BILATERAL ORTHOTOPIC LUNG TRANSPLANT IN TANDEM WITH CD3+ AND CD19+ CELL DEPLETED BONE MARROW TRANSPLANT FROM PARTIALLY HLA-MATCHED CADAVERIC DONORS

PROTOCOL RTB-003

Version 8.0/ January 15, 2021

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NIH National Institute of Allergy and Infectious Diseases

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Protocol Number: RTB-003	**Version Number/Date: 8.0/1.15.2021**
Protocol Title: Bilateral Orthotopic Lung Transplant in Tandem with CD3+ and CD19+ Cell Deple Bone Marrow Transplant from Partially HLA-Matched Cadaveric Donors	
IND/IDE Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)	

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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, 812 and in the International Conference for Harmonisation (ICH) document entitled *Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2)*. 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.

*[*The site Principal Investigator should sign and date at the indicated location below. A written signature/date is acceptable (e.g., scanned and sent via email as a PDF version). An electronic signature is also acceptable (e.g., sent via email as a PDF version).]*

_____

Site Principal Investigator (Print)

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Site Principal Investigator (Signature) **Date**

Protocol Synopsis

Title	Bilateral Orthotopic Lung Transplant in Tandem with CD3+ and CD19+ Cell Depleted Bone Marrow Transplant from Partially HLA-Matched Cadaveric Donors
Short Title	BOLT-BMT
Clinical Phase	Phase I/II
Number of Sites	Single center study
IND Sponsor/Number	NIAID/ 17497
Primary Objectives	The primary objectives are to evaluate the safety and efficacy of performing bilateral orthotopic lung transplantation followed by cadaveric, partially HLA-matched (≥2/6 HLA-match with an identical ABO blood type) CD3+/CD19+ depleted bone marrow transplant for patients with primary immunodeficiency and end-stage lung disease.
Secondary Objectives	The secondary objectives are to evaluate the feasibility and long-term complications associated with combined solid organ and BMT including the ability to initiate and successfully withdraw subjects from immunosuppression following BMT and to attain independence from treatment dose antimicrobial drugs.
Study Design	This is a single center, Phase I/II study in which subjects receive a cadaveric, partially HLA-matched lung transplant followed by a CD3+/CD19+ depleted bone marrow transplant from the same donor.
Primary Endpoints	**Safety Endpoint** Safety will be determined by assessing the presence or absence of the following adverse events during the study period (up to 2 years post-BMT): • Death • Engraftment syndrome • Engraftment failure • Grade 4 or 5 events potentially attributable to Rituximab **Efficacy Endpoints (subjects who receive both BOLT and BMT)** • BOS at 1-year post BOLT; supplemental oxygen requirement and/or ventilatory support (noninvasive/invasive) at 1-year post BOLT • ≥ 25% donor T-cell chimerism at 12 months post BMT. • For subjects with myeloid disorders (e.g. CGD), attaining ≥ 10% myeloid chimerism at 12 months post BMT. • For subjects with B-cell disorders, attaining ≥ 10% B-cell chimerism at 12 months post BMT.
Secondary Endpoints	**Secondary Endpoints (all enrolled subjects, as applicable)** The following secondary endpoints will be assessed:

	• Feasibility of proceeding to BMT within 6 months following lung transplantation. • Development of tolerance to both the host and pulmonary graft. • Long-term complications of combined solid organ and BMT. • Incidence of acute cellular rejection and graft failure post BMT. • Incidence of acute and chronic graft-versus-host disease (GVHD) following tandem lung and BMT. • Initiation weaning of immunosuppression by 1 year following BMT. • Time from BMT to withdrawal of immunosuppression. • Time from BMT to independence from treatment dose antimicrobial drugs. • For T cell lymphopenias, achieving age adjusted, low limit normal range lymphocyte count by 1-year post-BMT. • A significant development in chronic lung allograft dysfunction (as evidenced by a change in BOS stage, see pg. 19 for BOS table) or allograft failure at 1-year and up to 2 years post lung transplant for BOLT alone and BOLT-BMT subjects. • Incidence of Grade 4 or 5 adverse events possibly related to the use of Rituximab prior to the start of BMT conditioning.
Mechanistic Endpoints	The following mechanistic objectives will be evaluated: • The pace of immune reconstitution as well as the incidence and degree of mixed donor chimerism will be evaluated. • The incidence of mixed chimerism (>5% host cells) at Months 1, 3, 6, 12 and 24 months post BMT. • Improvement in immunologic marker(s) unique for the underlying PID syndrome by one year post BMT (see Appendix 5).
Accrual Objective	Up to 30 subjects will be consented in order to enroll 8 subjects who receive both BOLT and BMT.
Study Duration	Total of 5 Years (4-year accrual + 1-year minimum (up to 2 years) follow-up post-BMT).

Treatment Description	Subjects will undergo BMT utilizing CD3+/CD19+-depleted bone marrow with conditioning beginning no less than 8 weeks after BOLT.
	Conditioning regimen will be as follows:
	• Day -28 to -3: Hydroxyurea
	• Day -28, Day +35: Rituximab
	• Alemtuzumab:
	  ○ Day -21, Day -20: Subjects with prior ACR ≥ A2 and/or ≥B2R (see section 7.2.3 for further details) will receive two doses of Alemtuzumab **OR**
	  ○ Day -14: Subjects without prior ACR (see section 7.2.3 for further details) will receive a single dose of Alemtuzumab
	• Day -3 to -1: ATGAM
	• Day -2: Thiotepa

	• Day -1: TBI 250 cGy (200cGy for radiosensitive subjects) with lung shielding. Lung shielding will not be completed for subjects with previous ACR of ≥ A2 and/or ≥B2R. Additional organ shielding for fertility preservation will be allowed based upon the investigator's discretion.
Inclusion Criteria: Study Entry and Lung Transplant	Individuals must meet all of the following criteria in order to be eligible for this study. 1. Subject and/or parent guardian must be able to understand and provide informed consent. 2. Male or female, 10 through 45 years old, inclusive, at the time of informed consent. 3. Meet criteria for UNOS listing. 4. Patients must have evidence of an underlying primary immunodeficiency for which BMT is clinically indicated. Examples of such diseases include, but are not limited to: • Severe Combined Immunodeficiency • Combined immunodeficiency with defects in T-cell-mediated immunity, including Omenn syndrome and DiGeorge Syndrome • Severe Chronic Neutropenia • Chronic Granulomatous Disease • Hyper IgE Syndrome or Job Syndrome • CD40 or CD40L deficiency • Wiskott-Aldrich Syndrome • Mendelian Susceptibility to Mycobacterial Disease [6] • GATA2 Associated Immunodeficiency NOTE: A genetic diagnosis is recommended, but not required. 5. Patients must have evidence of end-stage lung disease and be candidates for bilateral orthotopic lung transplant as determined by the lung transplant team. 6. GFR ≥50 mL/min/1.73 m2. 7. AST, ALT ≤4x upper limit of normal, total bilirubin ≤ 2.5 mg/dL, normal INR. 8. Cardiac ejection fraction ≥ 40% or shortening fraction ≥26%. 9. Negative pregnancy test for females >10 years old or who have reached menarche, unless surgically sterilized. 10. All females of childbearing potential and sexually active males must agree to use a FDA approved method of birth control for up to 24 months after BMT or for as long as they are taking any medication that may harm a pregnancy, an unborn child or may cause birth defect. 11. Subject and/or parent guardian will also be counseled regarding the potential risks of infertility following BMT and advised to discuss sperm banking or oocyte harvesting.

Exclusion Criteria: Study Entry and Lung Transplant	Individuals who meet any of these criteria are not eligible for this study: 1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol. 2. Patients who have underlying malignant conditions. 3. Patients who have non-malignant conditions not requiring hematopoietic stem cell transplantation. 4. HIV positive by serology or PCR, HTLV positive by serology. 5. Females who are pregnant or who are lactating. 6. Allergy to DMSO or any other ingredient used in the manufacturing of the stem cell product. 7. Uncontrolled pulmonary infection, as determined by radiographic findings and/or significant clinical deterioration. NOTE: Pulmonary colonization with multiple organisms is common and will not be considered an exclusion criterion. 8. Uncontrolled systemic infection, as determined by the appropriate confirmatory testing e.g. blood cultures, PCR testing, etc. 9. Recent recipient of any licensed or investigational live attenuated vaccine(s) within 4 weeks of transplant. 10. 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.
Eligibility: Donor	Donor must be a ≥2/6 (A, B, DR) HLA-matched cadaveric organ donor with an identical ABO blood type as the recipient.
Eligibility: Bone Marrow Transplant	The decision to proceed with the BMT will be at the discretion of the lung transplant team following clearance by the bone marrow team based on the criteria below. The conditioning for the BMT will begin no less than 8 weeks following the lung transplant. 1. GFR >50 mL/min/1.73 m^2. 2. AST, ALT <4x upper limit of normal, Total bilirubin < 2.5 mg/dL. 3. Cardiac ejection fraction ≥ 40% or shortening fraction of at least 26%. 4. FVC and FEV1 ≥40% predicted for age and SpO_2 of >90% at rest on room air AND with clearance by the lung transplant team. 5. HIV negative by serology and PCR. 6. HTLV serology negative. 7. Absence of uncontrolled infection as determined by positive blood cultures and radiographic progression of previous sites in particular pulmonary densities during the past 2 weeks prior to chemotherapy. 8. Absence of clinically significant acute cellular rejection (<A2-A4 and/or B2R rejection). 9. Bone marrow processing (section 6.1.2) has been completed, and an appropriate stem cell product is available for administration (section 6.1.3). 10. Any medical issue or finding 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.
Study Stopping Rules	**A. Temporary Suspension of Enrollment** If any grade 4 or 5 adverse events that are at least possibly related to the use of rituximab prior to BMT, occur in at least 2 subjects, a safety-related suspension of enrollment will occur pending notification to the Institutional Review Board (IRB) and an expedited review of all pertinent data by the National Institute of Allergy and Infectious Diseases (NIAID) Medical Monitor and the NIAID Data Safety Monitoring Board (DSMB). **B. Temporary Suspension of Enrollment and BMT** If any of the following occur in the first 100 days post-BMT among the first three subjects receiving BMT, a safety-related suspension of enrollment or proceeding to BMT will be initiated pending notification to the IRB and an expedited review of all pertinent data by the NIAID Medical Monitor and the NIAID DSMB. • Death • Respiratory failure as a result of rejection or infection requiring mechanical ventilation for more than 48 hours. • Engraftment syndrome **C. Study Stopping Rule** The incidence of specific events will be continuously monitored throughout the study to determine if any of their observed subject-based incidence rates exceed a threshold incidence rate. This stopping rule will be triggered if **2 of 2 patients, 3 or more of 5 patients, or 4 or more of 8 patients** experience one of the following sentinel clinical events. • Death • Respiratory failure as a result of rejection or infection requiring mechanical ventilation for more than 48 hours • High grade (grade ≥A3) persistent or high grade recurrent acute lung rejection post BMT (including post immunosuppression withdrawal) • Grade 4 or greater lung infection post BMT • Engraftment failure/failure to achieve chimerism (<5% donor cells at day 100 following BMT, or <10% donor cells at day 180) • Engraftment syndrome If a stopping threshold is met, enrollment and proceeding to BMT will be halted pending expedited NIAID DSMB review.

Study Contacts: Participating Centers

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Table of Contents

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Glossary of Abbreviations

ABO	A, B, O blood types
AE	Adverse event
ALT	Alanine aminotransaminase
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
ATG	Anti-thymocyte globulin
ATGAM	Anti-thymocyte globulin, Horse
β-hCG	Human chorionic gonadotropin
BMT	Bone Marrow Transplant
BOLT	Bilateral orthotopic lung transplant
CFR	Code of Federal Regulations
CBC	Complete blood count
CD	Cluster of differentiation
GCD	Chronic granulomatous disease
CHP	Children's Hospital of Pittsburgh
CIBMTR	Center for International Blood and Marrow Transplant Research
CLIA	Clinical Laboratory Improvement Act
CORE	Center for Organ Recovery and Education
CMV	Cytomegalovirus
CRF	Case report form
CRSS	Composite Rejection Standardized Score
CSF	Cerebrospinal fluid
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DAIT	Division of Allergy, Immunology, and Transplantation
DLI	Donor Leukocyte Infusion
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
EBV	Epstein-Barr virus
EKG	Electrocardiogram

FDA	Food and Drug Administration
FEV1	Forced expiratory volume
FKBP	FK binding protein
FVC	Forced vital capacity
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor
GFR	Glomerular filtration rate
GVHD	Graft versus host disease
HCV	Hepatitis C virus
HepBsAg	Hepatitis B surface antigen
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HPC	Hematopoietic progenitor cell
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplant
HSV	Herpes simplex virus
HTLV	Human T-lymphotropic virus
HU	Hydroxyurea
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
IVIG	Intravenous Immunoglobulin
LFT	Liver Function Tests
MOP	Manual of Procedures
NAT	Nucleic Acid Testing
NIAID	National Institute of Allergy and Infectious Diseases
NK	Natural killer
NRM	Non-relapse mortality
OPO	Organ Procurement Organization

PBSC	Peripheral blood stem cell
PCR	Polymerase chain reaction
PCP	Pneumocystis carinii pneumonia
PRN	Pro re nata (as needed)
PTLD	Post-transplant lymphoproliferative disease
PI	Principal Investigator
PID	Primary Immunodeficiency Disease
Q	Quaque (every)
RIC	Reduced intensity conditioning
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute (culture medium)
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SCN	Severe congenital neutropenia
SOP	Standard Operating Procedure
STAT3	Signal transducer and activator of transcription 3
STR	Short tandem repeat
SUSAR	Serious Unexpected Suspected Adverse Reaction
TBI	Total body irradiation
TLI	Total lymphoid radiation
TNC	Total nucleated cells
UNOS	United Network of Organ Sharing
UPMC	University of Pittsburgh Medical Center

