significantly worse than outcomes following transplantation of other solid organs. Despite increasing use of T-cell depleting induction therapies, 5-year survival has not been significantly impacted[1]. Five year survival has only increased incrementally from 51% to 53% in the most recent report.

Bronchiolitis obliterans syndrome and morbidity/mortality

BOS is the primary and most significant cause of long term morbidity and mortality after human lung transplantation (LTx) and reports from two large centers have shown the prevalence of BOS is 60% to 80% in 5 years post LTx in adults [3-6]. The most recent statistics show 14% of pediatric patients develop bronchiolitis obliterans syndrome (BOS) within the first year after transplantation, which is unchanged from 5 years ago[1,7]. The incidence of BOS within 4 years of transplant ranges from 31% in infants < 1 year at transplant to 54% in children $\geq$ 12 at transplant and up to 80% between 5 and 10 years in adults [1,7-9]. Between 25 and 40% of lung transplant recipients will die directly or indirectly from BOS [1,3]. While retransplantation for late lung allograft failure is feasible, limited organ availability can preclude rapid retransplantation and, more importantly, the survival after a second pediatric lung transplant is significantly worse than the primary transplantation particularly if performed within one year of the primary transplant [10-13].

Immunologic basis for bronchiolitis obliterans syndrome

BOS is a fibroproliferative process leading to obliteration of tubular structures in the organ [9,14]. Putative etiologies of BOS include acute rejection, infection, reperfusion injury, drug toxicity, and lung denervation. However, the bulk of evidence suggests BOS is caused by an immunological injury to pulmonary epithelial and endothelial cells [15]. Current understanding, derived from animal models of skin, heart, islet, tracheal and more recently, heterotopic lung transplant rejection, are that allograft injury, including many forms of chronic allograft injury, are T cell dependent[16]. Together this body of literature indicates both naïve and memory T cells, either directly recognizing donor allogeneic MHC or indirectly recognizing donor derived allogeneic (and or minor antigenic, mH) peptides expressed in the context of recipient MHC are central mediators of allograft injury[16-18]. Increasing experimental evidence further indicates that transplantation-associated inflammation can bypass self-tolerance mechanisms resulting in expansion of autoreactive T (and B cells) that contribute to allograft injury[19-22]. T cells reactive to heart-expressed cardiac myosin (CM) participate in murine heart transplant rejection and T cells reactive to lung-expressed type V collagen (ColV) mediate lung transplant injury. In addition to the antigen specificity, the frequency of responding CD4 and CD8 T cells and their induced effector functions (including cytokine profiles and cytotoxic potential) contribute to the pathogenicity of the transplant-induced immune response[23,24].

While a pathogenic role for alloreactive and autoreactive T cells mediating human lung allograft injury including BOS is implied from animal studies, there is less direct evidence to support this contention from human lung transplant recipients. As one example, Wilkes, Burlingham and colleagues showed that ColV specific, IL-17 producing T cells are specifically detectable prior to the clinical recognition of BOS in adult lung transplant recipients.[25] Further, Mohanakumar showed expansion of both ColV-specific and donor-specific HLA class II IFNg- producing T cells and IL-17- producing T cells in adult lung transplant recipients with BOS.[26-28] Data from the CTOTC-03 study include the most comprehensive analysis of T cell immunity in children ever reported. Preliminary findings reveal donor reactive T cells, as well as kα1T-reactive T cells, are detectable in a significant minority of children prior to and following lung transplantation.

It is essential to note that T cells function optimally in the context of interactions with other immune cells, including B cells. T-B interactions are bidirectional. T cell expressed CD154 interactions with B cell expressed CD40, along with T cell derived cytokines, provide helper signals for B cells to undergo an isotype switch (e.g. from IgM to IgG). The resultant antibodies to mismatched donor-MHC and or autoantigen are key contributors to acute and chronic allograft injury[16]. Work from Mohanakumar and colleagues showed that antibodies to kα1T and ColV are strongly associated with lung transplant injury in adults[29,30].

Antibodies to MHC as well as autoantibodies can cause obliterative airway disease in mice.[31] As one example, Mohanakumar et al demonstrated that administration of anti-HLA in the absence of T cells could cause obliterative airway disease (OAD) in a transgenic murine model [32]. Recently his group has also shown that antibodies to kα1T when given intraperitoneally following syngeneic lung transplantations in a murine model of orthotopic left lung transplantation can result in OAD (abstract presented at ISHLT, Prague, 2012). Potential mechanisms of antibody mediated injury at the effector site, largely derived from studies in animals, include complement mediated injury, macrophage/NK cell mediated injury via FcR binding, and direct stimulation of the target cell by the antibody.

Vascular rejection and development of anti-HLA antibodies were associated with chronic allograft rejection in humans [33,34]. Mohanakumar and colleagues showed anti-HLA is associated with development of BOS after LTx in adults [5] . Based on the findings of shed donor HLA in the lungs following LTx[35], it is likely that donor HLA are processed and presented to T helper cells engaged in indirect recognition, production of cytokines and secretion of allo-Ab [36-38]. Mohanakumar's studies demonstrated T-cells from LTx recipients (LTxR) with BOS can recognize donor HLA-I & II peptides [36,39,40]. Moreover, this work showed anti-HLA can activate human airway epithelial cells (AEC) resulting in growth factor production which play an important role in BOS [41].

Independent of antibody production, B cells are also found within the lung at sites of inflammation/rejection and were shown to be associated with the late development of BOS. Intriguingly, while mice administered anti-MHC developed OAD associated with an increased number of lung infiltrating B cells ($2.3\pm0.6x10^6$ vs. $0.9\pm0.4x10^6$cells, $p<0.05$), mice lacking B cells did not develop OAD following administration of anti-MHC antibodies.[31,42] Testing whether B cell depletion prevents B cell infiltrates in the lung and thereby limits BOS in children is one goal of the proposed work.

B cells also process and present donor antigen with appropriate costimulatory signals to T cells, raising the intriguing hypothesis that B cell depletion could prevent T cell activation by removing a key antigen presenting cell (APC) in cellular immune responses. Experiments performed in mice[43,44] as well as in primates[45,46] support this hypothesis, as the absence or depletion of B cells prevented chronic heart allograft injury and the development of OAD in a murine

model[42] associated with diminished T cell activation. These experimental studies provide important rationale for our proposed work in children with lung transplants, in which we will test the clinical and mechanistic impact of B cell depletion on outcome and T cell alloimmunity respectively.

While the above discussion focuses on the pathogenic role of T-cells and B cells, both T-cells and B cells can function as regulatory/suppressor cells to control pathogenic immunity. Regulatory T-cells (Treg) have many phenotypes with the principal one being CD4+CD25+Foxp3+. These regulatory cells exert control over other T cells through multiple mechanisms including CTLA4 induced inhibition of APCs, secretion of immunomodulatory cytokines (TGFb, IL-10) and adenosine metabolism via CD39. Regulatory, IL-10 producing B cells have also recently been described in animals[47-50], and potentially in humans. [51-55] Regulatory B cells may directly inhibit pathogenic T cells but additionally have been reported to function in part by inducing/propagating Treg. Intriguing evidence from animal models and from spontaneously tolerant human kidney transplant recipients suggest that B cells are required for tolerance induction and that B cell depletion can occasionally precipitate rejection, potentially by removing Breg (and as a consequence Treg).[50,52,56]

B cell depletion with rituximab in pediatrics and transplanation

Rituximab is a chimeric mouse/human monoclonal antibody approved as early as 1997 for use in Non-Hodgkin's Lymphoma, Chronic Lymphocytic Leukemia, Rheumatoid Arthritis (RA) in combination with methotrexate in adult patients with moderately-to severely-active RA who have inadequate response to one or more TNF antagonist therapies, Wegener's Granulomatosis and Microscopic Polyangiitis in combination with glucocorticoids. Rituximab targets CD20, a cell surface expressed protein found immature, transitional, mature and memory B cells but is absent on antibody producing plasma cells. While its exact mechanism of action is not fully understood, multiple studies in adults with autoimmune diseases such as Systemic Lupus Erythematosis (SLE) or RA showed that rituximab markedly depletes CD19+HLA-DR+ [57] and memory CD19+CD27+ B cells.[58] Switched memory B cells (CD27+IgD-) are relatively spared compared to CD27-IgD+ naïve B cells and CD27+IgD+ unswitched B cells.[58] Further in RA patients, responders to rituximab therapy showed a significant decrease in CD19+CD27+ memory B cells compared to non-responders indicating that response may be impacted by the subset of B cells affected.[57] One important consequence of rituximab-induced B cell depletion is that the host B cell repertoire repopulates over the ensuing 3-6 months. This reconstitution process appears to recapitulate B cell ontogeny, where immature B cells undergo negative selection on stromal cells in the bone marrow and emerge with a transitional phenotype into the periphery.[59] Transitional B cells, characterized by expression of CD38hiCD24hiIgD+CD27-CD10+/-,[60] require survival signals provided by the cytokines BAFF and APRIL and are key intermediaries in the development of the mature B cell repertoire. Evidence suggests that the transitional B cell population contains Breg and that transitional B cells are subject to mechanisms of self-tolerance in the periphery.

2.2. Rationale for Selection of Investigational Product

Rituximab is a chimeric mouse/human monoclonal antibody approved as early as 1997 for use in Non-Hodgkin's Lymphoma, Chronic Lymphocytic Leukemia, Rheumatoid Arthritis (RA) in combination with methotrexate in adult patients with moderately-to severely-active RA who have inadequate response to one or more TNF antagonist therapies, Wegener's Granulomatosis and Microscopic Polyangiitis in combination with glucocorticoids. Rituximab targets CD20, a cell surface expressed protein found immature, transitional, mature and memory B cells but is absent on antibody producing plasma cells. While its exact mechanism of action is not fully understood, multiple studies in adults with autoimmune diseases such as Systemic Lupus Erythematosis (SLE) or RA showed that rituximab markedly depletes CD19+HLA-DR+ 57 and memory CD19+CD27+ B cells.58 Switched memory B cells (CD27+IgD-) are

relatively spared compared to CD27-IgD+ naïve B cells and CD27+IgD+ unswitched B cells.[58] Further in RA patients, responders to rituximab therapy showed a significant decrease in CD19+CD27+ memory B cells compared to non-responders indicating that response may be impacted by the subset of B cells affected.[57] One important consequence of rituximab-induced B cell depletion is that the host B cell repertoire repopulates over the ensuing 3-6 months. This reconstitution process appears to recapitulate B cell ontogeny, where immature B cells undergo negative selection on stromal cells in the bone marrow and emerge with a transitional phenotype into the periphery.[59] Transitional B cells, characterized by expression of CD38hiCD24hiIgD+CD27-CD10+/-,[60] require survival signals provided by the cytokines BAFF and APRIL and are key intermediaries in the development of the mature B cell repertoire. Evidence suggests that the transitional B cell population contains B reg and that transitional B cells are subject to mechanisms of self-tolerance in the periphery.

Rituximab has been safely used in pediatric patients

In pediatrics, rituximab is safe when used for multiple indications including B-cell leukemia/lymphoma, relapsing minimal change disease and focal segmental glomerulosclerosis, calcineurin-dependent nephrotic syndrome and progressive IgA nephropathy.[61] Treatment of post-transplant lymphoproliferative disease in solid organ transplant recipients with rituximab appears both promising and safe.[62] Further, rituximab has been reported in limited numbers of pediatric lung transplant recipients for treatment of PTLD, AMR and capillaritis.[63,64] Several protocols for hematopoietic stem cell transplant report safely using rituximab as adjunctive therapy to T-cell depleting induction; this is similar to the proposed intervention arm but in a novel population, pediatric lung transplant recipients. The safety of rituximab in pediatric transplantation is underscored by a recent review of 42 pediatric children's hospitals indicating that 30% of pediatric rituximab use is in pediatric transplant recipients.[65]

Rituximab has been used with some efficacy in transplant recipients to remove pre-existing antibodies.

In adult transplantation, trials evaluating rituximab as induction in sensitized kidney recipients, unsensitized kidney (RESTARRT) and heart recipients (CTOT-11) are ongoing. Induction with rituximab was successfully reported in a double-blind, placebo controlled study of adult kidney transplant recipients in Sweden without an increased incidence of post-transplant infection.[63] In pediatric renal transplantation, Chaudhuri and colleagues reported the resolution of post-transplant nephrotic syndrome recurrence (mediated by autoantibody to nephrin proteins) with rituximab.[64] Additionally, 4 weekly doses of rituximab was successfully used in trials as a treatment in combination with thymoglobulin and steroids for acute kidney rejection with B-cell infiltrates in pediatric recipients.[65] No increased risk of infection was observed. Furthermore, pediatric and adult kidney recipients who develop early post-transplant anti-HLA antibodies were randomized to receive 2 (adults) or 4 (children) doses of rituximab or placebo to assess for differences in chronic allograft function (CTOT-02). Data from kidney transplantation suggests that at least 2 doses are needed to achieve effective B-cell clearance. [69,70]

Use in adult pre-transplant desensitization and adult lung transplantation antibody therapy

While isolated B-cell depleting induction (without T-cell directed induction) was found to be inadequate with increased risk of acute T-cell mediated cellular rejection in one study,[71] B-cell depletion with rituximab in multiple adult studies has been shown to improve outcomes when used as part of ABOi desensitization protocols prior to kidney transplantation.[72,73] Rituximab has been employed successfully in adult lung transplantation for the same indication in case reports.[74,75] Furthermore, in adult lung transplant recipients found to have DSA and self-antigens (Ka1T and colV) rituximab was used as part of an antibody depleting regimen. Patients who cleared either DSA or self-directed antibodies were less likely to progress to BOS than patients with persistent antibody.[76,77] However,

only a subset of subjects cleared either DSA or auto-antibodies with the intervention suggesting that prevention of antibody development from the time of transplantation may be a more effective approach to BOS prevention.

Hypothesis and Dosing rationale

Taken together these studies suggest that rituximab can effectively deplete subsets of circulating B-cells and reduce antibody formation without significantly increasing infectious risk. Moreover its successful use in autoimmune disease suggests that, following reconstitution of the B-cell compartment, autoimmunity may not return. We hypothesize that, due to the injury associated with donor death and the transplant procedure, the early post-transplant period is critical for the development of adaptive immunity (both cellular and humoral). Therefore depletion of the B-cell population during this period will reduce or eliminate the development of adaptive humoral responses to donor antigens and exposed cryptic lung self-proteins and add to the reduction in cellular immunity by reducing the pool of B-cells available to serve as APCs.

We considered other potential interventions to affect antibody mediated pathology including intravenous immunoglobulin or bortezomib (which depletes plasma cells); however, rituximab was chosen due to the established safety profile in pediatric transplant patients coupled with the greatest potential biological impact (i.e. prevention of antibody development via depletion of specific B-cell subsets as opposed to strategies directed at antibody removal).

Thus our primary hypothesis is that induction with rituximab (in combination with Thymoglobulin) will improve outcomes following pediatric lung transplantation through a decrease in antibody development post-transplant.

The regimen of 375mg/m^2 was chosen based on manufacturer labeling for current indications, safety profile and historical pediatric experience with rituximab. In light of the information from prior studies using a single dose of rituximab a second dose is scheduled at day 12 post-transplant consistent with the study design of the adult heart transplant study (CTOT-11). We will assess the level of B cell reduction in the first 8 participants using flow cytometry to ensure B cell reduction in this patient population is equivalent to B cell reduction obtained in other studies.

2.3. Preclinical Experience

In the murine model for OAD previously established in the Mohanakumar lab, non-complement fixing MHC class I Abs were administered intra-tracheally to wild type mice and complement factor C3 knock out (C3KO) mice. Both groups developed OAD as evidenced by increase in cellular infiltration and fibrosis around the vessels and bronchioles (**Figure 1**).

This was associated with development of T-helper (Th)-17 type immune responses to ColV and Kα1T and up-regulation of profibrotic cytokines and growth factors. This indicates complement activation is not necessary in the pathogenesis of chronic rejection following lung transplantation.