Study Definitions Page

<table>
<tr><td>Bronchiolitis Obliterans Syndrome Classification System</td><td>
<table>
<tr><th>Stage</th><th>Pulmonary function criteria</th></tr>
<tr><td>BOS 0</td><td>FEV_1 > 90% baseline* & FEF_{25-75} >75% baseline*</td></tr>
<tr><td>BOS 0-p (potential)</td><td>FEV_1 81-90% baseline* &/or $FEF_{25-75} \leq$ 75% baseline*</td></tr>
<tr><td>BOS 1</td><td>FEV_1 66-80% baseline*</td></tr>
<tr><td>BOS 2</td><td>FEV_1 51-65% baseline*</td></tr>
<tr><td>BOS 3</td><td>$FEV_1 \leq$50% baseline*</td></tr>
</table>
*Baseline FEV_1 =average of 2 best values FEV_1 after transplant
*Baseline FEF_{25-75} =average of 2 best FEF_{25-75} values after transplant
Decline is determined by two measurements at least one month apart</td></tr>
<tr><td>Chronic GVHD</td><td>Defined per the National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group Report [36]. While 8 organ systems/sites are used to score cGVHD, the lungs will be excluded in this study due to the bilateral orthotopic lung transplant.</td></tr>
<tr><td>Chronic Lung Allograft Dysfunction (CLAD)</td><td>CLAD will be diagnosed locally based on ISHLT criteria for BOS or histologic evidence of obliterans bronchiolitis.</td></tr>
<tr><td>Composite Rejection Standardized Score</td><td>The composite rejection standardized score (CRSS) serves to characterize the burden of acute cellular rejection relevant to the allograft. In so doing, the vascular (A grade) and airway-centered (B grade) are combined and standardized by the number of samples obtained over the study period. The A grade ranges from 0-4 and the B grade ranges from 0-2; thus, the range of the CRSS is 0-6 (A0B0-A4B2R).</td></tr>
<tr><td>Donor Bone Marrow Engraftment</td><td>Donor bone marrow engraftment will be assessed by measuring, when feasible, chimerism as determined in whole blood and CD3+ T-cell fraction as performed by standard, clinical STR assay. For those enrolled subjects whose underlying immune deficiency affects phagocyte function or numbers, typically those with chronic granulomatous disease (CGD) or severe congenital neutropenia (SCN), whole blood chimerism will be the outcome measure. For all others, whose immune defect affects T-cell function or T-cell numbers, determining the percentage of donor CD3+ T-cell contribution (chimerism) will be the outcome measure. Nevertheless, for all patients both whole blood and CD3+ T-cell chimerism will be determined.</td></tr>
<tr><td>Engraftment Failure</td><td>Less than 5% donor cells at day 100 following BMT, or <10% donor cells at day 180.</td></tr>
<tr><td>Engraftment Syndrome</td><td>A syndrome which can occur post bone marrow transplant consisting of the following symptoms:
unexplained fever >38.5 for 3 consecutive days
erythrodermatous skin rash involving 50% or greater body surface area
weight gain >/= 5%
capillary leak syndrome as defined by the need for oxygen supplementation to keep resting room air saturation >92%</td></tr>
<tr><td>Lost to Follow-up</td><td>Subject who cannot complete study visits due to inability to reach the subject, subject relocation, etc.</td></tr>
</table>

<table>
<tr><td>Medical Monitor</td><td>The physician who is responsible for the safety aspects of this trial. The MM is responsible for making the final causal relationship assessment of each serious adverse event.</td></tr>
<tr><td>NIAID Project Manager</td><td>NIAID assigned project manager who is responsible for all day to day protocol related issues, including version control, consent review, etc.</td></tr>
<tr><td>Principal Investigator</td><td>Investigator awarded NIH funding for the grant.</td></tr>
<tr><td>Program Officer</td><td>NIAID official who oversees the scientific and budgetary aspects of the grant.</td></tr>
<tr><td>Pathological Classification and Grading of Pulmonary Allograft Rejection [41]</td><td>
<table>
<tr><th>Nomenclature</th><th>Classification</th><th>Grading</th></tr>
<tr><td>A</td><td>acute rejection</td><td>0 – none
1 – minimal
2 – mild
3 – moderate
4 – severe</td></tr>
<tr><td>B</td><td>airway inflammation</td><td>0 – none
1R – low grade
2R – high grade
X – upgradeable</td></tr>
<tr><td>C</td><td>chronic airway rejection</td><td>0 – absent
1 – present</td></tr>
<tr><td>D</td><td>chronic vascular rejection</td><td>Accelerated graft vascular sclerosis</td></tr>
</table>
</td></tr>
<tr><td>Protocol Mandated Procedures</td><td>Any procedure performed solely for the purpose of this research study (not site-specific standard of care).</td></tr>
<tr><td>Regulatory Affairs Officer</td><td>NIAID assigned officer responsible for regulatory aspects of study, including IND submissions and other communications with FDA, as applicable.</td></tr>
<tr><td>Site Principal Investigator</td><td>Lead investigator listed on the FDA 1572 at a particular center who is responsible for the conduct of the study at that center.</td></tr>
<tr><td>Study Termination</td><td>Subjects who are lost to follow up, withdraw consent, or die during the study. Data and specimens will no longer be expected from subjects who are terminated from the study.</td></tr>
<tr><td>Study Therapy</td><td>The investigational agents and all protocol required medications.</td></tr>
<tr><td>Tolerance</td><td>Successful withdrawal from systemic immunosuppression for 6 weeks with no increase in cGVHD score and stable or improving PFTs.</td></tr>
</table>

1 Study Hypotheses/Objectives

1.1 Hypotheses

Lung transplant prior to bone marrow transplant (BMT) would allow for restoration of pulmonary function prior to BMT, allowing patients to proceed to BMT, which would be curative for the patient's underlying immunodeficiency.

1.2 Primary Objective

The primary objectives are to evaluate the safety and efficacy of performing bilateral orthotopic lung transplantation followed by cadaveric, partially HLA-matched (≥2/6 HLA-match with an identical ABO blood type) CD3+/CD19+ depleted bone marrow transplant for patients with primary immunodeficiency and end-stage lung disease.

1.3 Secondary Objectives

The secondary objectives are to evaluate the feasibility and long-term complications associated with combined solid organ and BMT including the ability to initiate and successfully withdraw subjects from immunosuppression following BMT and to attain independence from treatment dose antimicrobial drugs.

1.4 Mechanistic Objectives

The mechanistic objectives are to evaluate the pace of immune reconstitution as well as the incidence and degree of mixed donor chimerism.

2 Background and Rationale

2.1 Background and Scientific Rationale

For many patients with primary immunodeficiencies, BMT is a curative, life-saving therapy, resulting in restoration of function in the immune system [2]. Although historically patients have received myeloablative conditioning prior to transplant, there has been increasing interest in reduced intensity conditioning for patients with primary immunodeficiencies in an effort to decrease toxicity and late effects following transplant. Using these regimens in patients with primary immunodeficiencies, the largest studies have shown overall survival rates of 69-94% at a median follow-up of 3-5 years; however, mixed chimerism is increased compared to myeloablative conditioning regimens. This can resolve with reduced immunosuppression, and if stable, may not preclude functional immune reconstitution [3, 4]. Patients with primary immunodeficiencies often develop pulmonary complications as a result of chronic or recurrent infections [6], and between 21-33% of patients with non-cystic fibrosis bronchiectasis have an underlying immunodeficiency [7, 8]. Potential BMT candidates with severe lung disease have historically been ineligible for transplant due to the unacceptably high risk of mortality associated with the conditioning regimen and the possibility of developing bronchiolitis obliterans syndrome following BMT, leading to further pulmonary compromise and overall futility. Conversely, patients inflicted with PID syndromes and concomitant pulmonary failure have been ineligible for BOLT as the underlying immunodeficiency would lead to recurrent infections of the newly transplanted lungs. These children and young adults have had palliative care as their only option.

Lung transplant prior to BMT would allow for restoration of pulmonary function prior to bone marrow transplant, decreasing the risk of the overall hematopoietic stem cell transplant procedure, which is curative for the patient's underlying immunodeficiency. Although lung transplantation is typically performed without HLA-matching criteria as long as ABO typing, size, and lung function are appropriate, enrolled subjects will receive partially HLA-matched lungs and bone marrow. Patients receiving lung transplantation are typically maintained on lifelong immunosuppression to reduce the risk of rejection by host T cells. Persistent engraftment of hematopoietic stem cells from a healthy, immune competent lung donor should result in the establishment of donor derived cellular immunity. A healthy donor-derived immune system should not only protect from infections but should also facilitate the graft survival of the transplanted autologous lungs and is expected to permit planned removal of immunosuppressive drugs to establish lifelong tolerance as we have recently demonstrated.

2.2 Rationale for Selection of Investigational Product or Intervention

The current protocol involves a cadaveric, partially HLA-matched lung transplant followed by a CD3+/CD19+ depleted bone marrow transplant using hydroxyurea, rituximab, alemtuzumab, anti-thymocyte globulin, thiotepa, and low-dose TBI (250 cGy) with lung-shielding as conditioning in patients with primary immunodeficiencies. This regimen minimizes the risk of toxicity to the transplanted lungs, and is heavily immunosuppressive, with anti-thymocyte globulin and alemtuzumab to reduce host NK cells along with T cells of either host or donor origin to minimize the risk of graft rejection and GVHD. The purpose of rituximab is to deplete host and donor B cells, potentially decreasing the risk of post-transplant lymphoproliferative disease (PTLD) developing from EBV-infected B cells and may reduce early de novo allosensitization to the lung graft [15, 37, 38, 39]. Furthermore, the use of Rituximab may

reduce the potential danger of pre-BMT allosensitization, which is associated with poor marrow graft function [40]. As noted above, there has been reported a low risk of GVHD in the haploidentical setting when using CD34 selected grafts [14, 15]. Depletion of CD3+ cells will remove potentially alloreactive T cells, the primary effectors of GVHD, from the graft, thereby decreasing the risk of GVHD. In addition, due to the risk of EBV-driven PTLD, we will perform CD19+ cell depletion to remove a reservoir of latent EBV infected B cells. Also, as opposed to CD34+ selection, which removes all but the CD34+ cells, this depletion process will retain large numbers of other immune cells, such as donor NK cells (which are important for facilitating engraftment), dendritic cells, granulocytes and monocytes in the graft [22], potentially providing additional protective immunity early after transplant. We anticipate that by processing the stem cell product in this manner in combination with aggressive serotherapy as part of the conditioning regimen, we will significantly decrease the risk of both GVHD and PTLD. As seen in other studies, this will allow for the development of a functional immune system from the engrafting donor-derived bone marrow [23], and protection from rejection and GVHD of the transplanted lungs as they will be HLA-identical to each other. In addition to the decreased risk of rejection in this setting, there is a high likelihood that if no GVHD were to develop, patients could achieve operational tolerance and be removed from all immunosuppressive medications.

2.3 Clinical Studies

2.3.1 Previous Experience with BOLT and BMT

Dr. Szabolcs designed a novel reduced-intensity conditioning regimen in 2009 and was the sponsor and PI of an FDA approved treatment plan (IDE: 14206) titled: Single-patient use of CliniMACS® CD3/CD19 depletion device for T cell depletion on cadaveric unrelated bone marrow before transplantation into a lung transplant recipient at Duke University. Bilateral orthotopic lung transplantation (December 2009) followed by cadaveric 4/8 HLA-matched (A, B, C, DR), CD3+ and CD19+ depleted bone marrow transplant (April 2010) from the same donor was successfully performed using a treatment plan with rituximab, alemtuzumab, antithymocyte globulin, hydroxyurea, thiotepa, and low-dose TBI as conditioning. Although 4 months later the patient developed severe gastrointestinal GVHD, there was rapid and complete response following a single dose of Infliximab and a short course of steroids. She has been off all immunosuppressive medications and bacterial/viral/fungal/PCP prophylaxis for >4 years without evidence of GVHD, rejection, or infections and is currently enrolled in college. This is the first ever-successful human case and it demonstrates the feasibility and immune consequences of tandem cadaveric lung and T-cell-depleted marrow transplantation from the same HLA-mismatched unrelated donor to create solid organ and recipient-specific tolerance in the absence of systemic immunosuppression.

While at Duke University, Dr. Szabolcs received FDA/IRB approval for a second patient to be treated by the same treatment plan, however, following his departure from Duke University, in November 2011 he transferred the sponsorship of the IND application (14643) to Dr. Gwynn Long from the Division of Cellular Therapy in the Department of Internal Medicine. BOLT was performed in April 2012 with dramatic clinical improvement in lung function and eventual nutritional rehabilitation, however, the externally harvested marrow dose was below the target of 1x 10e6CD34+/kg. Following receipt of the FDA/IRB exception approval to permit this deviation, the BMT was performed 3 months after BOLT in late July 2012 with ~0.3x 10e6/kg CD34+ cells. The patient engrafted rapidly without any severe AEs,

however, was found with 5% donor cells at engraftment that were rapidly lost by day +50 when only host cells were identified. In May 2013, the Duke PI reported no serious, expected (≥grade 3) adverse events that were possibly related to study intervention. There were no IND safety reports submitted during the period up to May 2013. He remained on FK506 for lung rejection prophylaxis however, developed allograft rejection and died in his home state in March 2014 due to VRE sepsis and renal failure.

Dr. Szabolcs and Dr. McDyer received FDA/IRB approval for an additional single case treatment (IND: 15221) titled: Single patient use of CliniMACS® CD3 and CD19 depletion device for T and B cell depletion on cadaveric unrelated bone marrow before transplantation into a lung transplant recipient with Job's Syndrome. This patient received BOLT under the care of Dr. John McDyer from a 2 of 6 HLA-matched cadaveric donor, however, declined to undergo BMT subsequently due to a protracted and difficult post-BOLT course complicated by early Pseudomonas pneumonia. The patient recovered however was found to have several right supra diaphragmatic Aspergillus abscesses that responded to 3 months of IV liposomal amphotericin. She did well until about 2 ½ years post-transplant when she developed recurrent pleural effusions and was found to have invasive Aspergillosis encasing her right pulmonary artery. Despite aggressive medical management she died just prior to her 3rd year post-transplant.

The fourth patient was the first to enroll in the Phase 1/2 clinical trial initiated in 2013 at the University of Pittsburgh (PI Paul Szabolcs) under IND 15414. This patient had intermittent neutropenia, FTT, and weight loss with recurrent pneumonia driven by alcaligenes, pseudomonas, acinetobacter, and aspergillus leading to progressive bronchiecatasis. She has two independent frameshift mutations in the IL7R genes explaining her T---, B+, NK+ SCID phenotype. A 2 of 6 HLA-matched donor was identified after >2 years on the waitlist and BOLT was performed in September 2015 at age 14 years. She did well and was noted only to have impaired movement of right hemi diaphragm causing restrictive pattern on PFTs and left pleural effusion, with subsequent thoracentesis of this transudate in November 2015. No pathogens were identified. So far, the routine post-BOLT biopsies have been free of ACR as of August 2016. She has thrived since BOLT, in fact her weight increased from 29kg pre---BOLT to ~ 38 kg by the time of her BMT. Accordingly, her BMI has increased from ~14.0 to 17.5 currently.

High resolution allele level typing identified HLA A0201 as a "true" match while the UNOS low resolution match at the HLA---DR locus was found to be mismatched at high resolution. The recipient allele is DRB1 11:01 versus HLA-DRB1 11:03 donor allele. The patient received the thawed CD3/19 –depleted cadaveric donor BMT on January 28, 2016. She engrafted on Feb 10, 2016 with 100% donor cells by STR analysis. She received cryopreserved marrow grafts from external harvest of iliac crest along with cells extracted from vertebral bodies (VB), however vertebral body derived marrow provided >90% of the collected CD34+ stem cell dose. Her post-BMT immune recovery is significant for prolonged T cell lymphopenia (<10 CD3+ T cells/ul) up to day+50. With autologous T cells surviving and detectable at Day+50 (host T cells were dominant @~75% recipient). Donor leukocyte infusion (DLI) was infused approximately 2 months after BMT obtained from cryopreserved unmodified cadaveric bone marrow containing 5x 10e4 viable CD3+ T cells/kg. Since then, T cell chimerism has risen >99% donor since DLI, with absolute CD3+ T cells detectable~ 200/ul, notably, almost all CD4+. The DLI given 2 months ago completely reversed the host T cell dominance, however, donor myeloid chimerism has declined since day+50, although, it seems to have been fluctuating, ~ 30% ± 5% . NK cell chimerism has remained ~ 75-80% donor origin. Significantly, she developed stage 2 skin GVHD covering 45---50% of her skin that

responded to ~ 4 days of 1 mg/kg prednisone, with a 5mg daily maintenance dose. Besides FK506 there have been no other IS drugs used since BMT.

2.3.2 Experience with Reduced-Intensity Conditioning in Primary Immunodeficiency

Reduced-intensity conditioning (RIC) regimens have been used successfully for patients with non-malignant conditions, including primary immunodeficiencies. These regimens are typically highly immunosuppressive, and typically utilize serotherapy with agents such as antithymocyte globulin or alemtuzumab in conjunction with other immunosuppressive agents such as fludarabine and decreased intensities of alkylating agents such as busulfan, melphalan, or cyclophosphamide [9]. Veys *et al*. reported successful use of reduced intensity conditioning, primarily fludarabine, melphalan and alemtuzumab, in 113 patients with primary immunodeficiency. In this group of patients, 81% had stable donor chimerism and overall survival was 82% at a median of 2.9 years post-BMT [3]. Similar results have been reported by other groups [10], suggesting that RIC regimens are effective for primary immunodeficiencies, particularly in patients with other comorbidities and recovery of some host-derived leukocytes does not diminish the curative potential of transplant. Long-term stable coexistence of recipient and donor hematopoietic cells in the absence of any immunosuppressive drugs and without clinical evidence of immune-mediated pathology is often referred as "*persistent mixed chimerism*". This phenomenon of tolerant state was already recognized long before the advances of RIC regimens. Despite undergoing myeloablative conditioning, a sizeable proportion of patients with β-thalassemia[11] or sickle cell disease[12] reconstitute with mixed host and donor hematopoietic cells. Interestingly, donor cells contributing in excess of 10% lead to transfusion independence and freedom from pathological symptoms.

2.3.3 Worldwide Experience with Bone Marrow Transplant HLA-Mismatched at 3 or More Loci

Haploidentical (3 of 6 HLA-matched) donors are an established source of stem cells, and have been used successfully using both myeloablative and reduced intensity conditioning. Some experienced centers are now reporting outcomes using haploidentical grafts similar to those seen in matched-unrelated donors [13]. In addition, using CD34 selected, haploidentical grafts with a myeloablative conditioning regimen, Aversa *et al*. reported a very low incidence of GVHD, despite the absence of GVHD prophylaxis, and treatment-related mortality of 36%, with most deaths due to infection [14]. Depletion of CD3+ and CD19+ cells in haploidentical grafts has also been used successfully in 61 patients with high-risk malignancy using a reduced-*intensity* conditioning regimen containing fludarabine, melphalan, thiotepa, and ATG. In this study, 56/61 patients engrafted with full donor chimerism at a median of 12 days post-transplant. In addition, there was rapid reconstitution of NK cells, with slower reconstitution of T and B cells. The incidence of grade II-IV GVHD was 46%, and was related to the extent of T-cell depletion, with more effective T-cell depletion resulting in less GVHD. The incidence of non-relapse mortality (NRM) was low, at 23% at 100 days and 42% at two years [15]. Chen *et al*. also reported more rapid immune reconstitution following CD3+ depleted haploidentical stem cell transplant using reduced-intensity conditioning regimens compared to myeloablative conditioning regimens [16]. Umbilical cord blood is also a commonly used stem cell source. Eapen *et al*. recently reported the results of mismatched umbilical cord blood transplantation. In her analysis of CIBMTR data, 328 patients received an umbilical cord blood transplant with at least 3 of 8 mismatched loci. Treatment mortality was 33% (83/253) for 3 mismatched loci, and 37% (28/75) for 4 mismatched loci [17].

2.3.4 Induction of Tolerance with Solid Organ and Bone Marrow Transplant

16 patients with at least 10/10 HLA-matched (A, B, C, DR, DQ), living renal and peripheral blood stem cell donors underwent renal transplant followed by conditioning with TLI and ATG with PBSC 11 days after renal transplantation. Eleven patients were taken off all immunosuppression, and no patients developed acute or chronic GVHD, while maintaining response to third-party alloantigens [18]. Similarly, researchers at Northwestern University and the University of Louisville have reported operational tolerance in renal transplantation using a preparative regimen of cyclophosphamide, fludarabine, and total-body irradiation (TBI) followed by renal transplantation and HLA mismatched mobilized peripheral blood stem cells. Using this approach, 5 of 8 patients engrafted and were able to be removed from all immunosuppression. No patients developed GVHD in this setting, even with as little as a 1/6 HLA match, meaning that only 1 of the 6 HLA antigens were a match between donor and recipient, and patients maintained response in mixed lymphocyte reaction to third party stimulator cells [19-21].

2.3.5 Institutional Experience with the CliniMACS® device

The CliniMACS® device has been used at the Children's Hospital of Pittsburgh of UPMC for the processing of hematopoietic stem cells since 2011. The primary use has been the selection of CD34+ stem cells from peripheral blood stem cell products for use in an IRB-approved protocol for autologous stem cell transplantation for patients with medically refractory Crohn's Disease. To date, this procedure has been performed on hematopoietic stem cell products from 2 patients. While the second patient has not received conditioning for transplant, the first one engrafted promptly on day 12. In addition, it has been used successfully for CD34+ selection in an FDA approved protocol IND 15245: Single Patient Use of Autologous Stem Cell Transplantation with CliniMACS® CD34-Selected Peripheral Blood Stem Cells (PBSC) in a Young Adult Patient with Relapsing Polychondritis. This subject engrafted on day +10. We have also successfully performed a CD3+ and CD19+ depletion for an FDA approved protocol (IND: 15221) titled: Single patient use of CliniMACS® CD3 and CD19 depletion device for T and B cell depletion on cadaveric unrelated bone marrow before transplantation into a lung transplant recipient with Job's Syndrome. This patient refused the BMT and subsequently died before her 3rd year BOLT anniversary.

2.3.6 Worldwide Experience with CD3/CD19 Depletion

Depletion of CD3+ and CD19+ cells in haploidentical grafts has also been used successfully in both pediatric and adult patients. Lang et al. reported on 49 pediatric patients aged 1-23 years with leukemia or myelodyspastic syndrome using fludarabine or clofarabine, melphalan, thiotepa and ATG or OKT3 as conditioning. In this group, 88% of patients achieved neutrophil engraftment within the first 28 days post-transplant, and the remaining patients engrafted after receiving a second infusion of CD3/CD19 depleted stem cells. Twenty-seven percent of patients developed grade II-IV GVHD, and while the majority of patients developed viremia for CMV, adenovirus, or BK virus, the mortality related to these infections was extremely low (2%), which was attributed to rapid immune recovery following transplant. The overall treatment-related mortality was 8% at 1 year and 20% at 5 years [27]. Additionally, Bader et al. reported on 59 patients who received a reduced-intensity conditioning regimen utilizing fludarabine, melphalan and thiotepa. Neutrophil engraftment was rapid, occurring at day +12 and immune reconstitution also occurred rapidly and was long-lasting. The treatment-related mortality in this group of patients was 11% at one year post-transplant [28]. In a much smaller study, Koh et al. reported 3 pediatric patients with severe aplastic anemia who received haploidentical CD3/CD19 depleted stem cells grafts following a reduced-intensity conditioning regimen utilizing fludarabine, cyclophosphamide and ATG. Similar to Lang et al, there was rapid immune recovery and low treatment-related mortality

[29]. This strategy of reduced-intensity conditioning and haploidentical transplant using CD3/CD19 depletion has also been successfully employed in the adult population. Bethge et al. reported on 29 adult patients with high-risk hematologic malignancy using a reduced-intensity conditioning regimen containing fludarabine, melphalan, thiotepa, and ATG. Engraftment was rapid with a median of 12 days, and 48% of patients developed grade II-IV GVHD. Treatment-related mortality was 20% at day 100 post-transplant [30]. Federmann et al. reported on 61 adult patients with high-risk malignancy using a similar reduced-intensity conditioning regimen containing fludarabine, melphalan, thiotepa, and ATG. In this study, 56/61 patients engrafted with full donor chimerism at a median of 12 days post-transplant. In addition, there was rapid reconstitution of NK cells, with slower reconstitution of T and B cells. The incidence of grade II-IV GVHD was 46% and was related to the extent of T-cell depletion, with more effective T-cell depletion resulting in less GVHD. The incidence of non-relapse mortality (NRM) was low, at 23% at 100 days and 42% at two years [15]. Thus, depletion of CD3 and CD19 positive cells is safe and is associated with rapid engraftment and immune reconstitution with a low risk of severe GVHD or treatment-related mortality.

3 Study Design

3.1 Description of Study Design

This is a single center, Phase I/II study in which subjects receive a cadaveric, partially HLA-matched lung transplant followed by a CD3+/CD19+ depleted BMT from the same donor. In this study, the investigators will use a ≥2/6 (A, B, DR) HLA-matched T cell depleted bone marrow transplant from a cadaveric organ donor with an identical ABO blood type as the recipient.

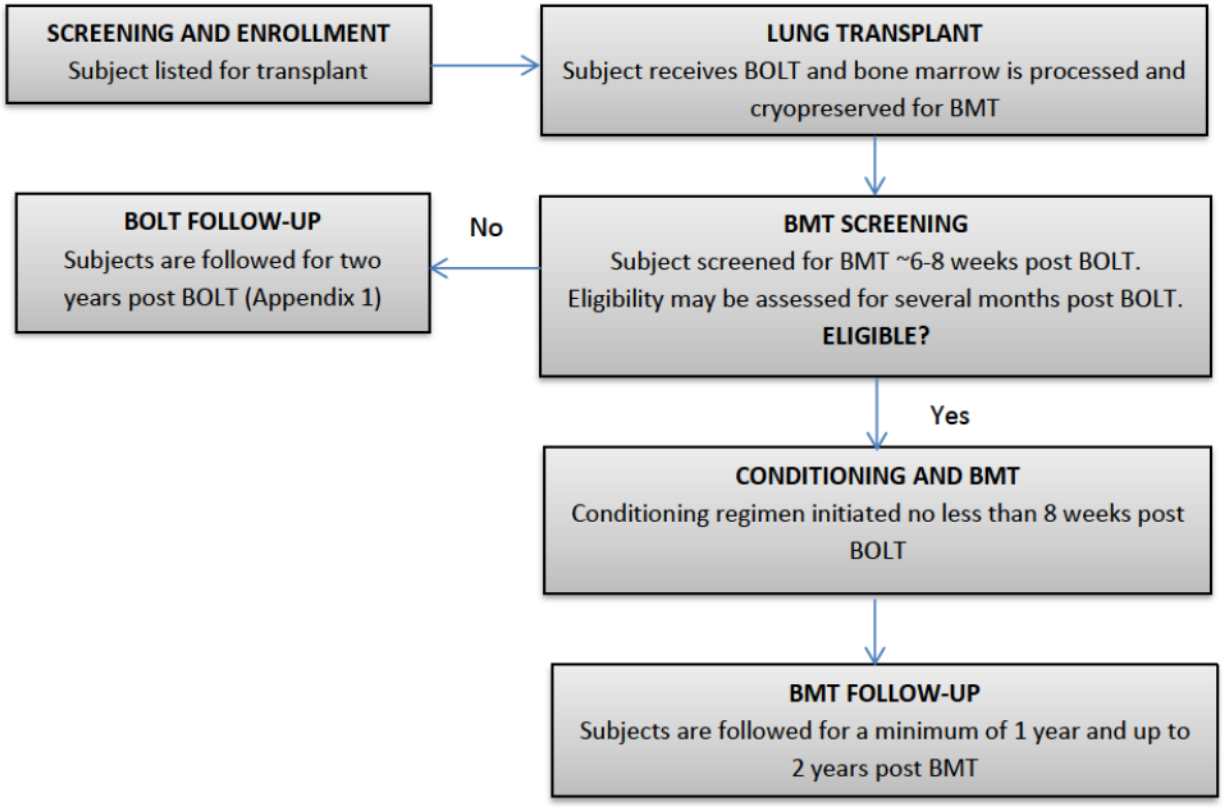
Subjects will undergo BOLT utilizing basiliximab induction or an alternate induction therapy based on the subject's underlying disease. Rituximab may be initiated prior to the lung transplant with tacrolimus as the ongoing maintenance immunosuppression.

Subjects will undergo BMT utilizing CD3+/CD19+-depleted bone marrow with bone marrow conditioning beginning no less than 8 weeks after BOLT. The conditioning regimen may be paused for up to 7 cumulative days if there are clinical complications e.g. infection. The regimen will resume from the point it was paused once the investigator and NIAID medical monitor agree it is safe to proceed.

The conditioning regimen will be as follows:

- Day -28 to -3: Hydroxyurea
- Day -28, Day +35: Rituximab
- Alemtuzumab:
    - Day -21, Day -20: Subjects with previous acute cellular rejection (see section 7.2.3 for further details) (ACR) of ≥ A2 and/or ≥B2R will receive two doses of Alemtuzumab OR
    - Day -14: Subjects without previous ACR of ≥ A2 and/or ≥B2R (see section 7.2.3 for further details) will receive a single dose of Alemtuzumab
- Day -3 to -1: ATGAM
- Day -2: Thiotepa
- Day -1: TBI 250 cGy (200cGy for radiosensitive subjects) with lung shielding. Lung shielding will not be completed for subjects with previous ACR of ≥ A2 and/or ≥B2R. Additional organ shielding for fertility preservation will be allowed based upon the investigator's discretion.