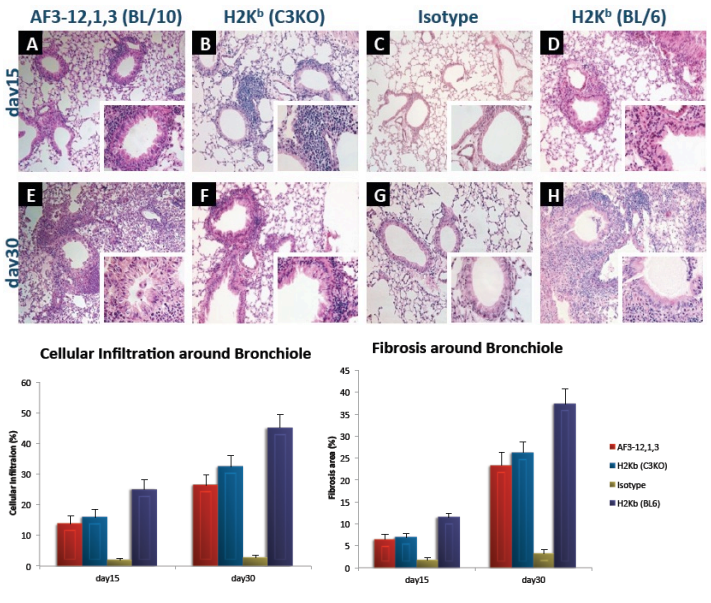


Figure 1: OAD lesions upon ligation with non-complement fixing Abs in murine alloimmune OAD model

2.4. Clinical Studies

The funded and ongoing CTOTC-03 trial is a prospective, observational study to evaluate the impact of respiratory viral infections on the development of allo-and autoreactivity in pediatric lung transplant recipients. The team has laboratory results on 36 patients thus far.

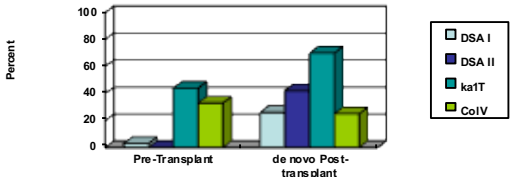


Figure 2: Percent + antibodies from those at risk

While positive class I (3%) or class II (0%) DSA pre-transplant were rare, significant numbers developed class I and class II DSA within 3 months post-transplant **(Figure 2)**.

Autoantibodies to ColV and kα1T were detectable prior to transplant in 33% and 44% of subjects, respectively. Post-transplant, de novo DSA and autoantibodies to both self-antigens occurred frequently, with kα1T most common. Correlations between antibodies and outcomes have not been assessed to date.

These results indicate both allo- and autoantibodies develop frequently and early following pediatric lung transplantation supporting our contention that induction therapy targeting B cells will be required to prevent and or eliminate these antibodies.

We analyzed recipient T cells for reactivity to donor antigens (when donor tissue was available) and to ColV and kα1T as putative autoantigens. We observed 9/26 (35%), 2/36 (6%) and 21/36 (58%) of the recipients exhibited significant frequencies of IFNg producing cells to donor and/or autoantigens (ColV and Kα1T) posttransplant, respectively.

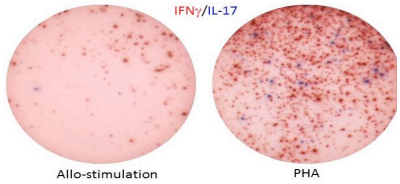


Figure 3: 2 color IFNg/IL-17 ELISPOT assay

To expand upon these techniques to test for IL-17 production (IL-17 has been associated with BOS in recent adult transplant recipients[25]) with limited available cells from pediatric patients we developed a 2 color IFNg/IL-17 ELISPOT assay (**Figure 3**).

CTOTC-03 tested for respiratory viral infections as a potential trigger for immune activation. We evaluated prospective serial nasopharyngeal (NP) and bronchoalveolar lavage (BAL) specimens and interrogated by respiratory multiplex PCR (Luminex xTAG) that identifies 17 viruses.

Preliminary epidemiologic data revealed 23 of 31 (74%) of subjects had at least one positive viral specimen. The median time to virus-positive specimens was 65 days post-transplant (range 1-599). Rhino/enterovirus was recovered most frequently (40 episodes) compared to coronavirus (3), hMPV (1), influenza (2), RSV (2), adenovirus (1), parainfluenza (1). Rhino/enterovirus occurred throughout the year but the other viruses were detected in the winter/spring months. Along with previous infectious diseases epidemiology, we have the capacity to monitor for and respond to changes in the rates of infectious episodes in our patient population.

Development of Abs to HLA following Lung Transplant and its impact on the development of BOS:

Since 2006, the Washington University adult lung program and the Mohanakumar lab instituted serial analysis for development of Abs to donor mismatched HLA during the post-transplant period. All adult LTxR had negative donor cross-matches. Of 116 patients serially analyzed, 65 developed DSA (56%), with 52 in the first 90 days, again supporting our contention that early intervention will be required to prevent induction. To test the hypothesis that antibodies contribute to late graft injury, the Mohanakumar group used IVIG (500 mg/kg x 6 monthly doses) and rituximab (375 mg/m^2 x 1 dose) for desensitization. Of the 44 patients treated, 27 (61%) cleared the DSA, and 17 (39%) had persistent DSA. An additional 17 patients were treated with IVIG alone and 11 (65%) cleared the DSA. More importantly, patient and graft survival was significantly better in successfully desensitized patients (Figure 4) providing strong correlative evidence that DSA participate in the development of allograft injury.[78]

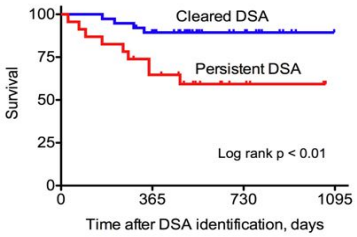


Figure 4: Improved survival following clearance of DSA.

A critical role for Abs to self-Ags in the pathogenesis of BOS:

Among adult LTxR who developed DSA (n=57) and subsequently cleared DSA following Ab directed therapy (n=34), 9 patients nonetheless developed BOS. We detected persistence of Abs to self-Ags, ColV and kα1T in most of these individuals, strongly implicating pathogenic roles for Abs to self-Ags. As shown in **Figure 5,** the LTxR who cleared both Abs to HLA and self-Ags demonstrated improved freedom from BOS in comparison to patients that cleared only DSA but not Abs to self Ags.

		Abs Kα1T		
		pre-Rx	post-Rx	
DSA Cleared (34/57)	BOS(+) (n=9)	9/9 696 ±137	8/9 635 ±152	p <0.01
	BOS(-) (n=25)	22/25 534 ±123	11/25 232 ±122	

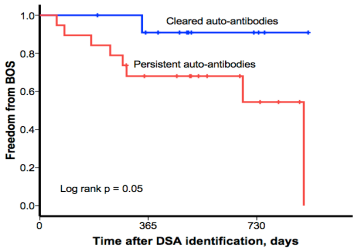


Figure 5: Increased incidence of BOS in DSA cleared LTx patients following Ab directed treatment when Abs to self-Ags persist

Taken together, these preliminary results strongly support our hypothesis that Abs to self–Ags play a critical role in the pathogenesis of BOS and our proposed study will assess both the impact of intervention and further determine significance and mechanisms of antibodies to self-antigens.

Successful post-transplant desensitization of Abs to self-Ags in adult LTxR:

To determine whether a post-transplant desensitization regimen for DSA with rituximab and IVIG has an impact on the Abs to self-Ags, we analyzed 123 LTxR who developed DSA. Forty-five LTxR had Abs to kα1T and 31 had Abs to ColV prior to desensitization. Following treatment 46.7% of the kα1T+ and 48.4% of the ColV+ became negative and another 25% demonstrated a significant decrease. This study demonstrates that rituximab-based desensitization of Abs to self-Ags is feasible.

Correlation between antibodies to self-Ags and pro-inflammatory milieu persists with continued Abs to self-Ags even after DSA clears: To determine the mechanisms by which Abs against HLA and self-Ags may contribute to the pathogenesis of BOS, were performed serum cytokine analysis using 25-plex Luminex assay. We analyzed sera from three groups of patients: group I DSA and Abs to self-Ags present (prior to therapy), group II cleared DSA but Abs to self-Ags persisted and group III cleared both DSA and Abs to self-Ags.

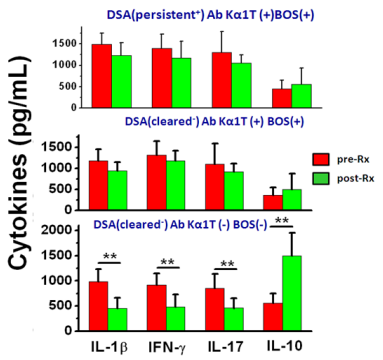


Figure 6: Increased Pro-inflammatory cytokines in LTx patients with persistent DSA and Abs to self-Ags correlated with BOS.

Results presented in **Figure 6** demonstrate that group I patients who had both DSA and Abs to self-Ag (n= 23) as well as group II that cleared DSA but persisted Abs to self-Ag (n=9) had high levels of pro-inflammatory cytokines. In contrast, group III cleared DSA and Abs to self-Ags (n=25) and did not develop BOS had decreased levels of pro-inflammatory cytokines (namely IL-1β (3.2 fold decrease), IL-17 (3.0), and IFN-γ (2.3)) and increased IL-10 (3.7 fold, $p<0.01$ for all) within 6 months. These results support persistent immune responses to self-Ags can lead to a pro-inflammatory milieu that can facilitate the development of BOS.

<u>Increased frequency of B cell with autoimmune phenotype in BOS</u>:

Data from mouse models and humans with autoimmune diseases suggest an important role for a specific subset of B cells in the pathogenesis of autoimmune diseases[79-81]. These B cells express CD11c, produce autoantibodies and can be induced to proliferate in response to TLR stimuli. To determine whether this subpopulation of B cells is increased in adult LTxR with BOS we determined the frequency of the autoimmune B cells (AIB) with phenotype of CD19+, CD11c+ B220+ in peripheral blood of LTxR with BOS and Abs to self-Ags. Results presented in **Figure 7** demonstrate a five-fold increase in the AIB (9.6±2.8% of B cells) over normal (1.6±0.7% of B cells). To determine whether those B cells are present in the lung allografts, BAL cells were characterized. Results demonstrate that the BOS(+) LTxR who had Abs to self-Ag demonstrate a 3.5 fold increase over BOS(-) LTxR (9.3±2.1% vs. 2.8±1.2% of B cells, n=6). It is of interest that BOS(+) anti self-Ag(-) LTxR showed similarly low frequency of AIB as in BOS(-) LTxR (data not shown). These results strongly favor our contention that AIB may play a role in immune responses to self-Ags and development of BOS, which will be tested in the mechanistic study.

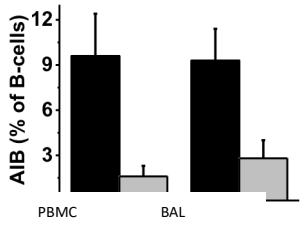


Figure 7: Increased frequency of AIB in PBMC and BAL.

<u>Circulating B cells in BOS(+) LTxR augment the frequency of self-Ag specific Th17 cells:</u>

To determine the role of B cells to activate self-Ag specific T cells , T cells isolated from BOS(+) self-Ab(+) or BOS(-) self Ab(-) LTxR were cultured with sub-optimal concentrations of kα1T or ColV (100ng/ml) with or without B cells. At the end of 2 weeks, the frequency of Th17 cells was analyzed by ELISPOT.

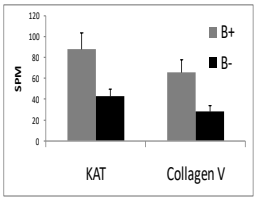


Figure 8: Frequency of Ag specific Th17 cells increases in the presence of B cells.

Results in **Figure 8** demonstrate that in the presence of B cells there was a significant increase in the frequency of Th17 cells specific for kα1T and ColV when compared without B cells. BOS(-) self-Ab(-) LTxR or cells from normal volunteers failed to respond.

<u>BOS(+) LTxR with significant increase in pro-inflammatory environment even without Abs to HLA, ColV and kα1T</u>:

Sera from LTxR with BOS but neither DSA nor Abs to self-Ags (ColV, Kα1T) were analyzed for serum cytokines using Luminex. LTxR who developed BOS (24%, 12/51) but did not develop Abs to HLA or ColV and kα1T had elevated levels of IL-17 (8.2), IFN-γ (3.7) and decreased levels of IL-10 (2.9) suggesting that induction of Th17 immune responses may play a role even in the absence of humoral responses to HLA, ColV and kα1T.

3. Study Design

3.1. Description of Study Design

This is a phase 2, prospective, multi-center, double-blind, randomized, placebo-controlled clinical trial in which 50 primary pediatric lung transplant recipients will be randomized (1:1) to receive either induction therapy with anti-CD20 mAb (375 mg/m^2) IV or placebo (IV day 0 and day 12 post-transplant) plus standard of care immunosuppression (thymoglobulin induction, tacrolimus or equivalent, MMF or equivalent, and steroids). The study will randomize 50 primary pediatric lung transplant recipients from seven participating centers. Subjects will be screened, consented, and enrolled while on the UNOS waitlist. When the recipient has received the transplant and is deemed hemodynamically stable, randomization will occur.

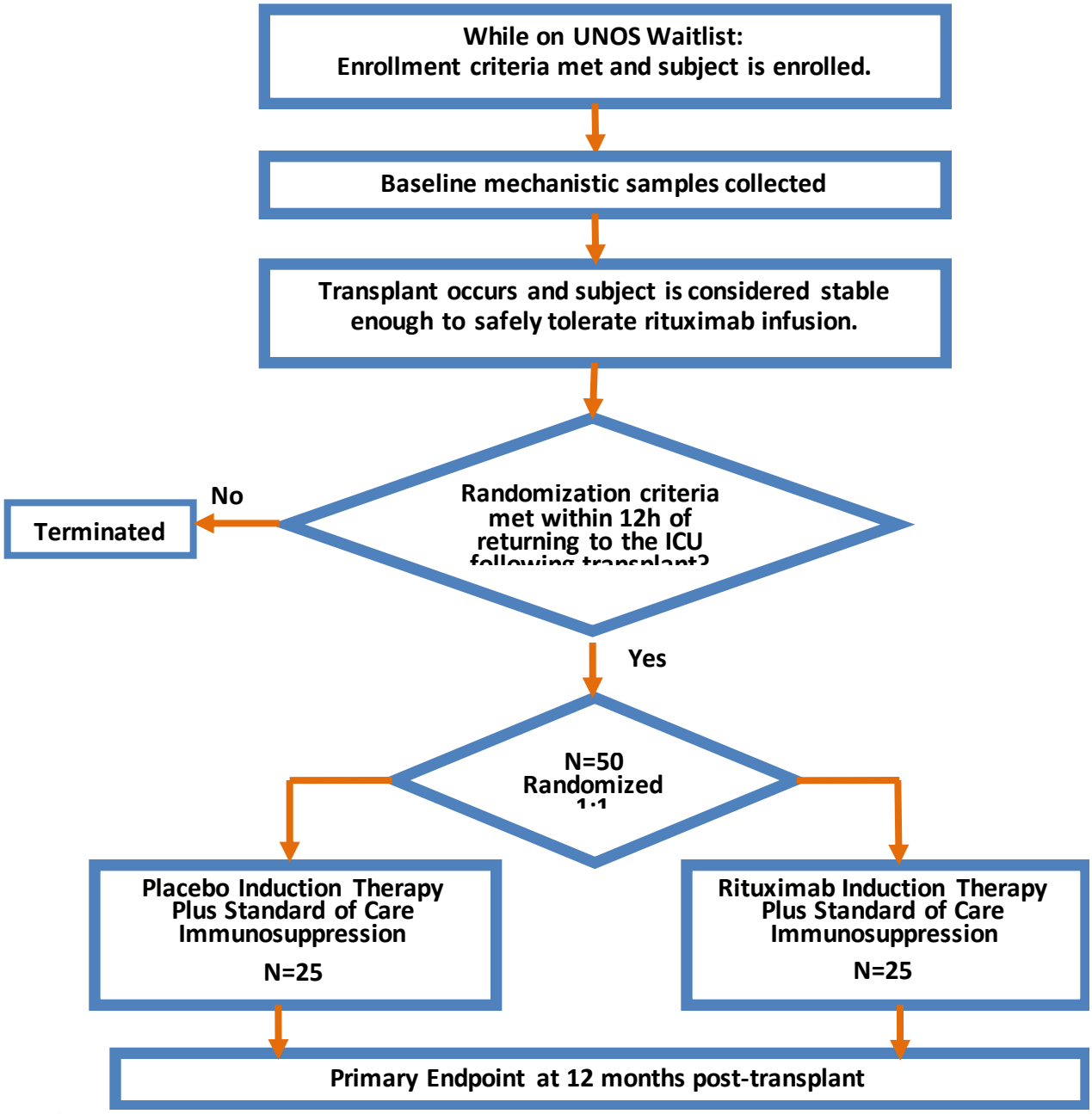


Figure 9: Study Design

3.2. Primary Endpoints

The primary endpoint is the earliest time to any of the following events during the follow-up period:

- Chronic Allograft Dysfunction
- Listed for Retransplant
- Death

3.3. Secondary Endpoints

The following *secondary clinical endpoints* will be assessed:

1. Post-transplant outcomes including:
a) Incidence of chronic allograft dysfunction, relisting and death during the follow-up period, which will be a minimum of 12 months post-transplant;
b) Incidence of Primary Graft Dysfunction;
c) Incidence of Grade A Acute Rejection during the follow up period, which will be a minimum of 12 months post-transplant;
d) Incidence of Antibody Mediated Rejection during the follow up period, which will be a minimum of 12 months post-transplant;
e) Incidence of tacrolimus variability threshold during the follow up period, which will be a minimum of 12 months post-transplant;
f) Percentage of participants meeting tacrolimus variability threshold who complete tacrolimus variability intervention;
g) Magnitude of change in standard deviation of tacrolimus levels following intervention.
2. Post-transplant safety outcomes including:
    a) Incidence and severity of infection episodes; See previous comment in the synopsis above
    b) Serious adverse events related to rituximab.

3.4. Mechanistic Endpoints

The following *mechanistic endpoints* will be assessed:

1. Incidence and kinetics of DSA and autoantibodies, specifically Collagen V (ColV) and k-alpha-1 tubulin (kα1T);
2. Frequency, kinetics, phenotype and function of peripheral B cells;
3. Frequency, kinetics and cytokine profiles of allo- and auto reactive T cells;
4. Incidence and quantity of B cells and B cell proximity to other cells in the graft tissue.

3.5. Stratification, Randomization, and Blinding/Masking

This is a double-blinded study; therefore, medication assignments will be blinded to the study participants as well as to the site clinical personnel. ***Only the site research pharmacist will have access to the unblinded randomization schedule for that site.*** In the event the subject undergoes a life-threatening reaction, then study subject and treating physician will be unblinded to the treatment assignment. In the event the subject undergoes repeat severe infusion or hypersensitivity reactions, despite appropriate treatment, then study subject and treating physician will be unblinded to the treatment assignment. IND safety reports will be reported to the FDA, DSMB, and IRBs in an unblinded fashion.

Randomization treatment assignments will be developed by the SACCC statisticians and stored on the SACCC server. The SACCC will maintain a central web-based randomization system. Once the subject is determined to be eligible based on the criteria, the site personnel will be provided the appropriate treatment assignment.

3.5.1 Procedure for Unblinding/Unmasking

Unblinding must be approved by the study Medical Monitor unless an immediate life threatening condition has developed and the Medical Monitor is not accessible. The site investigator will notify the protocol chair(s) and the study Statistical and Clinical Coordinating Center team of the unblinding event on the next business day. The emergency unblinding will also be reported to the Data and Safety Monitoring Board (DSMB).

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

Unblinding the study due to an approved interim analysis, final analysis, or study termination will require written approval from NIAID.

4. Selection of Participants and Clinical Sites

4.1. Rationale for Study Population

Although early outcomes in pediatric transplantation have improved in the past 2 decades, conditional survival beyond the early post-transplant period has not improved significantly, due primarily to the development of chronic allograft dysfunction. This is particularly evident for pediatric lung transplant recipients. In recent years, accumulating evidence has implicated antibodies as an important contributor to chronic allograft dysfunction. These include antibodies to donor histocompatibility antigens or cryptic self-antigens exposed during organ transplantation or subsequent graft injury. The mechanistic connection between antibody development and chronic allograft dysfunction in lung transplantation has yet to be determined. Moreover, effective therapies for prevention and management of pathologic antibodies for chronic allograft dysfunction have yet to be developed.

This study and the associated mechanistic studies will dissect the role B cells play in this process, including direct antibody mediated effects and indirect effects on cellular alloimmune and autoimmune response.

4.2. Enrollment Inclusion Criteria

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

1. Subject and/or parent guardian must be able to understand and provide informed consent;
2. Less than or equal to 21 years of age;
3. Candidate for a primary lung transplant (listed for lung transplant);
4. Adequate bone marrow function based on the following criteria:
    a. ANC: >1000mm3
    b. Platelets: >100,000/mm3
    c. Hemoglobin: >7 gm/dL
    d. AST or ALT< 2x Upper Limit of Normal unless related to primary disease
5. Female and male subjects with reproductive potential must agree to use FDA approved methods of birth control for 12-months after completion of treatment.

4.3. Enrollment 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. Multi-organ transplant;
3. Previous treatment with rituximab (Rituxan®);
4. History of severe allergic anaphylactic reactions to humanized or murine monoclonal antibodies;
5. History of severe reaction to previous therapy with IVIG;
6. History of Burkholderia cenocepacia;
7. History of anti-CD20 therapy;
8. Persistent hypogammaglobulinemia (IgG < lower level of normal for age based on local laboratory ranges or 400 gm/dL for >2 months) and/or IVIG replacement therapy;
9. Positive blood culture, sepsis or other disease process with hemodynamic instability at time of enrollment;

10. Any history of serologic positivity to HIV, HBsAg, HBcAb and HCV Ab;
11. History of malignancy less than 2 years in remission of malignancy (any history of adequately treated in-situ cervical carcinoma, or adequately treated basal or squamous cell carcinoma of the skin will be permitted);
12. Any condition, including psychiatric disorders, that in the opinion of the investigator would interfere with the subject's ability to comply with study requirements;
13. Participation in another investigational trial within 4 weeks of enrollment;
14. Currently lactating or plans to become pregnant during the timeframe of the study follow-up period;
15. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.

4.4. Randomization Inclusion Criteria

Individuals who meet all of the following criteria are eligible for randomization:

1. Serum IgG immunoglobulin level greater than lower level of normal for age based on local laboratory ranges or 400 gm/dL within 90 days prior to randomization;
2. Female subjects of childbearing potential must have a negative pregnancy test within 48 hours of transplant;
3. Negative for Hepatitis B infection (if at time of transplant, participant does not exhibit effective immunization, the participant should be re-tested).

4.5. Randomization Exclusion Criteria

Individuals who meet any of these criteria are not eligible for randomization:

1. Use of an induction agent other than Thymoglobulin®;
2. Renal insufficiency requiring hemodialysis or ultrafiltration;
3. Inability to obtain intravenous access;
4. Positive blood culture, sepsis or other disease process with hemodynamic instability at time of transplant;
5. Use of investigational agent(s) within 5 half-lives of the investigational drug or 4 weeks, whichever is longer;
6. Receipt of a MMR vaccine within 30 days prior to randomization;
7. Any condition that, in the opinion of the investigator, would interfere with the subject's ability to comply with study requirements.

4.6. Selection of Clinical Sites

The target population for this study consists of pediatric lung transplant recipients. Subjects will be enrolled at sites throughout the United States. The sites participating in the study have performed the majority of pediatric lung transplants annually in the United States. The target population therefore is a fair representation of the pediatric lung population.

5. Known and Potential Risks and Benefits to Participants

5.1. Risks of rituximab (Rituxan®)

No dose-limiting effects were observed in Phase I/II studies. Reported adverse events including fever, chills, headache, nausea, vomiting, rhinitis, asthenia, and hypotension, occurred primarily during rituximab infusions and typically responded to an interruption of the infusion and resumption at a slower rate.

Fatal Infusion Reactions: Severe and fatal cardiopulmonary events, including angioedema, hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, and cardiogenic shock, have been reported. These severe reactions typically occurred during the first infusion with time to onset of 30-120 minutes.

Cardiac Events: Patients with preexisting cardiac conditions, including arrhythmia and angina, have had recurrences of these cardiac events during rituximab infusions.

Tumor Lysis Syndrome: Tumor lysis syndrome, some with fatal outcome, has been reported and is characterized in patients with a high number of circulating malignant cells (≥25,000 ul) by rapid reduction in tumor volume, renal insufficiency, hyperkalemia, hypocalcemia, hyperuricemia, and hyperphosphatemia.

Renal Events: Rituximab has been associated with severe renal toxicity including acute renal failure requiring dialysis, and in some cases has led to death. Renal toxicity has occurred in patients with high numbers of circulating malignant cells (≥25,000/mm^2) or high tumor burden who experience tumor lysis syndrome and in patients administered concomitant cisplatin.

Mucocutaneous Reactions: Severe bullous skin reactions, including fatal cases of toxic epidermal necrolysis and paraneoplastic pemphigus, have been reported in patients treated with rituximab. The onset of reaction has varied from 1 to 13 weeks following rituximab exposure.

Nervous System:

a. **Posterior reversible encephalopathy syndrome (PRES)/Reversible Posterior Leukoencephalopathy Syndrome (RPLS):** Cases of posterior reversible encephalopathy syndrome (PRES)/reversible posterior leukoencephalopathy syndrome (RPLS) have been reported. Signs and symptoms include visual disturbance, headache, seizures and altered mental status, with or without associated hypertension. A diagnosis of PRES/RPLS requires confirmation by brain imaging. The reported cases had recognized risk factors for PRES/RPLS, including hypertension, immunosuppressive therapy and/or other concomitant therapies.

b. **Progressive multifocal leukoencephalopathy (PML):** PML is a rare disease caused by the reactivation of latent JC virus in the brain. Immunosuppression allows reactivation of the JC virus which causes demyelination and destruction of oligodendrocytes resulting in death or severe disability. Rare cases of PML, some resulting in death, have been reported in patients with hematologic malignancies who have received rituximab. The majority of these patients had received rituximab in combination with chemotherapy or as part of a hematopoietic stem cell transplant. Cases of PML resulting in death have also been reported following the use of rituximab for the treatment of autoimmune diseases. The reported cases had multiple risk factors for PML, including the underlying disease and long-term immunosuppressive therapy or chemotherapy. Most cases of PML were diagnosed within 12 months of their last infusion of rituximab.

Physicians should consider PML in any patient presenting with new onset neurologic manifestations. Consultation with a neurologist, brain MRI, and lumbar puncture should be considered as clinically indicated. In patients who develop PML, rituximab should be discontinued and reductions or discontinuation of any concomitant chemotherapy or immunosuppressive therapy should be considered.

Hematologic Events: In clinical trials, Grade 3 and 4 cytopenias were reported in 48% of patients treated with rituximab; these include: lymphopenia (40%), neutropenia (6%), leukopenia (4%), anemia (3%), and thrombocytopenia (2%). The median duration of lymphopenia was 14 days (range, 1 to 588 days) and of neutropenia was 13 days (range, 2 to 116 days). A single occurrence of transient aplastic anemia (pure red cell aplasia) and two occurrences of hemolytic anemia following Rituximab therapy were reported.

In addition, there have been a limited number of postmarketing reports of prolonged pancytopenia, marrow hypoplasia, and late onset neutropenia.

Infectious Events: Rituxan® induced B cell depletion in 70% to 80% of patients with NHL and was associated with decreased serum immunoglobulins in a minority of patients; the lymphopenia lasted a median of 14 days (range, 1-588 days). Infectious events occurred in 31% of patients: 19% of patients had bacterial infections, 10% had viral infections, 1% had fungal infections, and 6% were unknown infections. Serious infectious events (Grade 3 or 4), including sepsis, occurred in 2% of patients.

Hepatitis B Reactivation: Hepatitis B virus (HBV) reactivation with fulminant hepatitis, hepatic failure, and death has been reported in some patients with hematologic malignancies treated with rituximab. The majority of patients received rituximab in combination with chemotherapy. The median time to the diagnosis of hepatitis was approximately four months after the initiation of rituximab and approximately one month after the last dose.

Participants with pre-existing Hepatitis B infection will not be eligible to participate in this study.

Other Serious Viral Infections: The following additional serious viral infections, either new, reactivated or exacerbated, have been identified in clinical studies or postmarketing reports. The majority of patients received Rituxan® in combination with chemotherapy or as part of a hematopoietic stem cell transplant. These viral infections included JC virus (progressive multifocal leukoencephalopathy [PML]), cytomegalovirus, herpes simplex virus, parvovirus B19, varicella zoster virus, West Nile virus, and hepatitis C. In some cases, the viral infections occurred up to one year following discontinuation of Rituxan® and have resulted in death.

Bowel Obstruction and Perforation: Abdominal pain, bowel obstruction and perforation, in some cases leading to death, were observed in patients receiving Rituxan® in combination with chemotherapy for DLBCL. In post-marketing reports, which include both patients with low-grade or follicular NHL and DLBCL, the mean time to onset of symptoms was 6 days (range 1−77) in patients with documented gastro-intestinal perforation. Complaints of abdominal pain, especially early in the course of treatment, should prompt a thorough diagnostic evaluation and appropriate treatment.

Immunogenicity: Patients may develop a human anti-chimeric antibody (HACA) response with rituximab treatment. The clinical significance of this is unclear.

Pregnancy: B cell lymphocytopenia generally lasting less than 6 months can occur in infants exposed to rituximab in utero.

Immunization: Response rates may be reduced with non-live vaccines.

Additional Safety Signals: The following serious adverse events have been reported to occur in patients following completion of rituximab infusions: arthritis, disorders of blood vessels (vasculitis, serum sickness and lupus-like syndrome), eye disorders (uveitis and optic neuritis), lung disorders including pleuritis and scarring of the lung (bronchiolitis obliterans), that may result in fatal outcomes, and fatal cardiac failure.

See the rituximab Investigator Brochure for additional details regarding safety experience with rituximab.

5.2. Risks of Immunosuppression Medications

The safety and effectiveness of immunosuppression medications in pediatric patients is not established in controlled trials. However, the dose, efficacy and adverse event profile are not thought to be different from adults based on studies in the literature.

5.2.1 Thymoglobulin® [Anti-thymocyte globulin (rabbit)]

Risks associated with Thymoglobulin® include immune-mediated reactions, infections, reactivation of infection, malaise, dizziness, sepsis, thrombocytopenia, leukopenia, and increased incidence of malignancies, including lymphoma or post-transplant lymphoproliferative disease (PTLD). Occasional reactions are observed at the infusion site including pain, swelling and erythema.

The majority of pediatric lung transplant centers reporting to the International Society of Heart and Lung Transplantation (ISHLT) registry use antibody based induction immunosuppressive therapy.

5.2.2 CellCept® (Mycophenolate mofetil-MMF)

CellCept® (Mycophenolate mofetil - MMF) is approved (in combination with cyclosporine and corticosteroids) as an immunosuppressive agent 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, hypercholesteremia, hypokalemia, hyperglycemia, increased creatinine and BUN, cough, hypomagnesaemia, diarrhea, constipation, nausea, vomiting, respiratory infection, dyspnea, lung disorder, pleural effusion, tremor and insomnia.

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 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.

Use of CellCept® during pregnancy is associated with an increased risk of first trimester pregnancy loss and an increased risk of congenital malformations, especially external ear and other facial abnormalities including cleft lip and palate, and anomalies of the distal limbs, heart, esophagus, and kidney.

Females of reproductive potential will receive contraceptive counseling and use acceptable birth control during the study, and for 6 weeks after stopping CellCept®, unless the participant chooses abstinence (she chooses to avoid heterosexual intercourse completely).

For those females using CellCept® at any time during pregnancy and those becoming pregnant within 6 weeks of discontinuing therapy, the investigator or healthcare practitioner should report the pregnancy to the Mycophenolate Pregnancy Registry. The investigator or healthcare practitioner should strongly encourage the patient to enroll in the pregnancy registry.

Risks and benefits of CellCept® should be discussed with the patient. When appropriate, consider alternative immunosuppressants with less potential for embryofetal toxicity. In certain situations, the patient and her healthcare practitioner may decide that the maternal benefits outweigh the risks to the fetus. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

Additional information about MMF can be found in the package insert.