Figure 1. Study Design



3.2 Primary Endpoints

3.2.1 Safety Endpoint

Safety will be determined by assessing the presence or absence of the following adverse events during the study period (up to 2 years post-BMT):

- Death
- Engraftment syndrome
- Engraftment failure
- Grade 4 or 5 events potentially attributable to Rituximab

3.2.2 Efficacy Endpoints (all subjects who received both BOLT and BMT)

- BOS at 1-year post BOLT; supplemental oxygen requirement and/or ventilatory support (noninvasive/invasive) at 1-year post BOLT.
- ≥ 25% donor T-cell chimerism at 12 months post BMT.
- For subjects with myeloid disorders (e.g. CGD), attaining ≥ 10% myeloid chimerism at 12 months post BMT.
- For subjects with B-cell disorders, attaining ≥ 10% B-cell chimerism at 12 months post BMT.

3.3 Secondary Endpoints (all enrolled subjects, as applicable)

The following secondary endpoints will be assessed:

- Feasibility of proceeding to BMT within 6 months following lung transplantation.
- Development of tolerance to both the host and pulmonary graft.
- Long-term complications of combined solid organ and BMT.
- Incidence of acute cellular rejection and graft failure post BMT.
- Incidence of acute and chronic graft-versus-host disease (GVHD) following tandem lung and BMT.
- Ability to initiate withdrawal of immunosuppression by 1 year following BMT.
- Time from BMT to withdrawal of immunosuppression.
- Time from BMT to independence from treatment dose antimicrobial drugs.
- For T cell lymphopenias, achieving age adjusted, low limit normal range lymphocyte count by 1-year post-BMT.
- A significant development in chronic lung allograft dysfunction (as evidenced by a change in BOS stage, see pg. 19 for BOS table) or allograft failure at 1-year and up to 2 years post lung transplant for BOLT alone and BOLT-BMT subjects.
- Incidence of Grade 4 or 5 adverse events possibly related to the use of Rituximab prior to the start of BMT conditioning.

3.4 Mechanistic Endpoints

The following mechanistic endpoints will be evaluated:

- The pace of immune reconstitution as well as the incidence and degree of mixed donor chimerism will be evaluated.
- The incidence of mixed chimerism (>5% host cells) at Months 1, 3, 6, 12- and 24-months post BMT.
- Improvement in immunological marker(s) unique for the underlying PID syndrome by one-year post BMT (see Appendix 5).

3.5 Stratification, Randomization, and Blinding/Masking

This is an open label study and there will be no randomization or blinding as a part of this study.

4 Selection of Participants and Clinical Sites/Laboratories

4.1 Rationale for Study Population

Patients between the ages of 10 and 45 years of age, inclusive, with a primary immunodeficiency and end-stage lung disease without major toxicity to other organs or uncontrolled infections will be eligible for tandem BOLT and BMT from a ≥2/6 HLA-matched cadaveric organ donor with an identical ABO blood type as the recipient. Lung transplant prior to BMT (in patients with PID) would allow for restoration of pulmonary function prior to bone marrow transplant, decreasing the risk of the overall bone marrow transplant procedure, which is curative for the patient's underlying immunodeficiency.

4.2 Eligibility for Study Entry and Lung Transplant

4.2.1 Inclusion Criteria

Individuals must meet all of the following criteria in order to be eligible for this study.

1. Subject and/or parent guardian must be able to understand and provide informed consent.
2. Male or female, 10 through 45 years old, inclusive, at the time of informed consent.
3. Meet criteria for UNOS listing.
4. Patients must have evidence of an underlying primary immunodeficiency for which BMT is clinically indicated.

   Examples of such diseases include, but are not limited to:
   - Severe Combined Immunodeficiency
   - Combined immunodeficiency with defects in T-cell-mediated immunity, including Omenn syndrome and DiGeorge Syndrome
   - Severe Chronic Neutropenia
   - Chronic Granulomatous Disease
   - Hyper IgE Syndrome or Job Syndrome
   - CD40 or CD40L deficiency
   - Wiskott-Aldrich Syndrome
   - Mendelian Susceptibility to Mycobacterial Disease [6]
   - GATA2 Associated Immunodeficiency

   NOTE: A genetic diagnosis is recommended, but not required.
5. Patients must have evidence of end-stage lung disease and be candidates for bilateral orthotopic lung transplant as determined by the lung transplant team.
6. GFR ≥50 mL/min/1.73 m2.
7. AST, ALT ≤4x upper limit of normal, total bilirubin ≤ 2.5 mg/dL, normal INR.
8. Cardiac ejection fraction ≥ 40% or shortening fraction ≥26%.
9. Negative pregnancy test for females >10 years old or who have reached menarche, unless surgically sterilized.

10. All females of childbearing potential and sexually active males must agree to use a FDA approved method of birth control for up to 24 months after BMT or for as long as they are taking any medication that may harm a pregnancy, an unborn child or may cause birth defect.
11. Subject and/or parent guardian will also be counseled regarding the potential risks of infertility following BMT and advised to discuss sperm banking or oocyte harvesting.

4.2.2 Exclusion Criteria

Individuals who meet any of these criteria are not eligible for this study.

1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol.
2. Patients who have underlying malignant conditions.
3. Patients who have non-malignant conditions not requiring hematopoietic stem cell transplantation.
4. HIV positive by serology or PCR, HTLV positive by serology.
5. Females who are pregnant or who are lactating.
6. Allergy to DMSO or any other ingredient used in the manufacturing of the stem cell product.
7. Uncontrolled pulmonary infection, as determined by radiographic findings and/or significant clinical deterioration. NOTE: Pulmonary colonization with multiple organisms is common and will not be considered an exclusion criterion.
8. Uncontrolled systemic infection, as determined by the appropriate confirmatory testing e.g. blood cultures, PCR testing, etc.
9. Recent recipient of any licensed or investigational live attenuated vaccine(s) within 4 weeks of transplant.
10. 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.

4.2.3 Donor Eligibility

Donor must be a ≥2/6 (A, B, DR) HLA-matched cadaveric organ donor with an identical ABO blood type as the recipient. All donors will be screened and confirmed negative for COVID-19.

4.3 Eligibility for Bone Marrow Transplant

The decision to proceed with the BMT will be at the discretion of the lung transplant team following clearance by the bone marrow team based on the criteria below. The conditioning for the BMT will begin no less than 8 weeks following the lung transplant.

1. GFR >50 mL/min/1.73 m^2.
2. AST, ALT <4x upper limit of normal, Total bilirubin < 2.5 mg/dL.
3. Cardiac ejection fraction ≥ 40% or shortening fraction of at least 26%.
4. HIV negative by serology and PCR.
5. HTLV serology negative.

6. FVC and FEV1 ≥40% predicted for age and SpO2 of >90% at rest on room air AND with clearance by the lung transplant team.
7. Absence of uncontrolled infection as determined by positive blood cultures and radiographic progression of previous sites in particular pulmonary densities during the past 2 weeks prior to chemotherapy.
8. Absence of clinically significant acute cellular rejection (most recent evaluation <A2-A4 and < B2R rejection; see section 6.3.1).
9. Bone marrow processing (section 6.1.2) has been completed, and an appropriate stem cell product is available for administration (section 6.1.3).
10. Any medical issue or finding 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 Selection of Clinical Sites/Labs

Children's Hospital of Pittsburgh (CHP) of UPMC and Presbyterian Hospital of UPMC are world-renowned for lung transplantation. Adult patients will undergo BOLT at Presbyterian Hospital of UPMC while children will receive BOLT at CHP of UPMC as it is standard practice at both institutions. All subjects will receive the BMT at CHP of UPMC regardless of recipient age. CHP of UPMC has rich clinical experience in managing medically challenging adult patients in the Adult Congenital Heart Disease (ACHD) Center. Moreover, clinicians from each hospital will be able to follow enrolled subjects at all times due to the integrated health care system of the University of Pittsburgh Medical Center.

5 Known and Potential Risks and Benefits to Participants

5.1 Risks of CD3+ and CD19+ Depleted BMT

The risks associated with a CD3+ and CD19+ depleted BMT are similar to a standard BMT and include bone marrow depression, graft failure, Graft Versus Host Disease, Veno-Occlusive disease of the liver, interstitial pneumonia, disease recurrence, malignancy, central nervous system damage, serious infection(s), organ damage, and genetic disease transmission. Due to the HLA mismatch, the risk of graft failure may be slightly higher with this procedure than the standard BMT. Conversely, the risk of GVHD is expected to be lower with the CD3+ and CD19+ depletion. There could also be unforeseen risks that have not yet been identified.

5.2 Risks of Cryopreserved Donor Leukocyte Infusion (DLI)

The DLI is from non T-cell depleted fraction of cadaveric bone marrow and may be administered in subjects who experience a significant viral infection, a decline in donor chimerism, or show no evidence of CD3+ reconstitution after day 30. There is a risk of transmitted disease and/or infection, Graft Versus Host Disease, bone marrow depression, graft failure and engraftment syndrome as well as adverse effects related to the DMSO used for cryopreservation.

5.3 Risks of Granulocyte Infusion

Granulocytes (white blood cells) are a part of the immune system and aid in fighting bacterial and fungal infections.

Granulocytes are not protocol mandated but may be administered in subjects following BMT who experience severe infections, have been unresponsive to antibiotics, and/or neutropenia. Granulocytes are a white cell concentrate collected by donor apheresis.

There are risks associated with the transfusion of granulocytes. Mild reactions include rash, urticaria, fever, chills and headache. Less common reactions include respiratory distress or lung injury, exposure to blood borne microorganisms (bacteria, parasites) that could result in infection, possible effects on the immune system which may decrease the body's ability to fight infection, exposure to blood borne viruses such as Hepatitis B and shock. Extremely rare side effects are exposure to blood borne viruses such as Hepatitis C and Human Immunodeficiency Virus (HIV), cardiac events such as myocardial infarction, and death.

5.4 Risks of Other Protocol Specified Medications

5.4.1 Alemtuzumab® (Campath-1H®)

Alemtuzumab is an antibody against CD52, a non-modulating antigen present on the surface of essentially all T and B lymphocytes, the majority of monocytes, macrophages, and NK cells, and a subpopulation of granulocytes. The mechanism of action is expected to the antibody-dependent lysis following cell surface binding. Alemtuzumab binding was seen in lymphocyte tissues and the mononuclear phagocyte system. A proportion of bone marrow cells, including some CD34+ cells express variable levels of CD52.

Adverse Events: Cardiovascular: Chest pain, hyper-/hypotension, peripheral edema, tachycardia/SVT; Central nervous system: Depression, dizziness, drug-related fever, dysthesias, fatigue, headache, insomnia, malaise, neutropenic fever, somnolence, temperature change sensation; Dermatologic: Pruritus, purpura, rash, urticarial; Gastrointestinal: Abdominal pain, anorexia, constipation, diarrhea, dyspepsia, nausea, stomatitis/mucositis, vomiting; Hematologic: Autoimmune hemolytic anemia, autoimmune thrombocytopenia, lymphopenia, pancytopenia/marrow hypoplasia, positive Coombs' test without hemolysis, severe anemia, severe neutropenia, severe thrombocytopenia; Neuromuscular & skeletal: Back pain, myalgia, rigors, skeletal muscle pain, tremor, weakness; Respiratory: Bronchitis/pneumonitis, bronchospasm, cough, dyspnea, epistaxis, pharyngitis, rhinitis; Miscellaneous: Diaphoresis, infection (including sepsis, pneumonia, opportunistic infection; received PCP pneumonia and herpes prophylaxis);

Rare but important or life-threatening: Acidosis, acute renal failure, agranulocytosis, anaphylactoid reactions, angina pectoris, angioedema, anuria, ascites, asthma, bone marrow aplasia, cardiac arrest, cardiac failure, cerebral hemorrhage, coma, deep vein thrombosis, disseminated intravascular coagulation, gastrointestinal hemorrhage, hemolytic anemia, hemoptysis, hepatic failure, hyperthyroidism, hypoxia, interstitial pneumonitis, intestinal perforation, intracranial hemorrhage, malignant lymphoma, marrow depression, meningitis, MI, pancreatitis, paralysis, peptic ulcer, pericarditis, peritonitis, pneumothorax, polymyositis, progressive multifocal leukoencephalopathy, pseudomembranous colitis, pulmonary edema, pulmonary embolism, pulmonary fibrosis, renal dysfunction, respiratory alkalosis, respiratory depression, secondary leukemia, seizure (grand mal), splenic infarction, stridor, subarachnoid hemorrhage, syncope, toxic nephropathy, transformation to aggressive lymphoma, transformation to prolymphocytic leukemia, thrombocythemia, thrombophlebitis, ventricular arrhythmia, ventricular tachycardia

5.4.2 Anti-thymocyte Globulin, Horse (ATG, Atgam®)

ATG is a purified preparation of horse gamma-globulin which contains high concentrations of antibodies against human lymphocytes. The preparation may contain low levels of antibody which cross-react with human platelets, white cells, or red cells.

Adverse Events associated with ATG include: Cardiovascular: Bradycardia, cardiac irregularity, chest pain, edema, heart failure, hyper-/hypotension, myocarditis; Central nervous system: Agitation, chills, fever, headache, lethargy, lightheadedness, listlessness, seizure, viral encephalopathy; Dermatologic: Pruritus, rash, urticarial; Gastrointestinal: Diarrhea, nausea, stomatitis, vomiting; Hematologic: Leukopenia, thrombocytopenia; Hepatic: Hepatosplenomegaly, liver function tests abnormal; Local: Burning soles/palms, injection site reactions (pain, redness, swelling), phlebitis, thrombophlebitis; Neuromuscular & skeletal: Aches, arthralgia, back pain, joint stiffness, myalgia; Ocular: Periorbital edema; Renal: Proteinuria, renal function tests abnormal; Respiratory: Dyspnea, pleural effusion, respiratory distress; Miscellaneous: Anaphylactic reaction, diaphoresis, lymphadenopathy, night sweats, serum sickness, viral infection; Rare but important or life-threatening: Abdominal pain, acute renal failure, anaphylactoid reaction, anemia, aplasia, apnea, confusion, cough, deep vein thrombosis, disorientation, dizziness, eosinophilia, epigastric pain, epistaxis, erythema, faintness, flank pain, GI bleeding, GI perforation, granulocytopenia, hemolysis, hemolytic anemia, herpes simplex reactivation, hiccups, hyperglycemia, iliac vein obstruction, infection, involuntary movement, kidney enlarged/ruptured, laryngospasm, malaise,

neutropenia, pancytopenia, paresthesia, pulmonary edema, renal artery thrombosis, rigidity, sore mouth/throat, tachycardia, toxic epidermal necrosis, tremor, vasculitis, viral hepatitis, weakness, wound dehiscence

5.4.3 Hydroxyurea (HU, Hydrea®)

Hydroxyurea is a synthesized agent, which causes inhibition of ribonucleotide reductase. It is phase-specific, with its lethal effect in S phase. It readily passes the blood-brain barrier, achieving peak CSF levels at 3 hours. About 50% is degraded in the liver and excreted in the urine as urea and as respiratory carbon dioxide.