5.2.2 Prograf® (Tacrolimus)

Side effects of Prograf® (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. Risks of Study Procedures

5.3.1 Risk of Blood Draw (Venipuncture)

The amount of blood that may be drawn from pediatric subjects for research purposes will not exceed 3cc/kg per day or 7cc/kg per six 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 whether research blood tests can be performed.

5.3.2. Risk of Bronchoalveolar Lavage and Bronchoscopy

Bronchoalveolar lavage (BAL) will be obtained during standard of care bronchoscopies according to IPLTC guidelines. The risks associated with BAL collected for this study are no different than routine management.

5.3.3. Risk of Tacrolimus Standard Deviation Threshold

Non-adherence may be identified during the intervention. If non-adherence is identified, this may negatively affect the participant's candidacy for a second transplant.

The initial call for the intervention will be facilitated by the site coordinator at the transplant center. During this call the team at ICMMS will ask the participant or parent/guardian to provide contact information to the team at ICMMS for future calls. Any PHI shared with ISMMS may be disclosed.

5.4. Potential Benefits

Receipt of the study treatment (rituximab) may reduce the participant's risk of antibody-related complications from lung transplant. Participants may benefit by having their health status closely followed. In the future, other people may benefit from this study because the information learned from this study may influence future care of transplant patients.

6. Investigational Agent

6.1. Rituximab (Rituxan®)

Rituximab is a is a genetically engineered, chimeric, murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant pre-B and mature B cells. The antibody is an IgG_1 κ immunoglobulin containing murine light-and heavy-chain variable region sequences and human constant region sequences. Rituximab is composed of two heavy chains of 451 amino acids and two light chains of 213 amino acids (based on cDNA analaysis) and has an approximate molecular mass of 145 kD. Rituximab has a binding affinity for the CD20 antigen of ~8.0 nM.

6.1.1. Rituximab (Rituxan®)

6.1.1.1. Formulation, Packaging, and Labeling

Rituximab® is a sterile, clear, colorless, preservative-free liquid concentrate for intravenous (IV) administration. Rituximab is supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single-use vials. The products are formulated for intravenous administration in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection. The pH is adjusted to 6.5.

The vials provided to the pharmacy will have occluded, study-specific investigational agent labels.

6.1.1.2. Dosage and Preparation

Rituximab will be given as an intravenous infusion.

2 Doses: 375 mg/m^2 on Day 0 (within 12 hours of return to ICU following transplant) and Day 12 (+/- 2 days).

Rituximab should be prepared using appropriate aseptic techniques. Withdraw the necessary amount of Rituximab or Rituximab Placebo and dilute to a final concentration of 1 to 4 mg/mL into an infusion bag containing either 0.9% Sodium Chloride, USP, or 5% Dextrose in Water, USP. Gently invert the bag to mix the solution. Discard any unused portion left in the vial. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

Rituximab or Rituximab Placebo should be given as a **slow** intravenous infusion. **It should not be administered as an intravenous push or bolus.** Do not infuse rituximab concomitantly with another IV solution or other IV medications. Rituximab infusions should be made through a dedicated line.

Participants will be premedicated using:

- Tylenol® (Acetaminophen), 15 mg/kg (to a maximum of 1 gram) should be administered (by mouth, enteral or rectal administration) 30 to 60 minutes prior to the start of an infusion; and
- Benadryl® (Diphenhydramine) by mouth 30-60 minutes prior to the start of an infusion as described in **Table 1** or by IV 1 mg/kg (to a maximum of 50 mg) 30 minutes prior to the start of an infusion.

Age (years)	Diphenhydramine Oral Dose (mg)
2-6	6.25
6-<12	12.5
12+	25

Table 1: Diphenhydramine Pre-medication Dose

Premedication may attenuate infusion-related events. Since transient hypotension may occur during rituximab infusion, consideration should be given to withholding anti-hypertensive medications 12 hours prior to rituximab infusion.

6.1.1.3. Administration Guidelines

First infusion (Day 0): Rituximab should be administered within 12 hours of returning from the ICU following transplant, based on hemodynamic stability. Rituximab should be administered intravenously through a dedicated line at an initial rate of 0.5 mg/kg/hr (to a maximum rate of 50 mg/hr) for the first hour.

If hypersensitivity or infusion-related events do not occur, the infusion rate will be escalated by 0.5 mg/kg/hr every 30 minutes, to a maximum rate of 400 mg/hr.

If a hypersensitivity or infusion-related reaction event develops, the infusion rate should be temporarily slowed or interrupted. The infusion can continue at one-half the previous rate upon improvement of patient symptoms.

Subsequent Infusion (Day 12 +/- 2 days):

A second dose of rituximab will be administered to subjects considered stable enough to receive the infusion.

Individual Subject Stopping Rules:

Individuals who meet any of the criteria listed below will not receive the second dose of rituximab.

1. Serious adverse event causally related to the rituximab infusion;
2. Acute pulmonary infectious process with evidence of graft dysfunction;
3. Positive blood culture, sepsis or other disease process with hemodynamic instability;
4. Renal insufficiency requiring hemodialysis or ultrafiltration;
5. Inability to obtain intraveneous access;
6. Use of an investigational drug after the first dose of placebo or rituximab;
7. Any other event which in the opinion of the principal investigator may pose additional risk to the participant.

If the first infusion was well tolerated, the subsequent infusion may be administered intraveneously through a dedicated line at an initial rate of 1.0 mg/kg/hr (to a maximum rate of 100 mg/h) for the

first hour. If hypersensitivity or infusion-related events do not occur, the infusion rate will be escalated by 1.0 mg/kg/hr (maximum rate of 100 mg increase per hour) every 30 minutes, to a maximum rate of 400 mg/hr. If hypersensitivity or infusion-related event develops, the infusion should be temporarily slowed or interrupted. The infusion can continue at one half the previous rate upon improvement of patient symptoms.

If hypersensitivity or infusion-related events occurred during the first infusion, the subsequent infusion should be administered in the same manner as the first infusion.

6.1.2. Rituximab Placebo

6.1.2.1. Formulation, Packaging, and Labeling

Rituximab placebo is a sterile, clear, colorless, preservative-free liquid concentrate for intravenous (IV) administration. Rituximab is supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single-use vials. The product is formulated for intravenous administration in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection. The pH is adjusted to 6.5. The vials provided to the pharmacy will have occluded, study-specific investigational agent labels.

6.1.2.2. Dosage, Preparation, and Administration

Rituxmab placebo will be administered according to the guidelines for rituximab outlined in section 6.1.1.2.

6.2. Infusion Supervision (Rituximab and Rituximab Placebo)

Rituximab and rituximab placebo should be administered in a hospital environment where full resuscitation facilities are immediately available. Rituximab and rituximab placebo may be administered on an outpatient basis however patients may be hospitalized for observation at the discretion of the investigator.

The 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 SACCC using the appropriate case report forms.

Vital signs (temperature, blood pressure, pulse, and respiratory rate) and standard laboratory testing (CBC, basic metabolic panel) will be obtained prior to the start of **each** rituximab or rituximab placebo infusion. The local investigator will determine whether to hold the infusion due to lab abnormalities. Vital signs will be monitored every 15 minutes for the first hour of the infusion, every hour until the end of the infusion, and every hour thereafter for a total of 3 hours after the infusion. The IV line should remain in place for at least 1 hour following the infusion to enable the administration of drugs, if necessary. Additional vital signs may be obtained as clinically indicated. Fluid resuscitation will be provided as needed for changes in vital signs suggesting hydration would be beneficial.

6.3. Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) 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.

Records for receipt, storage, use, and disposition will be maintained by the study site. 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 unused study drug (unopened or within expiration date) may be returned to the manufacturer at the end of the study.

6.4. Toxicity Prevention and Management

Patients enrolled in this study will be evaluated clinically and with standard laboratory tests before and during their participation in this study. Safety evaluations will consist of medical interviews, recording of adverse events, physical examinations, blood pressure, and laboratory measurements. Subjects will be evaluated for adverse events, serious adverse events, and adverse events requiring study drug interruption or discontinuation at each study visit for the duration of their participation in the study.

6.5. Modification or Premature Discontinuation of Investigational Agent

Rituximab (Rituxan®) and Rituximab Placebo may be modified or prematurely discontinued for any participant for any of the following reasons:

- **General Infusion-Related Reactions.** If an infusion-related reaction develops, the infusion rate should be temporarily reduced or interrupted. The infusion may be continued at one-half the previous rate upon improvement of the patient's symptoms.
- **Life-Threatening Reaction.** In the event of a life-threatening reaction, the infusion should be discontinued and the patient should receive medical treatment. Patients who experience a life-threatening reaction will not receive additional Rituximab/Placebo, and will remain in the study, and be included in the ITT analysis. The study treatment assignment will be unblinded to the site personnel and the study participant. (Protocol section 3.5.1)
- **Severe Infusion Reaction.** The infusion should be interrupted for severe reactions, and supportive care measures should be instituted as medically indicated (e.g., IV fluids, vasopressors, oxygen, bronchodilators, diphenhydramine, and acetaminophen). The infusion should not be restarted until all the symptoms have disappeared. On the restarting of the infusion, the rate should be half of that which precipitated the reaction. Patients who experience repeat severe infusion reactions, despite appropriate treatment, will not receive additional Rituximab/Placebo, and will remain in the study, and be included in the ITT analysis. The study treatment assignment will be unblinded to the site personnel and the study participant. (Protocol section 3.5.1)
- **Hypersensitivity Reactions.** Hypersensitivity reactions (non-IgE-mediated reactions) may respond to adjustments in the infusion rate and in medical management. The infusion should be interrupted for severe hypersensitivity reactions and can be resumed at a 50% reduction in rate (e.g., from 100 mg/hr to 50 mg/hr) when symptoms have completely resolved. Treatment of these symptoms with diphenhydramine and acetaminophen (or their equivalents) is recommended; additional treatment with bronchodilators or IV saline may be indicated. In most cases, patients who have experienced non-life-threatening hypersensitivity reactions have been able to complete the full course of

therapy. Medications for the treatment of hypersensitivity reactions (e.g., epinephrine, antihistamines, and corticosteroids) should be available for immediate use in the event of a reaction during administration. Patients who experience repeat hypersensitivity reactions, despite appropriate treatment, will not receive additional Rituximab/Placebo, and will remain in the study, and be included in the ITT analysis. The study treatment assignment will be unblinded to the site personnel and the study participant. (Protocol section 3.5.1)

- **Cardiovascular.** Infusions should be discontinued in the event of serious or life-threatening cardiac arrhythmias. Patients who develop clinically significant arrhythmias should undergo cardiac monitoring during and after subsequent infusions. Patients should be monitored throughout the infusion and the immediate post-infusion period.

7. Other Medications

7.1. Immunosuppression Medications

7.1.1. Protocol-mandated

7.1.1.1 Thymoglobulin® [Anti-thymocyte Globulin (Rabbit)]

All subjects will receive Thymoglobulin® induction therapy. Thymoglobulin® will be administered for 5 days as an intravenous infusion at a dose of 1.5 mg/kg/day beginning on the day of transplant.

7.1.1.2 Tacrolimus (Prograf®)

Tacrolimus (Prograf®), or generic equivalent, will be administered to attain target trough levels outlined in Table 3. Suggested dosing is outlined in Table 2.

Timepoint	Tacrolimus Dose
Post-operatively (by 72 hours following transplant operation) *	0.01-0.04 mg/kg/day IV continuous infusion OR 0.06-0.1 mg/kg/day (maximum 5 mg per dose) via NG or G-tube divided into 2 equal doses OR 0.08 mg/kg/day sublingual divided into 2 equal doses
Once taking PO	0.1-0.3 mg/kg/day PO divided into 2 equal doses
*Dosing may be adjusted to every 8 hours in infants and young children if therapeutic levels are hard to achieve (due to rapid metabolism of the drug in this age group).	

Table 2: Tacrolimus Dose

Dosing may be adjusted for underlying medical conditions and/or interactions with other medications.

Target whole blood tacrolimus concentrations are as follows:

Timepoint	Target Trough Level After Transplant
First 2 Weeks Post-Transplant	10-20 ng/mL
Week 2-1-year Post-transplant	10-15 ng/mL
After 1 Year	6-10 ng/mL
The above given recommendations for therapeutic drug monitoring of tacrolimus may not be suitable for infants secondary to the different pharmacokinetics in infants.	

Table 3: Tacrolimus Trough Levels

7.1.1.3. Mycophenolate Mofetil

Mycophenolate Mofetil (CellCept®), or generic equivalent, will be administered at an initial pediatric dose of 600-700 mg/m^2 twice daily. The dose may be modified to an initial dose of 800-900 mg/m^2 twice daily for cystic fibrosis subjects at puberty and above. Initial adult dosing is 1000-1500 mg twice daily.

Mycophenolate Mofetil (CellCept®), or generic equivalent, dosing will be adjusted based on clinical complications. Dosing outside of the target dose range will not be considered a protocol deviation.

7.1.1.4. Steroids

Methylprednisolone/Prednisone will be administered according to each center's standard practice. After the first negative biopsy, prednisone will be tapered, according to each center's protocol, to reach a target dose of 0.1- 0.2 mg/kg/day by one year post-transplant.

7.2. Viral Prophylaxis and Therapy

Participating sites will follow IPLTC guidelines for viral prophylaxis and treatment.

7.2.1. Respiratory Syncytial Virus

Subjects less than 2 years of age will receive palivizumab per the local standard of care. Respiratory Syncytial Virus will be treated with ribavirin per the local center standard of care.

7.2.2. Cytomegalovirus

Sero Positive Donor and/or Recipient (D+/R+, D+/R-, D-/R+) will receive ganciclovir/valganciclovir prophylaxis, per the local standard of care, for a minimum of 6 months following transplantation.

7.2.3. Fungal

All subjects will receive fungal prophylaxis post-transplant according to local center standard of care. Fungal therapies will be determined by the local standard of care.

7.2.4. Vaccination

Efficacy and/or safety of immunization during periods of B cell depletion have not been adequately studied. It is recommended that a patient's vaccination record and possible requirements be reviewed. Per the investigator's discretion, the patient may have any required vaccination/booster administered at least 4 weeks prior to the initiation of study treatment. Review of the patient's immunization status for the following vaccinations is recommended: tetanus; diptheria; influenza; pneumoccocal polysaccharide; *Varicella*; measles, mumps and rubella (MMR); and hepatitis B. Patients who are considered to be at high risk for hepatitis B virus (HBV) infection and for whom the investigator has determined that immunization is indicated should complete the entire HBV vaccine series at least 4 weeks prior to participation in the study.

All participants eligible for randomization will be encouraged to receive the influenza vaccine per Center for Disease Control guidelines.

7.3. Prohibited Medications

The following is a list of prohibited medications:

- Induction agent, other than Thymoglobulin®;
- Other investigational agents;
- Rituximab (Rituxan®), except for treatment of PTLD or treatment of AMR as described in section 7.4.2;

- IVIG, except for treatment of post-transplant hypogammaglobulinemia or humoral rejection as described in sections 7.4 and 7.5.2.

7.4. Treatment for Hypogammaglobulinemia

Intraveneous immune globulin will be administered at 400-500 mg/kg every four weeks to patients who develop hypogammaglobulinemia (IgG less than lower level of normal for age based on local laboratory ranges or 400 mg/dL) following transplant. For subjects with severe hypogammaglobulinemia (IgG less than 200 mg/dL), an IV loading dose of 1 gram/kg will be administered. The maintenance IV dose of 400-500 mg/dL at four week intervals will be titrated to response. IgG trough levels will be measured prior to an infusion.

7.5. Diagnosis and Treatment of Rejection

Rejection will be defined according to the current ISHLT working formulation of pulmonary allograft rejection. Each transbronchial biopsy (TBBx) will be evaluated at the local center and all therapeutic decisions will be based on the local pathologist's findings.

7.5.1. Acute Cellular Rejection

Acute Cellular Rejection will be graded based on ISHLT guidelines[103] described in **Table 4**.

ACR Grade	ACR Classification
A0	None
A1	Minimal
A2	Mild
A3	Moderate
A4	Severe

Table 4: Grading for Acute Cellular Rejection

Acute Cellular Rejection (Grade A2 or greater) will be treated based on IPLTC guidelines described in **Table 5**.

Table 5: Treatment for Acute Cellular Rejection

Initial Treatment	Methylprednisolone 10-20 mg/kg/day (max = 1 g/day) for 3 to 4 days AND return to maintenance steroid dosing
Repeat biopsy in 2-4 weeks depending on histology and/or clinical status (treatment success = ≤ A2)	
Persistent/Recurrent acute rejection*	Antibody-mediated rejection should be excluded ***If same or lower ACR grade (i.e. A3 to A2):*** Repeat high dose (IV) steroid protocol outlined in initial treatment. ***If worsening lung function and/or ACR grade, severe ACR (Grade A4) or failure of two courses of steroids:*** Rabbit (rATG): 1.5 mg/kg/day,(max 150 mg/day) for 10-14 days
Alternatives for refractory ACR	One or more of the following may be used: 1. Campath 1H (Alemtuzumab) 2. Total lymphoid irradiation 3. Photopheresis 4. Methotrexate 5. Rapamycin

7.5.2. Antibody Mediated Rejection

Antibody Medicated Rejection (AMR) will be graded locally as shown in **Table 5**.

	DSA[1] or Autoantibody	Abnormal Histology[2]	Graft Dysfunction[3]
I: Latent humoral response	X		
II: Subclinical humoral rejection	X	X	
III: Humoral rejection	X	X	X

[1] Circulating antibody to HLA or other antigens expressed in lung tissue.

[2] Including neutrophilic capillaritis or septal margination,high grade (≥A3) or persistent / recurrent acute rejection, acute lung injury/diffuse alveolar damage, high grade (B2R) or persistent low grade (B1R) lymphocytic bronchitis, obliterative bronchiolitis, arteritis in the absence of acute rejection or other finding not explained by clinical circumstances (i.e. infectious causes thoroughly excluded)

[3] Unexplained (i.e. infectious causes thoroughly excluded) persistent (> 1 week) change in respiratory status manifested by two MAJOR OR one MAJOR plus one or more MINOR criteria listed below:

MAJOR CRITERIA:

I. Increase in resting respiratory rate ≥ 10%
II. Decrease in resting SaO2 ≥ 4%
III. Persistent drop in FEV1 of ≥ 10%

MINOR CRITERIA:

I. Daily repetitive cough
II. Auscultatory findings
III. Inadequate weight gain defined as a drop between 5-10 percentile points
IV. CXR or CT changes

Table 5: Antibody Mediated Rejection Diagnostic Grading

Study participants will be screened for donor specific antibodies (DSA) monthly for the first 3 months following transplantation and then every 3 months if negative. C4d immunohistochemistry will be performed on all TBBx. AMR will be treated as described below.

I: Latent humoral response	No treatment, consider monitoring DSA more frequently
II: Subclinical humoral rejection	1. Administer methylprednisolone 10-20 mg/kg/day (max = 1 g/day) for 3 to 4 days AND return to maintenance steroid dosing. 2. Follow-up TBBx performed 2-4 weeks following completion of therapy. The local investigator may consider open lung biopsy if graft dysfunction is present. 3. If subclinical humoral rejection persists on the follow up biopsy, plasmapheresis will be performed.
III: Humoral rejection	1. Pulse steroids (10 mg/kg) day 1-3. 2. Plasmapheresis x 5 days without IgG replacement. 3. Bortezomib (1.3 mg/M2) on days 1, 4, 8, & 11 (given after plasmapheresis) with IgG replacement after last dose of Bortezomib. 4. Rituximab (375 mg/m^2) weekly weeks 1-4 for patients >1 month post-transplant.

Table 6: Treatment for Antibody Mediated Rejection

Subjects will be monitored for clinical improvement following treatment of AMR. Sites will consider repeat PRA for DSA at 1, 4 and 8 weeks post intervention and/or repeat biopsy for persistent symptoms.

7.5.3. Chronic Allograft Dysfunction

Chronic allograft dysfunction is defined as BOS, Grade 0p and above, or histologic evidence of oblitereans bronchilitis. [103, 105]For participants old enough to perform spirometry, BOS will be diagnosed locally based on the 2001 ISHLT diagnostic criteria shown in Table 7. Local sites will rule out acute rejection, acute infection and airway stenosis or narrowing prior to diagnosing BOS. Spirometry will be supervised by a trained technologist at each site.

BOS Grade	Classification
0	FEV_1 > 90% of baseline and FEF_{25-75} > 75% of baseline
0p	FEV_1 81 to 90% of baseline and/or $FEF_{25-75} \leq$ 75% of baseline
1	FEV_1 > 66-80% of baseline
2	FEV_1 > 51-65% of baseline
3	FEV_1 > 50% or less of baseline

Table 7: Classification of BOS

For participants unable to perform a pulmonary function test, BOS will not be determined. Obliterative bronchiolitis (OB) will be used as the outcome in this population. OB will be identified by histology according to the ISHLT report.

Biopsy to evaluate for OB will also be obtained from participants with unexplained air trapping on high resolution expiratory CT images or ventilation perfusion scanning and participants with unexplained obstructive changes on infant PFTs.

Chronic allograft dysfunction will be evaluated and treated as described in **Table 8**.

BOS Grade 0p	1. Reassess for contribution of reflux and/or infection and treat as appropriate. 2. Perform bronchoscopy and transbronchial biopsy. 3. If no OB on transbronchial biopsy consider open lung biopsy if rapidly progressive and monitor study participant closely.
BOS progressing to Grade1 despite above interactions	1. Reassess for ongoing contribution of reflux and/or infection and treat as appropriate. 2. Re-biopsy (transbronchial or open lung biopsy). 3. Pulse with steroids (Methylprednisolone 10 mg/kg/day x 3 days) and use cytolytic therapy (Thymoglobulin®) in patients with lymphocytic inflammation in active OB lesions.
Evidence of OB on biopsy	Pulse with steroids and use cytolytic therapy (Thymoglobulin®; dosing as above) in patients with lymphocytic inflammation in active OB lesions.

Table 8: Evaluation and Treatment for Chronic Allograft Dysfunction

Subjects will be re-evaluated every 3 months following the diagnosis of chronic allograft dysfunction. If BOS grade progresses the local PI may consider:

- Photopheresis
- Changing sirolimus to methotrexate (5-15 mg/M2 PO/IM weekly) for study participants who are not candidates for photopheresis.
- Re-transplant evaluation if consistent with local center guidelines.

7.5.4. Primary Graft Dysfunction

Primary Graft Dysfunction (PGD) will be evaluated at 4 time points as defined by the ISHLT Working Group on Primary Lung Graft Dysfunction.[107]The ISHLT grading scheme for primary graft dysfunction (PGD) described in Table 9 is based on the P/F ratio and chest X-ray.

Grade	PaO_2/FiO_2	Radiographic infiltrates consistent with pulmonary edema
0	>300	Absent
1	>300	Present
2	200-300	Present
3	<200	Present

Table 9: ISHLT Recommendations for Grading of Primary Graft Dysfunction (PGD) Severity

If multiple blood gas values are available, the worst P/F ratio will be used for the purposes of this grading scheme.

The following caveats to the grading scheme will be used.

- Absence of infiltrates on chest radiograph is Grade 0, even if PaO_2/FiO_2 ratio < 300.
- If the subject is on nasal cannula for oxygen of FiO_2 < 0.3, the subject is graded as 0 or 1, based on chest radiograph.
- Any subject on extracorporeal oxygenation is Grade 3.
- Any subject mechanically ventilated with FiO_2 > 0.5 on nitric oxide beyond 48 hours from the time of transplant is Grade 3.

8. Study Procedures

8.1. Enrollment

This research study will be explained in lay terms to each potential research participant. As the part of the informed consent process outlined in CFR Title 21 Part 50, the investigator or physician listed on the Investigator of Record (FDA 1572) will conduct a face-to-face meeting with the study candidate to review all required elements of informed consent. The potential study participant and/or parent/guardian will sign an informed consent form before undergoing any screening study procedures. Age of assent will be determined locally. Once the informed consent process is complete, the participant is considered enrolled in the study. All enrolled participants will be assigned a unique participant number and their disposition must be accounted for at the end of the study.

8.2. Screening/Baseline Visit

At the time of listing, potential participants will be entered on a site specific screening log. Age at listing, diagnosis and reason the patient was not enrolled into the study, if applicable, will be captured on the screening log. If the patient and/or parent(s)/guardian(s) are in agreement to participate in the study then the informed consent document should be obtained while the participant is on the wait list.

During the screening period study personnel will review the subject's medical record for previous and current medical history, perform a physical examination, and record the subject's demographic information (age, gender, and race). Blood specimens will be obtained for screening and mechanistic studies as specified in Appendix 1. Pre-transplant blood may be collected up to 60 days prior to transplant surgery. Blood must be recollected every 60 days until the transplant occurs.

The baseline visit will occur at the time of transplant. During the baseline visit, donor demographics (age, gender, and race), cause of death, HLA, Blood Type, serum creatinine, cytomegalovirus (CMV), Epstein - Barr virus (EBV), Hepatitis B, Hepatitis C, and HIV information will be collected.

8.3. Randomization

Eligibility for randomization will be confirmed. Hematology (CBC with differential and platelet count) and serum chemistries will be obtained within 2 weeks of randomization.

Randomization will occur within 12 hours after returning to the ICU following transplant.

Randomization will only occur if there are still spaces available at the time of transplantation. If the targeted accrual has been reached at the time of transplantation the participant will be excluded from further procedures and randomization.

8.4. Study Assessments

Subjects enrolled in this study will be followed for a minimum of 12 months post-transplant. Follow up visits will continue as detailed in Appendix 1 until the last participant completes 12 months of follow up (up to 54 months). Subjects may have blood and BAL samples collected at the following timepoints post-transplant:

- Transplant/Randomization-Day 0
- Day: 12
- Weeks: 4-6
- Months: 2, 3, 6, 9, 12, 18 and 24

Clinical safety will be monitored through routine physical examinations and appropriate laboratory assessments. During this period participants will have repeated clinical/laboratory evaluations, as specified in the Schedule of Events (Appendix 1, Schedule of Events- Recipient).

Assessments for the development of adverse events, serious adverse events, infections, rejections, death, PTLD, relisted for transplant or re-transplantation and hospitalizations will be completed at each study visit. All events will be reported using a designated electronic case report form (eCRF).

Chronic allograft dysfunction, relisting for transplant and death data will be collected up to 54 months post-transplant.

Protocol Biopsies

Transbronchial biopsies will be performed routinely as part of standard of care. The histologic evaluations from routine biopsies at day 12, week 4-6, month 3, month 6, month 12, month 18, and at the time of a symptomatic episode will be evaluated by the local pathologist and interpretations recorded on electronic case report forms (eCRFs).

Tacrolimus Standard Deviation Marker

Variability of tacrolimus will be monitored continuously starting 3 months post-transplant, using the calculation of the degree of fluctuation of tacrolimus blood levels over time, described by the standard deviation (SD) of tacrolimus levels.[82] All outpatient levels obtained at least 3 months post-transplant will be used. In order to ensure the validity of tacrolimus trough levels, clinical sites will agree on quality control measures for obtaining trough levels. A rolling SD will be calculated for every patient using 1-year of tacrolimus levels (at least 3 trough levels). A SD of 2.0 or more (Shemesh et al. manuscript in preparation), an undetectable level or the absence of a tacrolimus trough level for 3 months will trigger a phone call among the site coordinator, the participant, parent(s)/guardian(s) and trained personnel at ISMMS to address barriers to adherence.

Individuals who meet the criteria listed below will not participate in the phone calls for the tacrolimus standard deviation marker:

1. Inpatient status

2. Any condition that, in the opinion of the investigator, may impact the quality or interpretation of the data obtained from the intervention.

The frequency of the calls is described in the table below.

Timepoint **(following SD of 2.0 or more or undetectable level)**	**Call Frequency**
Month 1	Weekly
Month 2	Monthly
Month 3-Month 24	Every other month

Table 10: Tacrolimus Standard Deviation Marker Call Schedule

Per the center clinician's discretion, calls may be escalated to 2 times per week for up to 1 month at any time during the intervention.

After one month, calls may revert to a weekly schedule for the following reasons:

- Participant discloses continuing non-adherence during the call;
- The most recent trough level is "undetectable";
- The parent/guardian and/or team are concerned about ongoing non-adherence;
- A deterioration in medical outcomes as determined by the Center that is not explained by physiological factors;
- Per the clinical discretion of the call center staff member (i.e., the call center and parents/guardians are working on a remedy to an identified barrier).

The team at the call center will determine when the participant will return to the schema outlined in **Table 10**.

With the consent of the participant, information obtained during the intervention will be shared with the participant's parent(s)/guardian(s) and clinical team.

Neurological Assessment

All participants who receive at least 1 dose of Rituximab (Rituxan®) or placebo will undergo a screening neurological exam at the time points specified in Appendix 1. Subjects who have objective findings will be evaluated by their local Neurology consult service.

8.5. Unscheduled Visits

"Unscheduled" visits will occur if a participant develops symptoms of a respiratory viral infection (RVI) or a bronchoscopy is performed for suspicion of rejection or infection. Nasopharyngeal swab and blood specimens will be obtained at the time of an "unscheduled" visit. BAL will also be collected if specimens are obtained during a bronchoscopy for diagnostic purposes.

8.6. Visit Windows

Study visits should take place within the time limits specified below: the designated visit windows *(i.e. +/- n days)* for each scheduled visit are also indicated on the Table of Events.

Visit No.	Visit	Visit Window
00	Baseline	Pre-transplant blood may be collected up to 60 days prior to transplant surgery. Blood must be recollected every 60 days.
01	Transplant	Infusion within 12 hours of returning to the ICU following transplant Labs within 72 hours of transplant
02	Day 12	+/-2 days
03	Week 4-Week 6	+6/- 7 days
04-05	Month 2-Month 3	+/- 7 days
06-07	Month 6-Month 9	+/- 14 days
08-10	Month 12-Month 24	+/- 30 days

Table 11: Visit Windows

8.7. Study Treatment Assignment Procedures

8.7.1. Randomization Procedures

Randomization treatment assignments will be developed by the SACCC statisticians and stored on the SACCC server. The SACCC will maintain a central web-based randomization system. Once the subject is determined to be eligible, the site personnel will enter the information in the web-based system, which will then generate an automatic email and/or faxed communication to the site staff indicating successful randomization. Only the research pharmacist will receive a communication identifying the treatment assignment.

8.7.2. Blinding

This is a double-blinded study; therefore, medication assignments will be blinded to the study participants as well as to the site clinical personnel. ***Only the site research pharmacist will have access to the unblinded randomization 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.

8.7.3. Unblinding Authorization

Emergency unblinding of a participant for safety purposes is to be handled through the site Investigators, the medical monitor, and the site pharmacist. Whenever possible the 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 on 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 medical monitor prior to the unblinding, however ultimately the site Investigator must act in the study subject's best interest.

8.7.4. Reporting Unblinding Events

Any unblinding event will require a full report on the Study Treatment Unblinding eCRF. The information captured on the eCRF will include 1) a brief description of the medical events which led to the decision to unblind, 2) whether the NIAID medical monitor was notified 3) the date and time of unblinding, and 4) the reason for unblinding the subject. If the unblinding occurred in a situation other than the infusion of the study drug, the eCRF should include 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 medical monitor of the trial was notified and that a written account (described above) was completed.

9. Mechanistic Assays

Biological specimens obtained for this study will be used for development of future assays or validation of current assays to evaluate the biological response after lung transplantation. The assays described in this section and their clinical correlation will constitute the focus of this protocol and are described in detail below.