Adverse Events associated with Hydroxyurea include: Cardiovascular: Edema; Central nervous system: Chills, disorientation, dizziness, drowsiness (dose-related), fever, hallucinations, headache, malaise, seizure; Dermatologic: Alopecia, cutaneous vasculitic toxicities, dermatomyositis-like skin changes, facial erythema, gangrene, hyperpigmentation, maculopapular rash, nail atrophy, nail discoloration, peripheral erythema, scaling, skin atrophy, skin cancer, skin ulcer, vasculitis ulcerations, violet papules; Endocrine & metabolic: Hyperuricemia; Gastrointestinal: Anorexia, constipation, diarrhea, gastrointestinal irritation and mucositis, (potentiated with radiation therapy), nausea, pancreatitis, stomatitis, vomiting; Genitourinary: Dysuria; Hematologic: Myelosuppression (anemia, leukopenia/neutropenia [common], thrombocytopenia; hematologic recovery: within 2 weeks); macrocytosis, megaloblastic erythropoiesis, secondary leukemias (long-term use); Hepatic: Hepatic enzymes increased, hepatotoxicity; Neuromuscular & skeletal: Peripheral neuropathy, weakness; Renal: BUN increased, creatinine increased, renal tubular dysfunction; Respiratory: Acute diffuse pulmonary infiltrates (rare), dyspnea, pulmonary fibrosis (rare) Note: renal impairment enhances toxicity of the drug.

5.4.4 Rituximab (Rituxan®)

Rituximab is a genetically engineered chimeric murine/human monoclonal antibody, which binds specifically to the antigen C20 (human B lymphocytes restricted differentiation antigen, Bp35) located on the surface of pre B and mature B lymphocytes of both normal and malignant cells. The antibody is an IfF1 kappa immunoglobulin containing murine light- and heave-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 and has an approximate molecular weight of 145 kD. It is produced in mammalian cell (Chinese Hamster Ovary) culture.

Adverse Events associated with Rituximab include: Cardiovascular: Flushing, hyper-/hypotension, peripheral edema; Central nervous system: Anxiety, chills, dizziness, fatigue, fever, headache, insomnia, migraine, pain; Dermatologic: Angioedema, pruritus, rash, urticarial; Endocrine & metabolic: Hyperglycemia; Gastrointestinal: Abdominal pain, diarrhea, dyspepsia, nausea, vomiting, weight gain; Hematologic: Anemia, cytopenia, lymphopenia, leukopenia, neutropenia, neutropenic fever, thrombocytopenia; Hepatic: ALT increased; Neuromuscular & skeletal: Arthralgia, back pain, muscle spasm, myalgia, neuropathy, paresthesia, weakness; Respiratory: Bronchospasm, cough, dyspnea, epistaxis, rhinitis, sinusitis, throat irritation, upper respiratory tract infection; Miscellaneous: Infusion-related reactions (may include angioedema, bronchospasm, chills, dizziness, fever, headache, hyper-/hypotension, myalgia, nausea, pruritus, rash, rigors, urticaria, and vomiting); infection (including bacterial, viral, fungal); night sweats; human antichimeric antibody (HACA) positive; Rare but important

or life-threatening: Acute renal failure, anaphylactoid reaction/anaphylaxis, angina, aplastic anemia, ARDS, arrhythmia, bowel obstruction/perforation, bronchiolitis obliterans, cardiac failure, cardiogenic shock, encephalomyelitis, fatal infusion-related reactions, fulminant hepatitis, gastrointestinal perforation, hemolytic anemia, hepatic failure, hepatitis, hepatitis B reactivation, hyperviscosity syndrome (in Waldenström's macroglobulinemia), hypogammaglobulinemia (prolonged), hypoxia, interstitial pneumonitis, laryngeal edema, lichenoid dermatitis, lupus-like syndrome, marrow hypoplasia, MI, mucositis, mucocutaneous reaction, neutropenia (late-onset occurring >40 days after last dose), optic neuritis, pancytopenia (prolonged), paraneoplastic pemphigus (uncommon), pleuritis, pneumonia, pneumonitis, polyarticular arthritis, polymyositis, posterior reversible encephalopathy syndrome (PRES), progressive multifocal leukoencephalopathy (PML), pure red cell aplasia, renal toxicity, reversible posterior leukoencephalopathy syndrome (RPLS), serum sickness, Stevens-Johnson syndrome, supraventricular arrhythmia, systemic vasculitis, toxic epidermal necrolysis, tuberculosis reactivation, tumor lysis syndrome, uveitis, vasculitis with rash, ventricular fibrillation, ventricular tachycardia, vesiculobullous dermatitis, viral reactivation (includes JC virus, cytomegalovirus, herpes simplex virus, parvovirus B19, varicella zoster virus, West Nile virus, and hepatitis C), wheezing.

5.4.5 Tacrolimus (ProGraf®)

Tacrolimus is a macrolide immunosuppressant produced by *Streptomyces tsukubaensis*. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12, inhibiting the phosphatase activity of calcineurin and resulting in the inhibition of T-lymphocyte activation (immunosuppression). The plasma protein binding of tacrolimus is approximately 99% and is independent of concentration over a range of 5-50 ng/mL. Tacrolimus is bound mainly to albumin and alpha-1-acid glycoprotein, and has a high level of association with erythrocytes. The $T_{½}$ in adult patients ranges from 11-19 hours. Following oral administration, the absolute bioavailability was 31 ± 21%. Tacrolimus is extensively metabolized by the mixed-function oxidase system, primarily the cytochrome P-450 system (CYP3A) in the liver and to a lesser extent in the intestinal mucosa. The main route of elimination is via the biliary tract and excretion in feces. Administration with food significantly decreases the rate and extent of absorption Drugs that stimulate or inhibit hepatic P450 enzymes will alter clearance of tacrolimus and close attention to potential drug interactions is crucial.

Adverse Events associated with Tacrolimus include hypertention, nausea, vomiting, immunosuppression, tremor, acute and chronic kidney injury, hypomagnesemia, hypo/hyperkalemia, seizure, confusion, somnolence, leukopenia, anemia, thrombocytopenia.

5.4.6 Thiotepa

Thiotepa is a cytotoxic agent of the polyfunctional type, related chemically and pharmacologically to nitrogen mustard. The radiomimetic action of thiotepa is believed to occur through the release of ethylenimine radicals which, like irradiation, disrupt the bonds of DNA. One of the principal bond disruptions is initiated by alkylation of guanine at the N-7 position, which severs the linkage between the purine base and the sugar and liberates alkylated guanines. Thiotepa is desulfurated by cytochrome P-450 enzymes such as 2B1 and 2C11 which catalyze the conversion of thiotepa to tepa. Tepa is less toxic than thiotepa and has been demonstrated to produce alkali-labile sites in DNA, rather than cross-links. These findings indicate that tepa reacts differently from thiotepa and produces monofunctional alkylation of

DNA. A second metabolite of thiotepa, a mercapturic acid conjugate, is formed via glutathione conjugation. Monochlorotepa is the third metabolite found in the urine. Following short intravenous infusion (less than 5 minutes), peak concentrations of thiotepa were measured within 5 minutes. At steady state, the volume of distribution was independent of dose and ranged from 0.3 to 1.6 liters per kilogram (L/kg). Approximately 4.2% of the original dose is eliminated in the urine within 24 hours as tepa. The elimination half-life of thiotepa ranges from 2.3 to 2.4 hours. The half-life of tepa ranged from 3 to 21.1 hours in one study.

Adverse events associated with Thiotepa include: Central nervous system: Chills, dizziness, fatigue, fever, headache; Dermatologic: Alopecia, contact dermatitis, depigmentation (with topical treatment), dermatitis, rash, urticaria; Endocrine & metabolic: Amenorrhea, spermatogenesis inhibition; Gastrointestinal: Abdominal pain, anorexia, nausea, vomiting; Genitourinary: Dysuria, urinary retention; Hematologic: Anemia, bleeding, leukopenia, thrombocytopenia; Local: Injection site pain; Neuromuscular & skeletal: Weakness; Ocular: Blurred vision, conjunctivitis; Renal: Hematuria; Respiratory: Asthma, epistaxis, laryngeal edema, wheezing; Miscellaneous: Allergic reaction, anaphylactic shock, infection; Rare but important or life-threatening: Acute myeloid leukemia (AML), chemical cystitis (bladder instillation), hemorrhagic cystitis (bladder instillation), myelodysplastic syndrome

5.5 Risks of Study Procedures

5.5.1 Blood Draw

Risks of blood draw or venipuncture are typically minimal with temporary local discomfort. More serious risks would include ecchymosis and, rarely, localized infection. The amount of blood that may be drawn from adult subjects for research purposes will not be more than 550 mL over an eight-week period. For pediatric patients, no more than 3 mL/kg may be drawn in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period. The additional amount of blood could contribute to the development of anemia. The subject's clinical condition will be taken into consideration to determine if research blood tests can be performed.

5.5.2 Bronchoscopy and Bronchoalveolar Lavage

Bronchoscopies will be performed, and research samples obtained, unless medically contraindicated, as part of routine post-transplant care. Study participants will not undergo research bronchoscopies, therefore the risks detailed below will be incurred regardless of study participation. Study specific risk is limited to the collection of the bronchial brushing and the research BAL sample.

The most common risks associated with bronchoscopy include minor bleeding, limited to the area of the bronchoscope and occurring during the procedure, sore throat, cough, fever, fatigue, and/or mild hoarseness. These are infrequent and usually disappear by the next day.

5.5.3 Bone Marrow Aspirate

A bone marrow aspirate will be performed prior to BMT in order to obtain a sample for research purposes. This test may be painful. There is also a small risk of infection or bleeding. The pain normally lessens within minutes to hours.

5.6 Potential Benefits

This protocol may provide no direct benefit. If the lung transplant and bone marrow transplant is successful, the subject's lung disease and immune deficiency may improve. The results of this study could influence the future care of transplanted patients.

6 Investigational Intervention

6.1 Cadaveric Bone Marrow Harvest and Preparation of CD3+/ CD19+ Hematopoietic Stem Cells

6.1.1 Bone Marrow Harvest

- Bone marrow will be harvested using standard bone marrow harvest needles from the anterior and posterior iliac crests. The local OPO will be contacted in advance and their verbal assent will be obtained notifying them that the bone marrow harvest team will arrive together with the lung procurement team from the University of Pittsburgh early so bone marrow harvest can be performed with external approach prior to the harvesting of other organs and while the heart is beating. The goal is to harvest between 10 and 25 mL/kg of donor weight (up to 40ml/kg recipient weight) of marrow prior to removal of internal organs.
- If sufficient bone marrow is harvested from the donor (>3.0 x 10^7 MNC/kg body weight), a reserve up to 5% may be frozen and stored as a safety measure should the recipient have an issue with engraftment or other complication(s) that necessitates a donor leukocyte infusion (DLI).
- Vertebral bodies will be removed for processing and stem cell extraction in the Stem Cell Laboratory at the Children's Hospital of Pittsburgh and the processed product will be saved as an adjunct stem cell unit as described by Donnenberg et al [24,25]. An internal approach may also be utilized for marrow harvest from the iliac crests after removal of internal pelvic organs.
- Marrow and cells will be suspended in RPMI with heparin [26].
- Bone marrow from the iliac crests and vertebral bodies will be independently collected, accessioned, stored, and transported.

6.1.2 Bone Marrow Processing

- Bone marrow from the iliac crest and the vertebral bodies will be independently processed and cryopreserved. Both products will undergo independent evaluation for release criteria.
- Bone marrow obtained from iliac crest and vertebral bodies will be depleted of CD3+ and CD19+ cells using the CliniMACS® device (Miltenyi Biotech) described in the institutional standard operating procedures (SOP).
- The CD3+/CD19+ depleted bone marrow cells will be tested for viability (TNC count), Potency (CD34+ cell count, CD3+ cell count), sterility and endotoxin level prior to cryopreservation.
- The CD34+ count and CD3+ count in the negatively selected products prior to cryopreservation will be used to calculate the total CD 34+ and CD3+ cells to be administered (see 6.1.3. BMT dosage and administration).
- Stem cell products will be cryopreserved per standard CHP stem cell lab procedure.

A small percentage of grafts, from the iliac crests or vertebral bodies, may be positive for contamination. If the graft release testing shows microbial contamination, the sterility results will be reviewed by the

Department of Infectious Diseases (ID) faculty. The Investigators will follow the recommendations of the ID faculty regarding the clinically appropriate prophylaxis or treatment of the subject that is necessary to continue with the BMT and this will be documented in the medical record. This is standard clinical practice within the Division of Bone Marrow Transplant and Cellular Therapies (BMTCT).

6.1.2 BMT Dosage and Administration

In order to proceed to conditioning for BMT, the following criteria should be met:

a. Target CD34+: ≥1x106 cells/kg

b. Target CD3+: <1x10^5 cells/kg

c. Target total nucleated cells (TNC) viability: ≥70%

d. Negative for all tested adventitious agents

e. Negative bacteria for 14-day culture

f. Endotoxin level: < 5 EU/kg body weight/hour of administration

Stem cell product will be used from the iliac crests and/or vertebral bodies unless the cellular product obtained from the vertebral bodies would result in failure to meet release criteria, or if the use of the cellular product from the vertebral bodies would result in exceeding the maximum allowable threshold for CD3+ cells. The cellular products will be infused separately and may be infused in any order however there should be approximately 2 hours between infusions.

Freshly thawed CD3 and CD19 negatively selected cadaveric hematopoietic stem cell product (bone marrow from iliac crests and/or vertebral bodies) will be diluted (3-4 fold) with low molecular weight dextran in sodium chloride with added human albumin and infused on day 0 of the treatment regimen via central line, per institutional guidelines. There will be no processing of the cells after thawing.

6.1.3 Cryopreserved Donor Leukocyte Infusion (DLI)

The source of DLI may be from either the iliac or vertebral bone marrow.

Donor Leukocyte infusion (DLI) from non-T cell depleted fraction of cadaveric bone marrow will be considered for those patients who demonstrate:

a) No evidence of CD3+ T cell reconstitution after day 30 post-BMT (10 CD3+ T cells per microliter as cut off value), or

b) Experience significant decline in donor chimerism (with a drop of at least 10% in myeloid or T cell fractions from the previous or peak value), or

c) Significant viral or other opportunistic infection where antigen specific DLI is not available.

Exclusion criteria for DLI: Any stage of visceral GVHD or Stage 2 or higher skin GVHD is an absolute contraindication for DLI. Previous receipt of DLI is not an exclusion.

6.2 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 CD3+ CD19+ depleted bone marrow, including the date and quantity of the biologic received, to whom the biologic was dispensed (participant-by-participant accounting), and a detailed accounting of any biologic that is accidentally or deliberately destroyed.

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

All records regarding the disposition of the investigational product will be available for inspection. Unused product will be de-identified and stored. They may be utilized for laboratory studies.

6.3 Toxicity Prevention and Management

6.3.1 Acute Cellular Rejection (ACR) Prior to the BMT

If the subject experiences ACR prior to receiving the BMT, the rejection episode must resolve to a pathology of <A2-A4 and <B2R prior to proceeding to the BMT [41]. ACR is treated per the institutional standard of care, which typically includes a depletion agent. Once the follow-up biopsy criteria have been met, the lung transplant team will assess the stability of the subject to determine BMT eligibility.

6.4 Premature Discontinuation of Investigational Agent

6.4.1 Premature Discontinuation of the BMT

The infusion of hematopoietic stem cells may be discontinued if the subject has a hypersensitivity reaction, any infusion related serious adverse event, or any other medical condition that, in the opinion of the investigator, would preclude continued participation. If the subject received any portion of the infusion, he/she will be followed per the main Schedule of Events outlined in Appendix 2.

7 Protocol Mandated Medications

7.1 Lung Transplant Immunosuppression

7.1.1 Induction Therapy

Basiliximab is the typical induction therapy and will be administered per the package insert and adjusted for age, if this is used for induction. Other induction therapies such as Alemtuzumab may be considered if there is justification based on the subject's underlying disease. This will be at the discretion of the lung transplant physician.

7.1.2 Maintenance Immunosuppression

In addition to the Tacrolimus outlined below, subjects may be on concurrent maintenance immunosuppressive medications post BOLT and subsequently post BMT as clinically indicated. This may include mycophenolate mofetil or mycophenolic acid as well as prednisone.

7.1.2.1 Tacrolimus

Tacrolimus will be started at the time of lung transplant and will be continued without interruption through the BMT conditioning and post-transplant period. Dose adjustment for twice daily dosing will aim for tacrolimus trough levels between 8-12 ng/ml if tolerated. Dose adjustments may be made on the basis of toxicity. When the subject is admitted on day -4 prior to BMT, tacrolimus may be administered IV to maintain a steady state of 12-18ng/ml or alternately, the subject may remain on oral tacrolimus at the discretion of the Investigator.

An alternate maintenance immunosuppressive medication(s) may be considered if the subject experiences a clinical complication or toxicity.

7.1.2.2 Rituximab

A single dose of rituximab will be administered just prior to the lung transplant or up to 48 hours post-transplant at a dose of 375 mg/m2. Then, as clinically indicated if there are signs of B cell recovery (≥ 10 cells/μL). Rituximab may be held as long as the subject has no B cells (< 10 cells/μL). Pre-medications for Rituximab will follow the institutional standard of care.

7.2 Conditioning Regimen for BMT

7.2.1 Hydroxyurea

Hydroxyurea will be given orally at a single daily dose of 30 mg/kg, rounded to the nearest pill size, starting on day -28 and continuing through day -3. Filgrastim at 2-5 mcg/kg dose will be administered as needed every 2-3 days if the ANC is <1500 cells/μL and/or for any other signs of bone marrow suppression. Hydroxyurea will be held for ANC <500 cells/μL and resumed when ANC >1000 cells/μL.

7.2.2 Rituximab

In preparation for BMT, Rituximab will be administered on day -28 at a dose of 375 mg/m^2 and then again at day +35 to prevent the development of PTLD in the setting of a T cell depleted graft. Pre-medications may be given as outlined above in Section 7.1.2.2.

7.2.3 Alemtuzumab

One dose of Alemtuzumab will be given IV at a dose of 0.5 mg/kg on day -14 to subjects without previous ACR. Subjects with previous ACR of ≥A2-A4 and/or ≥B2R will receive two doses of Alemtuzumab on Days -21 and -20 at 0.5mg/kg. Pre-medications for Alemtuzumab will follow the institutional standard of care.

7.2.4 Anti-thymocyte Globulin, Horse (ATG, Atgam®)

ATG (ATGAM) will be given IV on days -3 to -1 at a dose of 30mg/kg. Pre-medications for ATG will follow the institutional standard of care.

7.2.5 Thiotepa

Thiotepa will be given IV at a dose of 250 mg/m^2 diluted to a final concentration of 2 mg/mL in 0.9% sodium chloride given over 2 hours x 1 dose on day -2.

7.2.6 Total Body Irradiation (TBI)

TBI at 250 cGy (200cGy for subjects with a radiation sensitive condition) with lung shielding will be given on day -1; liver shielding will not be utilized.

Lung shielding will not be utilized for subjects with previous ACR of ≥ A2 and/or ≥B2R; this is intended to mitigate the potential effects of prior alloimmunity. Additional organ shielding e.g. gonadal shielding only for fertility preservation will be allowed based upon the investigator's discretion.

7.3 Growth Factor Support

Subjects will receive G-CSF 5 mcg/kg/day IV from day +1 until engrafted and then tapered according to institutional practice or as clinically indicated. Dose adjustment is permitted as clinically indicated.

7.4 Infection Prophylaxis

Infection prophylaxis follows the institutional standard of care at UPMC.

7.4.1 Pneumocystis jirovecii (PCP)

Trimethoprim/sulfamethoxazole will be initiated within the first approximately 2-3 weeks following lung transplant and continue until day -2 pre-BMT. It will resume post BMT at 3 days/week once ANC > 2000 and platelets > 50,000 and continue until 6 months post BMT or until off all immunosuppression, whichever comes later. Pentamidine, at 4 mg/kg IV, will be given every 3 weeks starting on Day 28 and continue until patients are able to take trimethoprim/ sulfamethoxazole. If a subject in unable to tolerate these regimens, other regimens may be substituted at the discretion of the study physician.

7.4.2 Herpes Simplex Virus (HSV) and/or Varicella Zoster Virus (VZV)

Oral acyclovir or valacyclovir will be initiated following the lung transplant per institutional guidelines and continue for 3 months or longer as clinically indicated. Following BMT, Acyclovir prophylaxis at 250 mg/m2/dose IV every 12 hours will be given to any subject whose pre-transplant serologies or PCR were positive for Herpes Simplex Virus (HSV) or Varicella Zoster Virus (VZV). Acyclovir will be continued for at least 1-year post bone marrow transplant, or longer as clinically indicated. The subject will be transitioned to oral acyclovir or valacyclovir once they are able to tolerate oral medications. If subjects

are taking an antiviral for another indication (e.g. CMV therapy) that provides adequate coverage for HSV and VZV, additional prophylaxis is not necessary.

7.4.3 Fungal Prophylaxis

Subjects will be maintained on appropriate fungal prophylaxis following lung transplantation, and will remain on caspofungin, voriconazole, or another appropriate agent until the CD4+ T-cell count is >200 or longer as clinically indicated.

7.4.4 Toxoplasmosis

All patients should have toxoplasma serology evaluated prior to BMT. Seropositive patients should receive prophylaxis with trimethoprim/sulfamethoxazole (or an appropriate substitute) before and after transplantation per the institutional standard of care.

7.4.5 Cytomegalovirus (CMV)

All subjects should be monitored for reactivation or primary infection based on the current institutional standard of care from post lung transplant through day 100 post BMT. Following lung transplant, all subjects will be on CMV prophylaxis based on the UPMC lung transplant guidelines. All subjects at risk for CMV disease (e.g., either recipient or donor seropositive for CMV) will receive IV ganciclovir or oral valganciclovir daily during BMT conditioning regimen until Day-2 followed by Foscarnet or Cidofovir as clinically indicated. Once engraftment occurs, subjects may be switched back to ganciclovir or valganciclovir.

7.4.6 Epstein-Barr Virus (EBV) and Adenovirus Infections

All subjects should be monitored for reactivation or infection based on the current institutional standard of care. This includes testing post lung transplant through day 100 post BMT.

7.4.7 Intravenous Immunoglobulin (IVIG)

Following BMT, IVIG at 500mg/kg/dose should be given every 2 weeks until day +100, then every 4 weeks until functional B cell recovery (production of IgM and IgA) according to institutional practice post-transplant.

7.5 Veno-occlusive Disease Prophylaxis

All subjects will receive ursodiol for prophylaxis of veno-occlusive disease (VOD) around the time of BMT. Ursodiol will be given orally from day -2 to day +100. Ursodiol may be held for patients unable to take oral medications. Subjects meeting criteria for VOD (hyperbilirubinemia, painful hepatomegaly, ascites, and fluid retention) will be treated according to local institutional guidelines. Moderate to severe VOD may be treated with defibrotide.

7.6 Granulocyte Infusion(s)

Granulocyte support is not protocol mandated but may be administered at the discretion of the BMT physician and in accordance with local institutional guidelines. Subjects will receive pulse oximetry throughout the infusion and continuous cardiac monitoring for 30 minutes prior to the granulocyte infusion through 60 minutes following the granulocyte infusion.

7.7 Prohibited Medications

Prohibited medications for this protocol, except as specifically indicated in this protocol include:

- Live vaccines

7.8 Withdrawal of Immunosuppression Post-BMT

Withdrawal of tacrolimus or the current maintenance immunosuppression will be considered no less than 12 months post BOLT and at month 9 post BMT if all the following criteria are met.

- >5% donor T-cell chimerism
- No evidence of Grade 2 or > acute GVHD and/or extensive chronic GVHD in the previous 3 months
- No lung rejection (grade A2 or higher) in the previous 3 months
- At the time of immunosuppression wean initiation, FEV1 ≥ 0.9*FEV1 of the max FEV1 during the prior 90 days

If the subject meets the withdrawal criteria, immunosuppression may be tapered by approximately 10% weekly or as clinically indicated. During this time, subjects will continue to receive surveillance biopsies and chest x-rays as clinically indicated, with regular PFTs and blood tests in accordance with the post lung transplant standard of care to monitor for signs of allograft rejection at the discretion of the study physician.

Any subject who experiences lung allograft rejection during immunosuppression weaning or after immunosuppression withdrawal will have maintenance immunosuppression reinstated, the rejection will be treated, and maintenance immunosuppression will not be discontinued during the protocol mandated follow-up period.

8 Study Procedures

8.1 Enrollment

The research study will be explained in lay terms to each potential research subject. The potential subject will sign an informed consent form before undergoing any study procedures. The subject and/or guardians will meet with a physician to discuss the treatment recommendations and alternative treatment options. The risks of both the lung and bone marrow transplant will be outlined separately, including the risks of toxicities associated with the procedures as well as their post-transplant medical therapies. Consent will be obtained using forms approved by the University of Pittsburgh Institutional Review Board. If the potential subject and/or parent/guardian(s) are not able to come to CHP to have the informed consent discussion in person (i.e. out of state residents, too ill to travel, etc.), the discussion may be conducted via teleconference with all of the same members (subject, parent/guardian, physician, research team). Consent documents will be signed at both locations as appropriate and the subject and/or parent/guardian will send their original signed consent to the research team. The original signed consents will be combined and a copy will be sent back to the subject and/or parent/guardian.

8.2 Screening/Baseline Visit Pre-Lung Transplant

The initial screening visit (Visit L-1) takes place prior to the lung transplant and begins when the subject signs the informed consent until all eligibility criteria are available. During the screening period, study personnel will review the subject's medical record for past and current medical history, perform a physical exam and collect any necessary lab work/study procedures as outlined in the Schedule of Events (Appendix 1). The subject may have had certain assessments conducted as a part of their routine clinical care and these results may be used if they fall within an acceptable window as outlined in the Schedule of Events.

8.3 Screening/Baseline Visit Pre-BMT

Subjects who consent to the study and receive a lung transplant must meet specific eligibility criteria in order move onto the bone marrow transplant. Study assessments are outlined in the Schedule of Events (Appendix 2). In addition, all eligibility as outlined in Section 4.3 must be met. This involves clearance from the lung transplant team in order to move onto the BMT. Subjects who do not meet eligibility and/or are not cleared by the lung transplant team cannot move onto the next portion of the study and will be followed according to Appendix 1. This also pertains to subjects who cannot be transplanted due to an insufficient donor bone marrow harvest.

Subjects may begin BMT conditioning no less than 8 weeks post lung transplant. Ideally subjects will receive their BMT no later than 9 months post-transplant; however, subjects may be considered beyond this time-point with the approval of the BMT Team.

8.4 Study Visits or Study Assessments

8.4.1 Post-Lung Transplant Study Assessments

Following lung transplant, subjects will be followed based on the institutional standard of care. This includes a bronchoscopy every 2-6 months and as clinically indicated as well as frequent pulmonary function tests (see Appendix 1). Data from these procedures will be collected as a part of the study and specimens will be collected for research as outlined in Appendix 1.

8.4.2 Follow-up Visits Post Bone Marrow Transplant

The BMT conditioning may occur as early as 8 weeks post lung transplant and up to several months post lung transplant depending on when the subject has met all criteria and has been cleared by the lung transplant team. Once the subject has been cleared to receive the BMT, the conditioning regimen will begin (Appendix 3).

Following the BMT, the subject will have study visits at Days 7, 14, 21 and then visits at Months 1, 2, 3, 6, 9, 12, 18 & 24. Study visits align with the routine post-transplant standard of care and may include but are not limited to the following assessments (see Appendix 2).

- Physical exams
- Lab work
- Pulmonary function testing
- Bronchoscopies
- GVHD assessment
- Chimerism studies
- Adverse Events
- Concomitant medications
- Chest x-rays
- Echocardiograms/EKGs
- Research blood collection

8.5 Unscheduled Visits

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

8.6 Visit Windows

Study visits should take place within the time limits specified below: the designated visit windows for each scheduled visit are also indicated on the Schedule of Events.