9.1. Humoral Laboratory Core

The Humoral Core Laboratory is located at the Washington University in St. Louis, MO (PI: Thalachallour Mohanakumar, MD)

9.1.1. Antibodies to Mismatched Donor HLA

We will measure serum anti-HLA antibodies using standardized reagents and protocols developed within the CTOTC/CTOT consortium.[85] Dr. Mohanakumar's laboratory will function as the core lab for this work and has been an integral participant in the cross validation studies performed by the consortium. Serum will be screened by Luminex panel of reactive antibodies (PRA) and if positive, tested for reactivity to single antigen beads. We will also perform flow crossmatch testing using donor cells as targets. This latter approach is important because it will permit us to assess whether there are donor reactive antibodies that are undetected by the flow bead panels. Such antibodies may react to HLA molecules which may be under represented in the beads (e.g. some of the HLA C alleles, or DP, DQ alleles) or may react with non-HLA antigens. As part of the mechanistic analysis, we will determine whether donor specific antibodies (DSA) are complement fixing using complement dependent lymphocytotoxicity assay against the donor cells[86] We will perform analyses/correlations with outcome using any alloantibody, donor specific alloantibody and either class I reactivity or class II reactivity as test variables. All assays are standard in the Mohanakumar lab and have been adapted to conform with CTOT/CTOTC mechanistic studies procedures. For initial evaluation, a test will be considered positive with >5% reactivity present. This variable can also be evaluated as a continuous variable as described in the statistical consideration below. [87-89]

9.1.2. Autoantibodies

For detection of antibodies to lung associated self-antigens the team will test for reactivity to well-defined autoantigens using ELISA methods which have been standardized in Mohanakumar's lab. Detected antibodies will be analyzed for their titer and immunoglobulin isotype. [90] Pre and post-transplant sera collected longitudinally will also be tested and titers determined. Development of antibodies and the amount of antibodies to K α1 tubulin, Collagen V and Collagen I will be determined. Collagens II, III and IV and cardiac myosin will be included in the panel as controls. Antigens will be obtained commercially or produced in house (kα1T). The Mohanakumar lab has cloned the human K-alpha 1 tubulin gene with a N-terminal histidine tag under the control of lac Z promoter. The protein is overexpressed in E coli by induction with IPTG and purified using metal affinity chromatography. The purified recombinant protein is passed through a polymyxin column to remove the contaminating endotoxins and the preparations used for the assays are free of detectable endotoxin by Limulus Amoebocyte Lysate assay.[91] This antigen has been used to detect autoantibodies and autoreactive T-cells in the CTOTC-03 trial. Polyclonal and monoclonal antibodies against the human kα1T have already been raised and are available for this study as controls. The titers of anti-kα1T and anti-ColV specific antibodies present in the normal subjects will be used to determine the cutoff values to determine positivity in the lung transplant recipients defined as weak positive (one standard deviation above the mean from normal subjects) and strong positive ($\geq$2 standards above the mean) responses. Sera obtained before transplantation will be used to determine the baseline titers of these

antibodies in the lung transplant recipients. The isotype specificity and isotype switching in these recipients will also be characterized in an ELISA using specific secondary antibodies that recognize IgM, IgG1, IgG2, IgG3 and IgG4. Therefore, all of the antibodies when detected will be defined for their complement fixing ability by C3d binding assay as well as by determining the Ig isotypes (IgG1,2,3 ,4 and IgM) using ELISA method.

9.2. Markers of Graft Injury Core Laboratory

The Core Laboratory is located at the Washington University in St. Louis, MO (PI: Thalachallour Mohanakumar, MD)

A portion of the serum aliquoted for humoral studies and supernatant from the bronchoalveolar lavage specimen will be used to perform cytokine, chemokine, and growth factors analysis studies.

9.2.1. Cytokines, Chemokines and Growth Factors

In an effort to determine mechanisms through which autoantibodies could injure the allograft, in-vitro assays will be used to test whether sera or isolated IgG that contains reactivity to either ColV or kα1T activates airway epithelial cells in culture. These assays have been developed in the Mohanakumar lab and have been published.[92] Primary airway epithelial cells isolated from the donor tracheas which are currently available in Mohanakumar's lab or commercially available human bronchial epithelial cells (ATCC and Cambrex Inc.) and human small airway epithelial cells (Cambrex Inc.) will be used to study the signaling mediated by the antibodies to Kα1T and ColV. Epithelial cells ($1X10^6$) will be exposed to various dilutions of the patient sera (or IgG) with and without anti-epithelial and collagen specific antibodies for 1 hr, 4 hrs and 24 hrs at 37 C. Up regulation of genes including TCF-5, HSP-27, HSP-90, c-Myc, NF-kB, VEGF, HB-EGF and TGF-b have been demonstrated upon incubation of airway epithelial cells (AECs) with serum from kα1T Ab(+)BOS(+) adult patients.[30] Production of cytokines, chemokines, and growth factors in the epithelial cells exposed to patient sera will be quantitated by Luminex. Expression and activation levels of the signaling intermediates c-Myc, PI3 kinase, paxillin, src, and FAK will also be quantitated in these cells by western blot analysis. Expression of adhesion molecules (ICAM, VCAM, selectins) on the cell surface will be measured by flow cytometry. If we find that the autoantibody containing serum specifically induces epithelial cell activation and one or more assays, we will isolate the autoreactive fraction of the IgG using (for example) kα1T conjugated beads and repeat the assays.

9.3. Molecular Immunology Studies

The Molecular Immunology Core Laboratory is located at the Mount Sinai School of Medicine in New York, NY (PI: Peter Heeger, MD).

RNA obtained from peripheral blood collection in PAXgene RNA tubes (PreAnalytiX, a Qiagen/BD Company) will be used for both mRNA Profiling/Gene Expression (PI: Peter Heeger, MD) and IgG VDJ Region studies (PI: Ignacio Sanz, MD). The Molecular Core Laboratory will isolate the RNA and aliquot a portion for both studies.

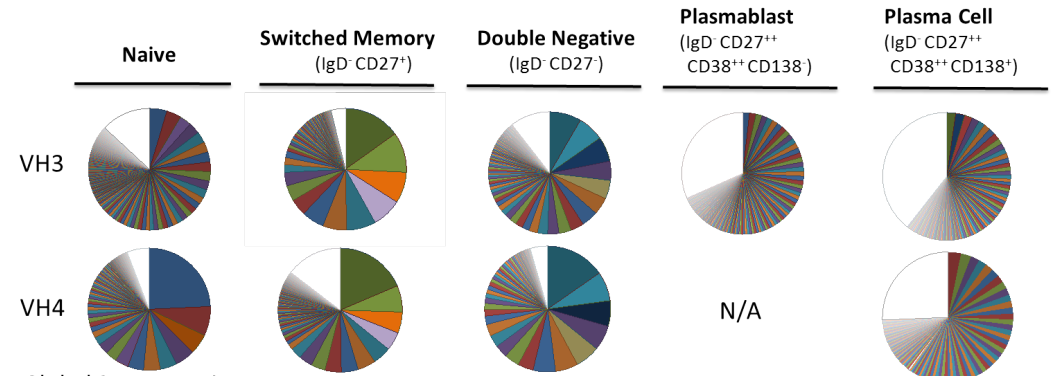
9.3.1. Gene Expression Profiles, mRNA Profiling in Blood and the Bronchoalveolar Lavage

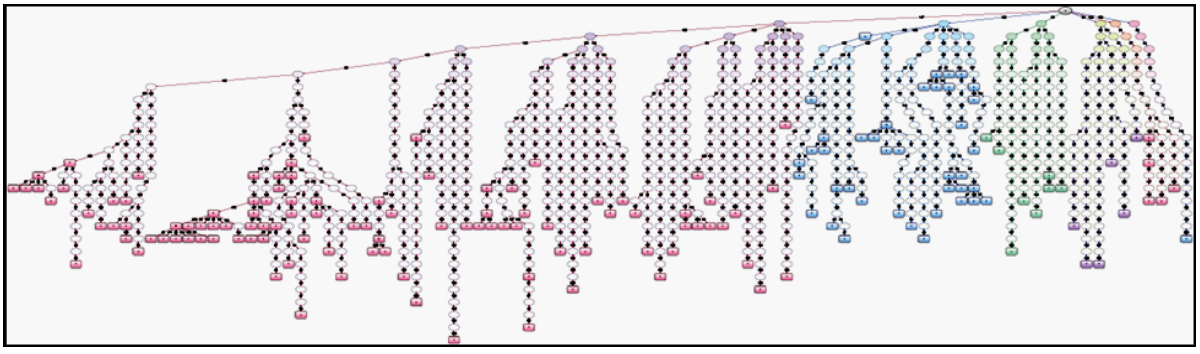
RNA will be stored at the Heeger laboratory and used for gene expression studies (RNA, miRNA). We will assess the transcriptional profile associated with rituximab based immunosuppression using array technology.

9.3.2. RNA Sequencing for IgG VDJ Regions

VH repertoire analysis will be performed using 454-based deep sequencing approaches well established in Dr. Ignacio Sanz laboratory (**Figure 9**). Briefly, 454 sequencing of all VH families will be performed using well validated primers in order to obtain unbiased representation of the B cell repertoire. Despite the lack of upfront fractionation of B cell subsets, memory repertoires can be reasonably deduced by the use of IgG-specific primers to analyze post-germinal center isotype switched cells. Up to 1 million reads per sample with an average length of 400 nucleotides (enough to span the entire VDJ rearrangement) will be obtained in a typical experiment. Sequences will be filtered using locally developed algorithms to eliminate non-Ig, artifactual and hybrid sequences. Approximately 75% of valid sequences will be collected and analyzed for clonal identity using identical CDR3 length (with a conservation of at least 70% sequence identity) in the context of identical VH, D and JH gene usage. Clonally related sequences will be analyzed using IgG Tree[93] (a collaboration between Dr. Sanz and Dr. Ramit Mehr (Bar-Ilan University) to establish genealogical trees indicative of antigen selected hypermutation. All sequences (whether clonally related or not) will be analyzed for their germline VH origin and their mutational divergence from the germline. Repertoire diversity and clonal relationships at a single time point and between longitudinal time points will be interrogated and expressed using different computational visualization tools.

Figure 9: B cell Repertoire Analysis





9.4. Cellular Immunology Studies

The Cellular Immunology Core Laboratory is located at the Mount Sinai School of Medicine in New York, NY (PI: Peter Heeger, MD).

Peripheral blood mononuclear cells will be isolated from blood collected in Cyto-Chex® BCT tubes (Streck). Streck Cell Preservative™ maintains cellular antigen expression, including cluster of differentiation (CD) markers and cell

morphology of biological samples for analysis by flow cytometry. In addition, the study will collect bronchoalveolar lavage specimen in a Streck Cell Preservative 10ml screw cap vial for B Cell Phenotyping analysis. Cell integrity is maintained at room temperature for up to 7 days in the cell preservative.

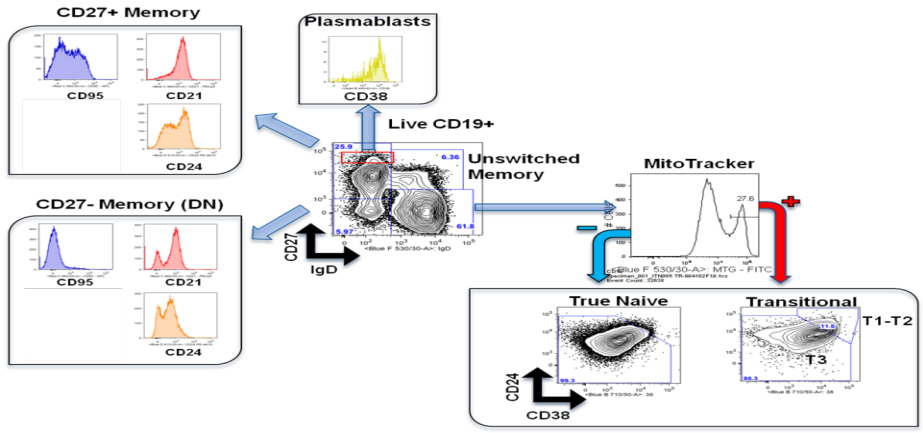
9.4.1. Peripheral Blood B cell Phenotyping

Peripheral blood phenotyping will be performed using standardized panels of antibodies (8 color). The efficacy of Rituxan® on peripheral blood CD20 positive B-cells will be assessed using flow cytometry in samples obtained at visit 03 (4-6 weeks post-transplant). The B cell phenotypes to be analyzed and a representative flow cytometry plot illustrating the proposed approach is included as **Table 12** and **Figure 10**.

Table 12: B-cell phenotypes

B cell Subset		Surface Phenotype	
Transitional	T1-T2	IgD+CD27- MTG+CD24hi CD38hi	
	T3	IgD+CD27- MTG+CD24int CD38int	
Naïve		IgD+CD27- MTG-CD24int CD38int	
Memory	Unswitched	IgD+ CD27+	
	Switched	Resting	IgD-CD27+CD21+CD95-CD24+
		Activated	IgD-CD27+CD21-CD95+CD24-
Switched CD27- memory (DN)		IgD-CD27-CD21+CD95-	
Antibody secreting cells	Plasmablasts	IgD-CD27++CD38++CD19+	
Autoimmune phenotype		CD19+, CD11c+ CD220+	

Figure 10: B cell phenotypes flow cytometry



B cell phenotyping will be performed along with analyses of T cells and APCs (see below). The team will quantify total B cells, naïve and memory B cells, plasma cells, transitional cells, B cells with an active "autoimmune" phenotype and Breg using the panels depicted in Table 7.[52 ,94, 95] Kinetic analysis will be performed for each patient and differences between the treatment group and the control group will be determined.

Antigen specificity analyses will focus on autoantigenic B cells reactive to ColV and Ka1T. Fluorescent labeled pentamers of kα1T using the recombinant kα1T produced in our lab will be used to identify the number of B cells specific for kα1T and their correlation with circulating Abs to kα1T and other self-Ags (ColV) will be analyzed.

9.4.2. B-cell Phenotyping in Bronchoalveolar Lavage

Cell surface markers of BAL samples will be analyzed for B cell subsets as outlined in Table 12 above, including quantifying the numbers of transitional B cells and those with an autoimmune phenotype (CD19, CD11c and CD220). The effect of B cell depletion on induction of immune response to self-Ags will be determined by monitoring the number and frequency of B cells and Abs to HLA (Luminex) and self-antigens (ELISA). We expect that the depletion of B cells by rituximab will result in a decrease in the frequency of CD19+CD11c+CD220+ B cells. Further, this decline will correlate to a decrease in Abs to HLA as well as Abs to self-Ags. Additionally, based on preliminary data, we expect that BOS+ pediatric lung recipients will have an increase in these CD19+CD11c+CD220+ B cells.

9.4.3. Peripheral Blood T-Cell Phenotyping

T-cell phenotyping will be performed by flow cytometry simultaneously with the B cell phenotyping outlined above and using validated panels for multicolor flow (**Table 13**), to assess CD4 and CD8 T-cell subsets and thymic emigrants.

Table 13: Example of flow phenotyping for CD4 T-cells

T-Cell Subset	Surface Phenotype
Naïve	CD4+ CD45RO+ CD27+
Central Memory	CD4+ CD45RA+ CD27+
Effector Memory	CD4+ CD45RO+ CD27-
Tregs	CD3+ CD20- CD4+ CD25+ CD127L
Naïve recent thymic emigrants	CD3+ CD20- CD4+ CD8- CCR7+ CD45RA+ CD31+
Recent thymic emigrant Treg	CD3+ CD20- CD4+ CD8- CD45RA+ CD31+ FoxP3+

9.4.4. T-Cell function by ELISPOT

Peripheral blood mononuclear cells (PBMC) and plasma will be isolated from whole blood collected in a Sodium Heparin Vacutainer Tube. The PBMCs will be used to perform T-Cell Function assays (PI: Peter Heeger, MD) and the plasma will be shipped to the Washington University in St. Louis (PI: Thalachallour Mohanakumar, MD) and used for humoral immunology assays.

For T-cell autoreactivity, PBMCs collected longitudinally immediately prior to transplant, and then post-transplantation and will be tested for their reactivity against self-antigens collagen V, Kα1Tand col IV as control. (All of these proteins are available as reported above and are endotoxin free). Because cellular immunity to autoantigens is anticipated to be relatively low frequency, we will use the highly sensitive cytokine ELISPOT as an initial approach for detection and enumeration of frequency of cells secreting specific cytokines following stimulation with various self-antigens described above. We have the ability to perform 2 color IFNg/IL17 ELISPOT assays for autoantigen reactivity, which will permit us to maximize our ability to obtain information from small amounts of blood (Figure 3 above). We will assess cellular alloreactivity by cytokine ELISPOT and if sufficient cells are available, by CSFE dilution and intracellular cytokine staining.

Donor B cell lines (primary expansion using CD40L transfected fibroblasts and IL-4 as described (and unmatched 3P control lines) will be made under GLP conditions in the Heeger lab.[17] IFNγ ELISPOT assays will be the primary readout. Assays for 2 different cytokines will be performed as part of the mechanistic study. Again, we will study IFNg and IL-17 production as prototypic proinflammatory cytokines and IL-4, IL-5 and IL-10 as prototypic type Th2

cytokines. Aliquots of samples will be stored in sufficient cells for potential future testing against additional autoantigens.

9.5. Immunohistochemistry

The Immunohistochemistry Core Laboratory is located at the Mount Sinai School of Medicine (PI: Michael J. Donovan, MD).

M Donovan, MD, PhD, ISMMS is an innovator in systems pathology in which he has developed computer assisted approaches for morphometric and multicolor image analysis of IF stained tissues. Using these published methods multicolor IF staining for B cells (CD20), T-cells (CD4, CD8), Treg (CD4, foxp3), and macrophages (CD68) will be performed on representative histology slides. Computer assisted image analysis will be used to determine the numbers of each cell type within the area being studied and to assess correlations among cell subsets. Examples demonstrating feasibility are shown in **Figure 11.**

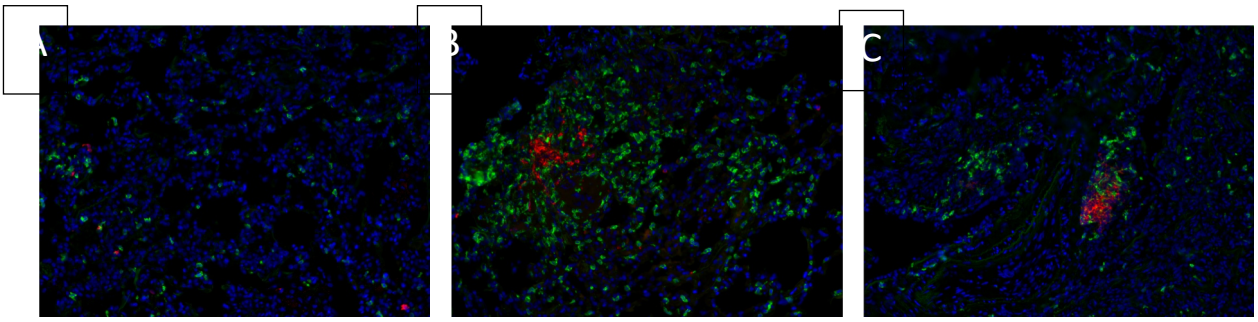


Figure 11: Detection of B cell infiltrates in paraffin embedded transbronchial biopsy samples from the CTOTC-03 trial. Representative 2 color IF staining for CD20 (red) and CD8 (green) showing A) rare T or B cells in an ISHLT grade A0B0 biopsy, B) diffuse T and B cell infiltration near an airway in an ISHLT grade A0B1C biopsy with bronchiolitis obliterans, and C) prominent B cell infiltrate adjacent to a vessel in an ISHLT grade A2B1 biopsy from a patient who later developed bronchiolitis obliterans

Results will be correlated with the graded score of the biopsy, the presence or absence of AMR features (capillaritis, C4d capillary loop staining) and outcome, including effects of treatment for acute rejection and the long term development of BOS.

9.6. Microbiology and Viral Detection Core

The Microbiology and Viral Detection Core Laboratory is located at the Washington University in St. Louis, MO (PI: Gregory Storch, MD).

9.6.1. Viral Detection by PCR in Bronchoalveolar Lavage (BAL) and Nasopharyngeal Swabs (NP)

Bronchoalveolar Lavage (BAL) samples will be obtained during scheduled surveillance or unscheduled symptomatic bronchoscopies. Nasopharyngeal swab (NP) samples will be collected during scheduled surveillance and at symptomatic episodes that do not clinically require bronchoscopy. With all bronchoscopies, samples for bacterial, fungal, and viral testing will be taken even if infection was not initially suspected. Mycobacterial testing will be performed if indicated. After processing for immediate clinical testing by conventional methods at local clinical sites with immediate reporting of infectious episodes, the remainder of the samples will be stored at -70^{o}C and batch shipped to the Microbiology and Viral Detection Core Laboratory. If we find that the batched analysis by this highly sensitive method detects potentially

treatable infection that are not detected by the individual clinical sites (which did not occur to date in CTOTC-03), we will adjust the design so that it is performed in real time so as to provide the clinically relevant information to the sites for therapeutic intervention.

9.6.2. Viral Detection in Whole Blood by Quantitative PCR

CMV has been epidemiologically linked to acute and chronic rejection including initial data from our research team showing an increased risk of early mortality in children with CMV viremia.[97]EBV, associated with post-transplant lymphoproliferative disease (PTLD), has also been identified as a potential co-factor for outcome. Due to known inter-laboratory variability in quantitative measurements of CMV and EBV viral load,[98,99] whole blood will be collected and sent to the Microbiology and Viral Detection Core.

Whole blood will be stored for potential evaluation of additional interacting viruses to include HHV-6.

10. Biospecimen Storage and Future Use

Biological specimens obtained under this protocol may be used in future assays to reevaluate biological responses as additional research tests are developed over time. These specimens will be collected at time points already scheduled for the core mechanistic studies outlined in the Schedule of Events (Appendices 1 and 2), 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-C 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.

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

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

11.1. Participant Completion

All participants will be followed for a minimum of12 months and up to 54 months after transplantation.

11.2. Participant Stopping Rules and 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.
5. The study is stopped by the site, the sponsor (NIAID), or the Food and Drug Administration.

11.3. Participant Replacement

Participants who withdraw or are withdrawn will not be replaced if they have received at least one dose of rituximab.

11.4. Follow-up after Early Study Withdrawal

Participants who receive at least one dose of rituximab will follow the schedule of events-Appendix 1.

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 DAIT/NIAID. Appropriate notifications will also be made to site principal investigators, Institutional Review Boards (IRBs) 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 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)

For this study, an adverse event will include any untoward or unfavorable medical occurrence associated with:

- **Study therapy regimen**: Rituximab or Rituximab Placebo
- **Study mandated procedures**:
  - Bronchoscopy: Any AE occurring within 24 hours after a study related bronchoscopy.
  - Blood Draw: Any AE occurring within 24 hours after a study related blood draw.

12.2.1.1 Suspected Adverse Reaction (SAR)

Any adverse event for which there is a reasonable possibility that the investigational drug [or investigational study therapy regimen] 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)).

12.2.2 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator Brochure or 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 Investigator Brochure or package insert as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation (21 CFR 312.32(a)*]*

12.2.3 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or DAIT/NIAID, it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID*,* 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.

The NIAID medical monitor will review each SAE and assess the relationship of the event to the study procedure. Events meeting serious criteria that occur outside of these parameters should also be reported if the investigator deems a possible association with a protocol mandated procedure. 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) version 4*.* 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 and Principal Investigator 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:

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.

Events grade 1 and 2 will not be collected. Events grade 3 or higher will be recorded on the appropriate AE electronic case report form except for hematologic cytopenias. Only hematologic cytopenias meeting serious criteria outlined in 12.2.3 and occurring from the time of first rituximab infusion to 6 weeks following the last rituximab infusion will be collected and reported to the sponsor. Outside of this period, hematologic cytopenias meeting grade 3 and higher will be collected and reported to the sponsor.

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/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 in Table 14.

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.

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

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

12.4.2 Collecting Adverse Events

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.

12.4.3 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.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the 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 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:

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).

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 FDA 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 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 shall be instructed to stop taking study medication. The investigator shall counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject shall continue until the conclusion of the pregnancy.

The investigator shall report to the SACCC 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 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 - an SAE shall be submitted to the *SACCC* using the SAE reporting procedures described above.

12.7 Reporting of Other Safety Information

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

12.8 Review of Safety Information

12.8.1 Medical Monitor Review

The *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 site(s) 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*).

12.8.2 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. In addition, satisfaction of any of the following **stopping rules** in study subjects at any time of follow-up in the treatment arms will trigger an *ad hoc* DSMB Safety Review:

- Any occurrence of confirmed PML.
- Incidence of death of 30% or more subjects.
- Incidence of at least mild acute rejection of 35% or more.
- Incidence of humoral rejection of 25% or more.
- Incidence of primary graft dysfunction of 50% or more.
- Incidence of PTLD of 5% or more.
- Incidence of infections of any type requiring hospitalization of 40% or more.

The thresholds for stopping rules involving subject-level incidence rates have been formulated based on an analysis of adverse events in the CTOTC-03 (NCT00891865) observational study of subjects who are similar to those in the control arm of this study. They will be evaluated routinely by the SACCC with the occurrence of any of the triggering events throughout follow-up of subjects within either randomized treatment arm. 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.

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 within either randomized treatment arm:

Table 15: Minimum Numbers of Subjects with the Event in Either Randomized Treatment Arm that meet the Stopping Rule for Death with a Threshold of Concern of 30%

Number of Subjects with Event	Number of Randomized Subjects	Cumulative Incidence Rate	Lower 95% Confidence Limit
4	4	100.00	47.29
5	6	83.33	41.82
6	8	75.00	40.03
6	10	60.00	30.35
7	12	58.33	31.52
8	14	57.14	32.50
9	16	56.25	33.34
10	18	55.56	34.06
10	20	50.00	30.20
11	22	50.00	31.13

Table 16: Minimum Numbers of Subjects with the Event in Either Randomized Treatment Arm that meet the Stopping Rule for Mild Acute Rejection with a Threshold of Concern of 35%

Number of Subjects with Event	Number of Randomized Subjects	Cumulative Incidence Rate	Lower 95% Confidence Limit
4	4	100.00	47.29
5	6	83.33	41.82
6	8	75.00	40.03
7	10	70.00	39.34
8	12	66.67	39.09
9	14	64.29	39.04
10	16	62.50	39.10
11	18	61.11	39.22
11	20	55.00	34.69
12	22	54.55	35.25

Table 17: Minimum Numbers of Subjects with the Event in Either Randomized Treatment Arm that meet the Stopping Rule for Humoral Rejection with a Threshold of Concern of 25%

Number of Subjects with Event	Number of Randomized Subjects	Cumulative Incidence Rate	Lower 95% Confidence Limit
4	4	100.00	47.29
4	6	66.67	27.13
5	8	62.50	28.92
6	10	60.00	30.35
7	12	58.33	31.52
7	14	50.00	26.36
8	16	50.00	27.86
9	18	50.00	29.12
9	20	45.00	25.87
10	22	45.45	27.13

Table 18: Minimum Numbers of Subjects with the Event in Either Randomized Treatment Arm that meet the Stopping Rule for Primary Graft Dysfunction with a Threshold of Concern of 50%

Number of Subjects with Event	Number of Randomized Subjects	Cumulative Incidence Rate	Lower 95% Confidence Limit
5	4	100.00	54.93
6	6	100.00	60.70
7	8	87.50	52.93
9	10	90.00	60.58
10	12	83.33	56.19
11	14	78.57	53.43
12	16	75.00	51.56
13	18	72.22	50.22
15	20	75.00	54.44
16	22	72.73	53.15

Table 19: Minimum Numbers of Subjects with the Event in Either Randomized Treatment Arm that meet the Stopping Rule for PTLD with a Threshold of Concern of 5%

Number of Subjects with Event	Number of Randomized Subjects	Cumulative Incidence Rate	Lower 95% Confidence Limit
2	4	50.00	9.76
2	6	33.33	6.28
3	8	37.50	11.11
3	10	30.00	8.73
3	12	25.00	7.19
3	14	21.43	6.11
3	16	18.75	5.31
4	18	22.22	7.97
4	20	20.00	7.14
4	22	18.18	6.46

Table 20: Minimum Numbers of Subjects with the Event in Either Randomized Treatment Arm that meet the Stopping Rule for Infection Requiring Hospitalization with a Threshold of Concern of 40%

Number of Subjects with Event	Number of Randomized Subjects	Cumulative Incidence Rate	Lower 95% Confidence Limit
4	4	100.00	47.29
5	6	83.33	41.82
6	8	75.00	40.03
8	10	80.00	49.31
9	12	75.00	47.27
10	14	71.43	46.00
11	16	68.75	45.17
12	18	66.67	44.60
13	20	65.00	44.20
14	22	63.64	43.91

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

12.8.2.2.1 Temporary Suspension of *enrollment and drug dosing* for *ad hoc* DSMB Safety Review

A temporary halt in enrollment, randomization and administration of rituximab/rituximab placebo will be implemented if an ad hoc DSMB safety review is required. In the event a study participant has already received the first dose of rituximab/rituximab placebo, every attempt will be made to expedite review such that a prompt decision can be made regarding the appropriate management for these subjects.

13.Statistical Considerations and Analytical Plan

13.1 Overview

In this randomized controlled clinical trial, we will compare primary pediatric lung transplant recipients given rituximab induction therapy plus standard immmunosuppression to subjects given a placebo induction therapy plus standard immmunosuppression and assess whether rituximab induction therapy improves outcomes in this population.

13.2 Endpoints and Safety Outcomes

The primary endpoint will be the earliest time to any of the following events during the follow up period: Chronic Allograft Dysfunction, listing for retransplantation, and death.

The following secondary clinical endpoints will be assessed during the post-transplant follow-up period, which will be a minimum of 12 months:

- incidence of chronic allograft dysfunction, listing for retransplant or death,
- incidence of Primary Graft Dysfunction,
- incidence of Grade A Acute Rejection,
- incidence of Antibody Mediated Rejection,
- incidence of TVT,
- percentage of participants identified to participate completing the TVI,
- and magnitude of change in standard deviation of tacrolimus levels following intervention.

The following secondary clinical post-transplant safety outcomes will be assessed: incidence and severity of infection episodes, and serious adverse events related to rituximab.

The following mechanistic endpoints will be assessed: incidence and kinetics of DSA and auto-antibodies, specifically Collagen V (ColV) and k-alpha-1 tubulin (kα1T); frequency, kinetics, phenotype and function of peripheral B cells; frequency, kinetics and cytokine profiles of allo- and auto reactive T cells; the incidence and quantity of B cells and B cell proximity to other cells in the graft tissue.

13.3 Measures to Minimize Bias

Study treatments will be randomly assigned to eligible subjects and the treatment assignment of each subject will be double-blinded. In addition, the central laboratories that perform the mechanistic assays will be blinded to both the treatment assignment and clinical status of all study subjects.

13.4 Analysis Plan

The overall focus of our statistical analysis of the clinical portion of the study will be on the estimation of hazard ratios (HR) and risk ratios (RR) and their two-sided 90% confidence intervals comparing the rituximab and placebo groups on each endpoint of the study. Focusing on estimates and confidence intervals will allow us to assess the range potential efficacies as best we can within the limitations imposed by studying such a rare pediatric population.

Further detail describing the analysis plan will be contained in a Statistical Analysis Plan (SAP) that will be maintained by the SACCC and updated as necessary.

13.4.1 Analysis Populations

Intent-to-Treat (ITT) Sample. All randomized subjects who receive at least a portion of the initial rituximab/placebo infusion will constitute the ITT sample. This sample will be used for evaluation of the primary and secondary clinical endpoints. Subjects will be analyzed in the group to which they were randomized, regardless of compliance with the protocol or withdrawal from the study.

Per-Protocol (PP) Sample. Subjects from the ITT sample who complete the full induction protocol, with complete administration of the prescribed dose at both the initial and subsequent infusions, will make up the PP sample. This sample will capture subjects who both tolerate rituximab well and recover as expected from their transplant.

Safety Sample. All subjects from the ITT sample will constitute the safety sample.

13.4.2 Primary Analysis of Primary Endpoints /Outcomes

We will use competing-risks Cox proportional hazards models to compare treatment groups on the time to endpoint, right-censored at the end of the two-year follow-up period or at time of loss to follow-up. This model, as opposed to a time-to-first-event model, will allow us to estimate a separate hazard function for each type of outcome as well as the overall outcome, and thus assess whether the effect of rituximab is the same for all outcome types[100]. Our focus will be on the estimates of these hazard ratios (HR) and their two-sided 90% confidence intervals, with HR < 1 indicating rituximab efficacy. We will assess whether HR is constant throughout follow-up, or if the effect of rituximab is confined to early or late portions of the study periods through the use of time-dependent covariates. Model diagnostics will include assessment of the proportional hazards assumption and of each observation for influence on model parameters. The model will be run on both the ITT and PP populations, as events allow.