BOLT Visit Windows

Time Post-Transplant	Visit # (BOLT Appendix 1)	Visit Window
Months 4-23	L-1, 1a, 1b, etc.	+/- 7 days of each bronchoscopy
Month 24	2	+/- 28 days

BMT Visit Windows

Time Post-Transplant	Visit # (BMT Appendix 2)	Visit Window
Days 7-21	1-3	+/- 3 days
Months 1-5	4-6	+/- 7 days
Months 6-11	7-8	+/- 21 days
Months 12-24	9-11	+/- 28 days

9 Mechanistic Assays

9.1 Chimerism

Chimerism will be assessed using PCR based STR assay from whole blood, CD33+ enriched myeloid cells, and CD3+ T-cells in the clinical HLA lab pre-BOLT and 3 months post BOLT. After BMT it will be tested at months 1, 3, 6, 12 and then yearly. Small research specimens from BAL and matched blood samples will be subjected to high-resolution melt profiling (HRM) PCR assays interrogating donor origin in leukocyte subsets. If feasible, FACS sorted CD123+/CD11c- plasmacytoid DC and CD4+/CD127-/CD49d-/CD25bright "T-regs" will be tested. When donor-recipient specific reagents are available, HLA-specific antibodies will interrogate for donor origin without the need for FAC sorting.

9.2 Global Cellular Immunity

Testing for global/systemic immunity will be performed from peripheral blood.

Lymphocyte subsets, including percentage and absolute numbers of CD3+, CD4+, CD8+, CD19+, and CD16/56+ cells along with NKT cells and gamma delta T cells.

Disease-specific assessments of immune function in patients with T-cell lymphopenia (SCID, CID in particular) normalization of CD4+, CD8+ T cell counts are indicative of successful BMT. Demonstration of IL-7 R alpha expressing T cells will be a donor-specific marker of immune correction in IL-7 R alpha null SCID. Similarly, activation induced CD40 ligand expression will demonstrate correction of CD40L deficiency, independent of the chimerism studies. FACS based neutrophil oxidative burst testing will demonstrate correction for patients with CGD and restored IL-17, IL-22 secretion in response to CD3/CD28 and microbial stimulation in patients with STAT3 deficient Job's syndrome. The presence of IgA in patients with CVID along with IVIG independence to maintain normal IgG levels are also specific markers.

B cell recovery: 8 color B cell FACS panel will monitor B cell reconstitution through appropriate developmental stages.

9.3 Thymic Function

9.3.1 T-cell Recovery

TREC assay, TCRVbeta, TCRVgamma/delta, BCR repertoire (all established, up and running in the Szabolcs Lab).

9.3.2 Flow Cytometry

Assessment of thymus function and memory, including 8 color flow cytometry for naive, central memory, effector memory phenotypes. Cell turnover in subsets will be measured by staining for intracellular markers of proliferation and apoptosis (Ki-67, activated Casp3) (all established, up and running in the Szabolcs Lab).

9.4 Pathogen Specific Immunity

Pathogen-specific immunity will be tested with microbial antigens that subjects have been exposed to prior to transplant. These may include but not necessarily limited to Aspergillus, Pseudomonas, Staphylococcus, Candida, adenovirus, influenza, RSV, and herpes viruses (HSV, CMV, and EBV). The proposed assays are:

- Cytokine flow cytometry (CFC) or Tetramer staining (McDyer Lab) to detect pathogen specific Th1, Th17 cells, provided lymphocyte numbers permit this assay. HLA-specific MoAbs will test for donor origin of these cells in cases of mixed T cell chimerism.
- Luminex based multiplex cytokine detection in microculture supernatants.
- ELISPOT assay to enumerate IFN-secreting antiviral cells if CFC or Tetramer staining not feasible
- Antigen specific humoral immunity will be tested once patients are off IVIG (~ 6-12 months after BMT) measuring titers before and after vaccination with DTaP and hepatitis B vaccines.
- Intracellular flow cytometry or Luminex/BioPlex cytokine detection will identify IL-17, IFN-γ, IL-22, TNF-α secreting cells in BAL, in response to Pseudomonas, Aspergillus, Candida, and other pathogens, previously encountered by enrolled subjects. At times of lymphopenia, BioPlex and ELISPOT will replace FACS assays.
- Viral/pathogen specific tetramer stain, CFC if applicable and feasible to detect memory responses in virus-specific CD4+ and CD8+ cells in both PBMC and BAL cells, as previously described. We will assess for the presence of ex-vivo multifunctional (T cells that produce IFN-γ, TNF-α, IL-2, granzyme B, CD107a (marker of cytotoxic degranulation) and at 6 days in conjunction with assessment of proliferation using CFSE-dilution. An inability to proliferate and produce multiple cytokines would warrant the evaluation for potential exhaustion or apoptosis of T cells, via PD-1, Fas or other mechanisms as previously published. T cell phenotyping (e.g. CD27, CD28, CD57) in conjunction with CMV class I tetramer (or other class I tetramers available) staining at days 0 and 6 (assessing proliferation) are also routine performed.

9.5 RNA Sequencing (bronchial brushings)

There are RNAseq approaches to measure T cell reconstitution in primate lung epithelium (both human and non-human) to assess the integrity of Th1, Th2, Th17, Th22 responses in lung using clinical bronchial brushings. Preliminary data in humans show that this analysis can also measure the presences of specific T cell receptor genes and T cell effector cytokines in the lung mucosa. Thus, this will be a robust way to measure immune reconstitution as well as resolution of frustrated hyper immune responses in the mucosa as has been shown to occur in CGD, moreover, as these patients undergo regularly scheduled bronchoscopy, we can assess mucosal immune reconstitution in serial samples.

9.6 Tolerance Assays

New assays with purified potent APC (monocyte-derived or CD34+ -derived dendritic cells from donor and host bone marrow) have been developed to establish very sensitive in vitro models capable to monitor for alterations in alloreactivity High resolution sequence based TCR Vbeta repertoire analysis will monitor for presence, kinetics, and quantity of alloreactive clones through serial time points that are

calendar driven and/or prompted by taper of immunosuppression. Additional assays will be prompted by new onset lung rejection or GVHD.

At fixed points, we will quantitate and characterize purified T cells, either from donor, host or both (in case of mixed T cell chimerism), alloreactivity in modified mixed lymphocyte cultures (MLC). Micro cultures of one-way MLC will be set up against host dendritic cells to study GVH reactions. In cases of mixed T cell chimerism in parallel microcultures T cells will be tested against donor APCs as well. Responders will be purified T cells from circulation or from BAL isolates if feasible.

We will analyze and compare the donor-host ratio of T cells, APCs in the circulation and also from BAL.

We will analyze the numbers, turnover, and donor-host origin of Tregs, quantitate the expression of FoxpP3 and Helios and will measure HLA-G expression intensity of circulating myeloid DC (mDC). HLA-G expression was found to correlate with FoxP3 expression in the tolerant subset of pediatric liver SOT recipients (46). HLA-specific antibodies should assist in the simultaneous identification of CD123+plasmacytoid DC, (pDC) and CD123-/CD11c+ mDC subsets along with their host-donor origins. Interestingly, in a CHP patient cohort, high pDC/mDC ratio has been observed to predict successful withdrawal of IST in liver transplant patients. We will test donor pDC/mDC ratio from donor blood obtained at the time of organ procurement and then serially to test for similar correlation with tolerance in the lung+bone marrow transplant setting.

Alloreactive T cell responses will be categorized as either pro-inflammatory based on (IFNγ, TNFα, IL-17, GM-CSF) or anti-inflammatory (IL-10, TGFbeta) against the allogeneic host-recipient target. The former profile has been associated with rejection and/or GVHD, while the latter has been described to promote active tolerance with no associated cytotoxicity between donor and recipient.

Supernatants will be analyzed by BioPlex for Th1/Tc1, Th2/Tc2, Th17, Tr1, and Treg signature cytokines.

In parallel, microcytoxicity assays (Europium labelled targets added to MLC) will examine cytolytic potential of the same population and correlate with the cytokine signature, as previously published.

When testing bulk populations, the cytokine profile and cytotoxicity assays will be performed with or without Treg depletion, triggered by IL-2-immunotoxin and with or without IL-10 receptor blockade to test for Tr1 cell activity.

9.7 Expression Profiling

From recipient PBMC drawn pre-BOLT, fractionated cells will be frozen for RNA analysis and unfractionated cells will be cryopreserved. Similarly, BOLT donor marrow MNC or PBMC will be fractionated into major lymph subsets (CD4+, CD8+, CD19+) and cryopreserved to make them accessible for future studies that may involve identification of gene signatures and immune pathways of tolerance/alloreactivity. Any remaining lymphocytes and DCs may be cryopreserved for gene expression profiling assays.

10 Biospecimen Storage

Biological specimens (i.e., whole blood, plasma, serum, tissue, BAL fluid and bone marrow) 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 central mechanistic studies, in order to allow specimens to be stored for use in new assays that have yet to be optimized or conceived, or assays performed by other Investigators 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 subjects 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 subject'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 or longer depending on funding.

11 Criteria for Participant and Study Completion and Premature Study Termination

11.1 Participant Completion

A subject has completed the study once they complete the required two-year follow-up. For subjects who receive both BOLT and BMT, all study visits in both Appendix 1 and Appendix 2 will be completed. For subjects who do not receive the BMT, only visits in Appendix 1 will be completed.

11.2 Premature Termination

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 no longer eligible for transplant.
3. 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).
4. The participant dies.
5. The Investigator no longer believes participation is in the best interest of the participant.

11.3 Participant Replacement

Complete enrollment is a total of 8 subjects who receive both BOLT and BMT. If a subject receives BOLT but does not proceed to BMT, an additional subject may be enrolled in order to meet complete enrollment. Subjects who receive BOLT but do not proceed to BMT will be followed according to Appendix 1 and the reason for not receiving a BMT will be recorded in the clinical database.

11.4 Follow-up after Early Study Withdrawal

Any subject who receives BOLT and/or BMT will be asked to complete the 2-year follow-up period. If a subject is prematurely terminated e.g. withdraws consent, they will be asked to complete the next scheduled visit for a final disposition but will no longer be followed on protocol. If a subject withdraws consent to specific assessments/procedures but agrees to certain follow-up, they will remain in the study.

11.5 Study Stopping Rules

11.5.1 Temporary Suspension of Enrollment

If any grade 4 or 5 adverse events that are at least possibly related to the use of rituximab prior to BMT, occur in at least 2 subjects, a safety-related suspension of enrollment will occur pending notification to the Institutional Review board (IRB) and an expedited review of all pertinent data by the National Institute of Allergy and Infectious Diseases (NIAID) Medical Monitor and the NIAID Data Safety Monitoring Board (DSMB).

11.5.2 Temporary Suspension of Enrollment and BMT

If any of the following occur in the first 100 days post-BMT among the first three subjects receiving BMT, a safety-related suspension of enrollment or proceeding to BMT will be initiated pending notification to the IRB and an expedited review of all pertinent data by the NIAID Medical Monitor and the NIAID DSMB.

- Death
- Respiratory failure as a result of rejection or infection requiring mechanical ventilation for more than 48 hours.
- Engraftment syndrome

11.5.3 Study Stopping Rule

The incidence of specific events will be continuously monitored throughout the study to determine if any of their observed subject-based incidence rates exceed a threshold incidence rate. This stopping rule will be triggered if **2 of 2 patients, 3 or more of 5 patients, or 4 or more of 8 patients** experience one of the following sentinel clinical events.

- Death
- Respiratory failure as a result of rejection or infection requiring mechanical ventilation for more than 48 hours
- High grade (grade ≥A3) persistent or high grade recurrent acute lung rejection post BMT (including post immunosuppression withdrawal)
- Grade 4 or greater lung infection post BMT
- Engraftment failure/failure to achieve chimerism (<5% donor cells at day 100 following BMT, or <10% donor cells at day 180)
- Engraftment syndrome

If a stopping threshold is met, enrollment and proceeding to BMT will be halted pending expedited NIAID DSMB review. The study regimen will be deemed ineffective or too toxic if an exact one-sided 80% confidence interval for the true rate of events excludes values of 0.30 or lower. The probability of concluding that the therapy is too toxic is 0.10 if the true rate of unacceptable toxicity is 20%, and 0.69 if the true rate is 50%.

12 Safety Monitoring and Reporting

12.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly (per Section 12.5, Reporting of Serious Adverse Events and Adverse Events) to the sponsor DAIT/NIAID. Appropriate notifications will also be made to the Institutional Review Board (IRB) 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:

- Bilateral orthotopic lung transplant
- Immunosuppressive regimen: induction with basiliximab (or alternate therapy), tacrolimus and Rituximab
- BMT conditioning regimen: Hydroxyurea, Rituximab, Alemtuzumab, ATGAM, Thiotepa, and TBI
- CD3+ and CD19+ depleted BMT
- Donor Leukocyte Infusion (DLI)
- Immunosuppression withdrawal
- Bronchoscopy/ BAL
- Bone marrow aspirate

12.2.2 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)).

12.2.3 Unexpected Adverse Event

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

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

12.2.4 Serious Adverse Event (SAE)

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

1. Death.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or Sponsor [add DAIT/NIAID or other Sponsor, *if applicable],* its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Any instance of COVID-19, regardless of grade, will be reported as a Serious Adverse Event.

Elective hospitalizations or hospital admissions for the purpose of conducting a protocol mandated procedure are not to be reported as an SAE unless the 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 4.0. 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 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.

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 Case Report Form. Final determination of attribution for safety reporting will be determined by the IND Sponsor. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided in Table 12.3.2.

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

Table 12.3.2 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 the initial Rituximab infusion (or BOLT induction if a subject did not receive pre-transplant Rituximab) 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 electronic adverse event case report form 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 NIAID as the IND sponsor via email notification and case report form. Timely reporting of adverse events is required by 21 CFR and ICH E6 guidelines.

The site investigator 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. When an event meeting serious criteria is entered into the clinical database, an automatic email notification will be generated to the study team, including the NIAID Medical Monitor. The site will then enter the details of the serious adverse event into the DAIT CRIS Interchange within 72 hours of discovering the event.

For serious adverse events, all requested information on the AE/SAE case report form 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 case report form will be updated and re-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 the IND sponsor and study medical monitor. There are two options for reporting to the health authorities:

12.5.2.1 Annual Reporting

NIAID as the IND Sponsor, 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 IND Annual 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 IND sponsor shall report any suspected adverse reaction that is both serious and unexpected. The IND 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 IND 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.

The IND sponsor shall notify the FDA 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

The investigators shall report adverse events, including expedited reports, in a timely fashion to the respective IRB in accordance with applicable regulations and guidelines.

12.6 Pregnancy Reporting

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

Information requested about the delivery shall include:

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

All pregnancy complications that result in a congenital abnormality, birth defect, miscarriage, and medically indicated abortion - an SAE shall be submitted to the DAIT/NIAID using the SAE reporting procedures described above. In addition, the FDA will be notified as appropriate.