13.4.3 Supportive Analyses of the Primary Endpoints

We will also model the time-to-first-event model within the Kaplan-Meier framework for the primary outcome, as needed, given the likelihood of little to no events for one or more of the 3 outcomes.

Secondary analyses will model each component of the outcome separately with the other components as risk factors, as allowed by the number of events and the sample size.

13.4.4 Analyses of Secondary Endpoints and Safety Outcomes

All secondary endpoint analyses will use the ITT sample and will be assessed during the post-transplant follow-up period, which will be a minimum of 12 months.

Separately for each of the secondary incidence endpoints, we will compare the rituximab and control groups on the proportion of patients in each group with at least one episode of the endpoint by estimating the relative risk of the endpoint with its two-sided 90% confidence interval. The absolute risk difference with 90% confidence interval will also be estimated. These methods assume no, or very low rates of, death and re-transplant in the study period. However, if meaningful numbers of subjects have these outcomes, the risk ratio for each incidence endpoint can be estimated through a Cox proportional hazards model with the incidence endpoint as the outcome and right-censoring at death, re-transplant, or other loss to follow-up, following the modeling process described above for the primary endpoint.

Among subjects who are identified as qualifying for and agreeing to participate in the TVI, we will compare the rituximab and control groups on change in standard deviation of tacrolimus levels following the TVI (described in sections 5.3.3 and 8.4) using an analysis of covariance (ANCOVA) model, in which the standard deviation of tacrolimus levels following intervention is the outcome, and study group and pre-intervention standard deviation of tacrolimus levels are the predictors. The group effect with its 90% confidence interval will be estimated.

For safety outcomes, we will analyze each type of infection separately (bacterial, fungal, or viral, including RVI, CMV, and EBV), as well as for all types of infections combined. We will first determine the incidence of bacterial, fungal and viral infections in the rituximab and control groups. 'Incidence rate' will be the number of infections per patient-month, and we will focus on the estimates and associated 90% confidence intervals for the ratio of such rates (RR), where RR > 1.0 indicates greater infection rates for rituximab. Of course, infection events are biologically related within patient, thus, relative to perfect independence of events, greater proportions of patients will experience more episodes of the same type of infection and greater proportions of patients will experience no episodes. Accordingly, a Poisson regression models offset for months of follow-up and adjusted for overdispersion will be used to compare groups on the rate of each type of infection and any infection.

Serious adverse events related to rituximab will be reported by their incidence rate per patient and per infusion with 90% confidence intervals. Adverse events will be summarized by treatment group, with 90% confidence intervals.

13.4.5 Analyses of Exploratory Endpoints

We will correlate pre and/or post-transplant serum reactivity to each autoantigen and to donor HLA with our primary endpoint to prospectively and independently test the hypothesis that autoantibodies can be used as predictors of incipient injury. We will also correlate the presence of serum antibodies with positive C4d staining of the graft tissue (to be collected as part of the clinical component of the study).

To test the hypothesis that rituximab prevents induction of de novo autoantibodies and or DSA, we will compare titers of autoantibodies (or DSA) between the rituximab and control groups. Development of antibodies to either DSA or self-antigens or both and the kinetics of antibody development (time course following transplantation) will be correlated with clinical evidence of AMR and BOS as determined in the

clinical part of study described above and the ISHLT criterion. While the absolute numbers of patients in each group with preexisting autoantibodies or DSA is anticipated to be small (thus limiting power) we will compare and report pre vs post-transplant titers in each case to determine if rituximab impacts preexisting antibody.

The results will be analyzed for development of PGD, development of DSA, diagnosis of AMR and BOS. In addition we will also determine any correlation which may exist between the strength of antibodies detected for DSA, HLA and each antibody to self-antigens either alone or cumulatively and clinical outcomes such as PGD, AMR and BOS.

We will analyze DSA and each type of antibody separately. The DSA and autoantibody data will consist of binary (positive/negative) or ordinal (negative/weak positive/strong positive) responses measured repeatedly at scheduled and unequally-spaced intervals. With these features of the data and at this sample size, it may not be feasible to obtain estimates of treatment effect at each time-point using a single statistical model with rituximab and time main effects and their interaction as covariates. Therefore we will describe the treatment effect over the course of the follow-up period.

The hazard ratio for the association between development of antibodies and clinical outcomes (the endpoint and its components, and the secondary incidence endpoints) will be estimated with its 90% confidence interval using a Cox proportional hazard model with presence or absence of antibodies as time-dependent repeated measures covariates, stratified by treatment group. We will model the association of each antibody on each outcome separately and in combination.

We will also estimate odds ratios with 90% confidence intervals for the associations between each of the various serum antibodies and C4d staining of the graft tissue. We will estimate these odds ratios at each time point between each pair of antibodies and each antibody and C4d result, both cross-sectional, and cumulative, in which case we would treat any subject with a current or prior positive result as positive for that particular antibody.

13.4.6 Descriptive Analyses

Selected subject baseline clinical and demographic characteristics, as well as use of concomitant medications and study subject disposition will be summarized using standard descriptive statistics for continuous and categorical variables.

13.5 Interim Analyses

No interim analyses are planned. However, as described in section 12.8.2., the DAIT Transplant DSMB will periodically review safety data in open and/or closed sessions.

13.6 Sample Size Considerations

Planning this study indicates a sample-size analysis that is congruent with our focus on estimates and confidence intervals. Accordingly, simulated study data using a Weibull distribution with parameters chosen to match our expectation that the outcome rate in the control group will be 74% at one year and 53% at two years, with hazard ratios of 0.40 and 0.33. For the purposes of the study only large group differences are clinically relevant, and therefore this effect size is reasonable.

Sample-sizes between N = 25 + 25 and N = 30+ 30 are feasible, as argued elsewhere. We also looked at N = 35 + 35, but acknowledge this probably not obtainable.

5000 individual trials were run for each combination of total sample size (N = 50, 60, 70) and true hazard ratio (0.40, 0.33). Each trial's dataset was analyzed using a standard Cox proportional hazards model and the estimated log (HR) and its standard error were obtained. Using the criteria given in **Table 21**, each trial's estimate and upper confidence limit was rated as being "excellent," "good," "fair," or "lacking" in supporting the rituximab efficacy. The obtained proportions are given in **Table 22**.

Table 21: Criteria for Estimating Upper Confidence Limit

Evidence for Efficacy	Estimated Hazard Ratio	Upper 90% Confidence Limit
"Excellent"	<0.55	<0.85
"Good"	<0.60	0.85 – 1.10
"Fair"	<0.70	1.10 – 1.20
"Lacking"	Any	>1.20

Table 22: Upper 90% Confidence Limits by HR and Sample Size

Sample Sizes	25+25		30+30		35+35	
True Hazard Ratio	**0.04**	**0.33**	**0.04**	**0.33**	**0.04**	**0.33**
Evidence for Efficacy						
"Excellent"	0.39	0.50	0.46	0.59	0.51	0.64
"Good"	0.22	0.21	0.23	0.21	0.24	0.20
"Fair"	0.07	0.06	0.06	0.05	0.06	0.04
"Lacking"	0.33	0.24	0.25	0.16	0.19	0.12

In addition, although the primary analysis is based on a Cox regression model, a simple power and sample size assessment using the less efficient log-rank test indicates that 25 subjects in each group will provide greater than 80% power to detect a HR of 0.45 for the treatment effect using a one-sided test at the 0.05 level of significance.

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. Protocol Deviations

15.1. 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 (Protocol Violation) - A Protocol Violation 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.

15.2. Reporting and Managing Protocol Deviations

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

Deviations that impact the ability to assess study outcomes will be collected for this study. Upon determination that a protocol deviation has occurred, the study staff will notify the SACCC by completing a Protocol Deviation eCRF. NIAID, the study PI and DSMB will review deviations on a regular basis. The site will be responsible for reporting deviations to the local IRB according to site-specific guidelines.

16. Ethical Considerations and Compliance with Good Clinical Practice

16.1. 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. Any amendments to the protocol or to the consent materials will also be approved by the IRB before they are implemented.

16.2. 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 investigator or designee listed on the FDA 1572 will review the consent and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants (or their legally acceptable representative) will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in participants' primary language. A copy of the signed consent form will be given to the participant.

The consent process will be ongoing. The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

16.3. 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.

17.Publication Policy

The CTOT-C policy on the publication of study results will apply to this trial.

18. References

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Appendix 1. Schedule of Events (Recipient)

Transplant Study Day		**-1**	**0**	**12**										**Unscheduled Visit within 3 months of transplant**	**Unscheduled Visit greater than 3 months from transplant**
Transplant Study Week					**4-6**										
Transplant Study Month						**2**	**3**	**6**	**9**	**12**	**18[6]**	**24[6]**	**25-54[6]**		
Visit Number		**00**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**			
Informed Consent, Screening	Original retained, copy provided to study participant	X													
Assessment of Eligibility Criteria	Enrollment and Randomization Inclusion/Exclusion Criteria	X	X												
Demographics	Date of birth, Race, Ethnicity and Gender	X													
Medical and Respiratory History	Body Systems	X													
Physical Exam	Height, weight, temperature, blood pressure, pulse, heart rate, screening neurological assessment		X	X	X	X	X	X	X	X	X	X		X	X
Pulmonary Function Test	Age Restrictions ***(collect if performed as standard of care)***	X		X	X	X	X	X	X	X	X	X		X	X
Imaging	Chest X-Ray, Chest CT, Perfusion Scan ***(collect if performed as standard of care)***			X	X	X	X	X	X	X	X	X		X	X
Medications	Concomitant, Immunosuppressive and Prophylactic Medications	→	→	→	→	→	→	→	→	→	→	→		→	→
Assessments of Events	AE/SAE, PGD, Infection, Rejection, Hospitalization, PTLD, or Re-transplant	→	→	→	→	→	→	→	→	→	→	→		→	→
Assessment of Endpoint Events	Chronic Allograft Dysfunction, Death, Re-Listed	→	→	→	→	→	→	→	→	→	→	→	→	→	→
Blood Type/HLA Typing	A, B, O, Rh factor, Class I (A, B, C), Class II (DR, DP, DQ) if available	X													
Panel Reactive Antibody	Current	X													
PRA	Highest PRA or cPRA ***(within 12 weeks of transplant)***		X												
Crossmatch	T cell and B cell		X												
Pregnancy Test	Serum or Urine ***(within 48 hours of transplant)***		X		X		X	X	X	X	X				
Hematology Panel	CBC (including differential and platelets)		X	X	X	X	X	X	X	X	X	X		X	X
Trough Levels	Tacrolimus ***(all tacrolimus trough levels collected as SOC will be collected after 3 months post-transplant)***		X	X	X	X	X	X	X	X	X	X		X	X
Chemistry Panel	Glucose, BUN, creatinine, uric acid, total bilirubin, alkaline phosphatase, LDH, total protein, SGOT (AST), SGPT (ALT), calcium	X	X	X	X	X	X	X	X	X	X	X		X	X
CMV/EBV	***Only collect if performed as standard of care, serology collected at baseline and viral***	X	X	X	X	X	X	X	X	X	X	X		X	X

Transplant Study Day		**-1**	**0**	**12**									**Unscheduled Visit within 3 months of transplant**	**Unscheduled Visit greater than 3 months from transplant**
Transplant Study Week					**4-6**									
Transplant Study Month						**2**	**3**	**6**	**9**	**12**	**18[6]**	**24[6]**	**25-54[6]**	
Visit Number		**00**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**		
	load at post-transplant visits													
Serology	HIV, HBsAg, HBcAb, HCV Ab ***(within 12 months of transplant)***	X												
Immunoglobulin Levels	IgG	X	X	X	X	X	X	X	X	X	X	X		X	X
Pathology	Local Biopsy Histology Scoring (ISHLT), including c1q fixation and C4d			X	X		X	X	X	X	X			X	X
Rituximab or Placebo (375 mg/kg)			X	X											
	HUMORAL ANTIBODY CORE – WUSTL (PI: THALACHALLOUR MOHANAKUMAR)														
Serum- Anti-HLA Ab, Non-HLA Ab, Crossmatch Testing		X	X	X	X	X	X	X	X	X	X	X		X	X
Serum- Autoantibodies		X	X	X	X	X	X	X	X	X	X	X		X	X
	MARKERS OF GRAFT INJURY CORE – WUSTL (PI: THALACHALLOUR MOHANAKUMAR)														
Serum- Cytokine/Chemokine/GF					X		X	X	X	X	X	X		X	X
Bronchoalveolar Lavage (BAL) – Supernatant for Cytokine Analysis			X	X	X		X	X	X	X	X			X	X
	MOLECULAR IMMUNOLOGY CORE – ISMMS (PI: PETER HEEGER)														
Blood-mRNA Profiling, Gene Exp.					X		X	X	X	X	X	X			
Bronchoalveolar Lavage – Pellet for Gene Expression Profiles			X	X	X		X	X	X	X	X			X	X
RNA Sequencing for IgG VDJ Regions (I. Sanz, Emory)								X	X	X	X				
	CELLULAR IMMUNOLOGY CORE – ISMMS (PI: PETER HEEGER)														
Blood- T and B Cell Phenotyping (Flow Cytometry)		X			X		X	X		X	X				X
Bronchoalveolar Lavage –in Cell Preservative: Phenotyping B Cells			X	X	X		X	X	X	X	X			X	X
T Cell Function (ELISPOT) and Plasma to Archive		X						X		X	X				X
	IMMUNOHISTOCHEMISTRY CORE –ISMMS (PI: MICHAEL DONOVAN)														
Immunohistochemistry: CD4, FoxP3, CD20 and CD8, CD20 and CD68: (5 unstained slides: Minimum of 3 slides prepared with 2 sections each representative of more than one tissue section)				X	X		X	X	X	X	X			X	X
	VIRAL DETECTION CORE –WUSTL (PI: GREGORY STORCH)														
Bronchoalveolar Lavage – Whole specimen for Viral Detection			X	X	X		X	X	X	X	X			X	X
Nasal Swab-Viral PCR			X	X	X		X	X	X	X	X			X	X
Whole Blood Quantitative Viral Studies (CMV/EBV)		X	X	X	X	X	X	X	X	X	X	X		X	X

Transplant Study Day		**-1**	**0**	**12**										**Unscheduled Visit within 3 months of transplant**	**Unscheduled Visit greater than 3 months from transplant**
Transplant Study Week					**4-6**										
Transplant Study Month						**2**	**3**	**6**	**9**	**12**	**18[6]**	**24[6]**	**25-54[6]**		
Visit Number		**00[1]**	**01[2]**	**02[3]**	**03**	**04**	**05**	**06**	**07[4]**	**08**	**09**	**10**			
	BIOSPECIMEN REPOSITORY – ISMMS (PI: PETER HEEGER)														
Blood to Archive		X													

1. Visit 00: Samples may be collected up to 60 days prior to transplant surgery.
2. Visit 01: Samples may be collected up to 72 hours following transplant.
3. Visit 02: Samples may be collected 1-2 weeks after transplant.
4. Visit 07: The month 9 visit will occur if the subject is seen as part of the local standard of care at this timepoint.
5. Unscheduled Visits: BAL obtained if performed by the clinical site for diagnostic purposes. For visits occurring greater than 3 months blood will be collected if visit is conducted at the transplant center.
6. All subjects will complete 12 months of follow-up. A sub-set of subjects will also complete Month 18 – Month 54 visits.

Appendix 2. Schedule of Events (Donor)

Transplant Study Day		**-1**	**0**	**12**									**Unscheduled Visit within 3 months of transplant**	**Unscheduled Visit greater than 3 months from transplant**
Transplant Study Week					**4-6**	**8**	**12**							
Transplant Study Month						**2**	**3**	**6**	**9**	**12**	**18**	**24**		
Visit Number		**00**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**		
GENERAL ASSESSMENTS														
Demographics	Age, Race, Ethnicity, Gender		X											
Donor Information	Cause of Death		X											
LOCAL LABORATORY ASSESSMENTS														
Blood Type	A, B, O, Rh		X											
HLA Typing	I (A, B, C), II (DR, DP, DQ)		X											
Viral Panel	CMV IgG, EBV IgG, HBsAg, HBcAb, HCV Ab, HIV		X											
MECHANISTIC ASSAYS – ISMMS (PI: PETER HEEGER)														
T Cell Function (ELISPOT)	Blood or Spleen		X											
Blood to Archive	Biospecimen Repository-ISMMS		X											