12.7 Reporting of Other Safety Information

An investigator shall promptly notify the site IRB as well as the DAIT/NIAID 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 NIAID Medical Monitor shall receive monthly reports from the Principal Investigator compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study site on the appropriate case report form.

In addition, the NIAID Medical Monitor shall review and make decisions on the disposition of the SAE and pregnancy reports (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 NIAID 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, and summaries of the number and percent of patients reporting adverse events for each phase of therapy (BOLT, post-lung transplant, BMT, post-BMT) and overall.

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 protocol chair or DAIT/NIAID. In addition, if any stopping rules are met (Section 11.2) the DSMB will perform an ad hoc review.

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

13 Statistical Considerations and Analytical Plan

13.1 Overview

This is a prospective, single arm (non-randomized) study evaluating the feasibility, safety and efficacy of BOLT followed by BMT for primary immunodeficiency patients with pulmonary failure.

13.2 Endpoints

13.2.1 Primary Endpoints

13.2.1.1 Safety Endpoint

Safety will be determined by continued enrollment without exceeding the stopping rules as determined by observing the cumulative incidence of transplant related mortality and engraftment failure.

13.2.1.2 Efficacy Endpoints

- For all subjects receiving lung transplant, absence of severe allograft dysfunction using BOS scoring system at 1-year post lung transplant.
- For all subjects, ≥ 25% donor T-cell chimerism at 12 months post BMT.
- For subjects with myeloid disorders (e.g. CGD), ≥ 10% myeloid chimerism is also required at 12 months.
- For subjects with B-cell disorders, ≥ 10% B-cell chimerism is also required at 12 months.

13.2.1.3 Secondary Endpoints

The following secondary endpoints will be assessed:

- Feasibility of proceeding to BMT within 6 months following lung transplantation.
- Development of tolerance to both the host and pulmonary graft.
- Long-term complications of combined solid organ and bone marrow transplant.
- Acute cellular rejection and graft failure post BMT.
- Acute and chronic graft-versus-host disease (GVHD) following tandem lung and BMT.
- Time from BMT to withdrawal of immunosuppression.
- Time from BMT to independence from treatment dose antimicrobial drugs.
- For T cell lymphopenias, achieving age adjusted, low limit normal range lymphocyte count by 1-year post-BMT.
- A significant development in chronic lung allograft dysfunction (as evidenced by a change in BOS stage, see pg. 19 for BOS table) or allograft failure at 1-year and up to 2 years post lung transplant for BOLT alone and BOLT-BMT subjects.
- Incidence of Grade 4 or 5 adverse events possibly related to the use of Rituximab prior to the start of BMT conditioning.

13.2.1.4 Mechanistic Endpoints

- Pace of reconstitution of immunity.
- Incidence of mixed chimerism (>5% host cells) at months 1, 3, 6, 12 and 24 post-transplant.

- Improvement in immunologic marker(s) unique for the underlying PID syndrome by one-year post BMT (see Appendix 5).

13.2.2 Measures to Minimize Bias

This is a single-arm study without randomization or blinding. Samples will be identified by study ID rather than patient name when feasible, for privacy and for objectivity of laboratory staff.

13.3 Analysis Plan

13.3.1 Analysis of Safety and Efficacy

Analysis of safety data: All reported adverse events (defined in 12.3.1) will be coded using the NCI common toxicity criteria (version 4.0). The number and percent of patients reporting adverse events will be quantified for each phase of therapy (BOLT, post-lung transplant, BMT, post-BMT) and overall. Within each phase of therapy, and separately for each patient over all phases of therapy, summaries will include only the highest grade experienced by each patient for each adverse event. The rate of reported serious adverse events (defined in section 12.2.4) will be reported separately.

Efficacy will be analyzed as a case series, with rates for key efficacy criteria reported with 90% Wilson (score) confidence intervals. Deaths will be counted as treatment failures. For example, if 6 patients receiving BOLT + BMT have myeloid non T cell disorders, 1 dies within 12 months of BMT, and 3 display ≥10% myeloid chimerism at 12 months post-BMT, the efficacy rate for this group would be reported as 3/6 = 0.50 (90% CI 0.22 – 0.78).

13.3.2 Stopping rules for safety:

13.3.2.1 Temporary Suspension of Enrollment and BMT

Prior to and following lung transplant, a safety-related suspension of enrollment will occur pending notification to the IRB and an expedited review of all pertinent data by NIAID Medical Monitor and the NIAID DSMB if specific clinical events occur as outlined in Section 11.5 of the protocol.

The study regimen will be deemed ineffective or too toxic if an exact one-sided 80% confidence interval for the true rate of events excludes values of 0.30 or lower. The probability of concluding that the therapy is too toxic is 0.10 if the true rate of unacceptable toxicity is 20%, and 0.69 if the true rate is 50%.

Donor bone marrow engraftment will be assessed by measuring, when feasible, chimerism as determined in whole blood and CD3+ T-cell fraction as performed by standard, clinical STR assay at the University of Pittsburgh Medical Center's HLA-Laboratory. For those enrolled subjects whose underlying immune deficiency affects phagocyte function or numbers, typically those with chronic granulomatous disease (CGD) or severe congenital neutropenia (SCN), whole blood chimerism will be the outcome measure. For all others, whose immune defect affects T-cell function or T-cell numbers, determining the percentage of donor CD3+ T-cell contribution (chimerism) will be the outcome measure. Nevertheless, for all patients both whole blood and CD3+ T-cell chimerism will be determined.

13.3.3 Analysis Populations

The study sample for safety and feasibility will be patients who receive BOLT, including those who do not proceed to BMT. Most efficacy analyses only consider patients who receive both BOLT and BMT. Additional patients who are enrolled but do not proceed to BOLT or BMT will be described in study reports, but not included in study analyses.

13.3.4 Analyses of Secondary and Other Endpoint(s)/Outcome(s)

Efficacy endpoints will be described as a case series and summarized for all patients based on study status. For example, absence of severe allograft dysfunction will be summarized for all patients receiving BOLT; BMT endpoints will be summarized for those receiving BMT as well as BOLT.

Rate of improvement in immunological markers, specific to the underlying PID, will be analyzed by descriptive statistics. Since several diseases will likely be assessed, each patient will serve as their own control, comparing post-BMT measures to values pre-BOLT and pre-BMT (see Appendix 5). Additionally, accounting for unique PID diseases, immune outcomes on our patients with specific PID syndromes (e.g. CGD, SCID) will be compared to outcomes reported in the literature for pediatric lung transplant patients, and for patients without lung failure who undergo BMT alone for the identical PID syndromes.

The primary efficacy endpoint is the absence of severe allograft dysfunction (BOS grades 2 or 3), using the BOS scoring system, at 1-year post lung transplant. However, it is anticipated that patients undergoing BOLT before the end of year 3 will have 2 years of follow-up to assess for BOS, for which the reported incidence of dysfunction is 30% [31].

Additional secondary efficacy endpoints include whether or not weaning of immunosuppression has been initiated by 1 year following BMT, the timing of post-BMT initiation of weaning from immunosuppression, and the timing of post-BMT independence from treatment dose antimicrobial drugs. As another secondary endpoint regarding lung allograft outcomes, acute cellular rejection (ACR) episodes will be assessed, and the burden of ACR will be described using the cumulative rejection scoring system (CRSS). Here again, rough comparisons among lung transplant recipients are available with approximately 50% of patients having at least one episode of ACR in the first-year post-lung transplant [32].

In addition to ongoing review of clinical records by research staff, these efficacy outcomes will be queried specifically every 3 months (when chimerism is assessed). For example, if immunosuppression weaning has not been initiated, the treating physicians will be asked which of the 3 weaning criteria have not been met (>5% donor T-cell chimerism; lack of grade 2+ GVHD for 3 months; no lung rejection for 3 months).

13.3.5 Analyses of Mechanistic Endpoints

Chimerism will be assessed using PCR based STR assay from whole blood, CD33+ enriched myeloid cells, and CD3+ T-cells in the clinical HLA lab at months pre-BOLT and 3 months post BOLT. After BMT it will be tested months 1, 3, 6, 12 then yearly. These values will be summarized graphically and interpreted in the context of other clinical and laboratory results.

Spurious correlations are a concern when multiple assays are evaluated for a small number of patients or time points. Since a comprehensive alpha spending strategy is infeasible to apply in a way that would yield equivalent standards for testing each patient's immune functioning, the emphasis will be on

whether results from multiple assays are consistent with the hypothesized mechanism. For example, if there is a detectable response by identifying circulating tetramer positive cells in a SCID or CID patient with past CMV or adenovirus exposure who had no such cells detected prior to BMT, one can expect in parallel either normalization of CD4+ and CD8+ T cell counts or at least a significant improvement compared to pre-BMT values. Clinical responses such as resolution of viremia, and or ability to reduce or stop antiviral medications are expected to correlate but likely with notable time delay as clinical decisions are not to be influenced by the proposed mechanistic study data. Experimental findings in the lung mucosa will be qualitatively compared to assays performed on PBMCs, as has been previously done by Dr. McDyer's group [33, 34, 35]. Longitudinal clinical and research laboratory assay results will be displayed graphically, with normal functioning levels indicated when known.

Statistical analysis will be performing using GraphPad Prism, SAS/STAT (SAS Institute, Inc., Cary, NC) and R (R Foundation for Statistical Computing, Vienna, Austria) software. A two-tailed p-value of less than 0.05 will be considered statistically significant. As no assumption will be made regarding the Gaussian distribution of measured variables, the non-parametric tests of Wilcoxon signed-rank, Mann-Whitney-Wilcoxon, and Spearman's rank correlation will be primarily used for comparing distributions of paired measures and independent groups (such as samples from BOLT/BMT recipients vs. repository samples or in vitro Tolerance assay conditions with or without Treg depletion, IL-10 R blockade, etc). For many assays, control data from either normal donors or other age-matched lung transplant control patients (such as patients undergoing lung transplant for cystic fibrosis) will be available for comparison. When appropriate, linear mixed-effects models will be fitted to longitudinal data.

13.3.6 Descriptive Analyses

Baseline demographic, disease, and prior treatment characteristics of each participant will be reported and summarized for the safety and efficacy analysis populations. Measures of central tendency (e.g., means, medians) and variation (range, standard deviation) will be presented for continuous variables and percentages will be reported for discrete variables. The descriptive statistics may also be stratified by underlying primary immunodeficiency, if possible.

13.4 Interim Analyses

13.4.1 Interim Analysis of Efficacy Data

There is no planned interim analysis of efficacy data.

13.4.2 Interim Analysis of Safety Data

Interim analyses of safety data will be prepared for and reviewed by the DSMB annually, and as needed (described in section 12.8.2).

13.4.3 Futility Analysis

The stopping rules discussed in section 11.5 constitute a futility analysis.

13.4.4 Statistical Hypotheses

1) The BOLT+BMT approach will be considered feasible if at least half of patients enrolled complete both BOLT and BMT procedures (although enrollment will not be capped at 16)
2) The BOLT+BMT approach will be safe enough not to trigger the stopping rules in section 11.5

For each efficacy endpoint, at least half of patients treated will have a favorable outcome, for which a 90% Wilson confidence interval will include a 70% success rate.

13.5 Sample Size Considerations

With enrollment and follow-up over a 5-year period, we expect a sample size of 8 patients to receive both BOLT and BMT. This sample size, with additional safety and feasibility data from patients who do not receive BMT, should be adequate for detecting a high rate of BMT failure (see stopping rules, **Section 11.5**), with a 69% probability of triggering the stopping rule if the underlying rate of BMT failure is 50%. For binary efficacy variables, point estimates of the rate of treatment success will be provided with 90% Wilson confidence intervals of width <50 percentage points (see **Section 13.3.1**).

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 the relevant health authorities e.g. Food and Drug Administration (FDA). 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 as directed by the study Sponsor. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

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 local institutional Review Board (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 form 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 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 publication guidelines and policies stipulated in the grant will apply to this protocol.

18 References

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Appendix 1. Schedule of Events (Recipient: Relative to BOLT)

Time points (Months)	Screening	Day 0 (BOLT)	Surveillance Bronchoscopy Visits	24	For Cause
Visit #	**L(-1)**	**L0**	**L-1, 1a, 1b, etc.**	**L-2**	**FC**
Visit Window (Days)			**7 days**	±28	
General Assessments					
Informed consent	X				
Demographics	X				
Medical history	X				
Inclusion/Exclusion	X				
Transplant Data		X			
Bronchoscopy/BAL/transbronchial biopsy/bronchial brushings[8]			Initial bronchoscopy within 2-3 weeks of transplant. Subsequent bronchoscopy frequency will be approximately q2-6 months and as clinically indicated.		X
Physical Exam	X	X	Collected within 30 days of each bronchoscopy.		X
Pulmonary Function Testing including FEV1 and FVC (Spirometry)[5]	X		At a minimum, PFTs will be collected within 14 days of each bronchoscopy; however, all available PFT data will be collected for the study.		X
Chest x-ray	X		Collected within 14 days of each bronchoscopy.		X
Imaging of Abdomen, Chest and Pelvis (CT, MRI or Ultrasound)	X				
CT or MRI Head (if clinically indicated)	X				
Echocardiogram and EKG	X				
Adverse Events	X	→	→→→	→	X
Concomitant Medications	X	→	→→→	→	X
Laboratory Assessments					
CBC w/ Differential	X				
Chemistry Panel[1]	X				
Donor Specific Antibodies[9]	X		As clinically indicated. Most recent results will be collected with bronchoscopies.		X[9]
HLA Testing	X				
Pregnancy test[3]	X				
EBV/CMV/Adenovirus PCR Testing	X		Most recent test results, along with date tested, with each bronchoscopy.		
HIV/Hepatitis[2]	X				

Time points (Months)	Screening	Day 0 (BOLT)	Surveillance Bronchoscopy Visits	24	For Cause
Visit #	L(-1)	L0	L-1, 1a, 1b, etc.	L-2	FC
Visit Window (Days)			7 days	±28	
QUIGs (IgA, IgE, IgM, IgG)	X				
DiGeorge Panel[6]	X		Collected as clinically indicated based on the subjects underlying immune deficiency.		
Chimerism (whole blood STR assay, CD3+T-cell STR assay, CD33+ myeloid)	X				
Lymphocyte Subset[7]	X		May be repeated every 2-4 months post BOLT or as clinically indicated.		
Mechanistic Assays					
Szabolcs Lab					
Chimerism, FACS or HRM	X				X[10]
Global Cellular Immunity[4]	X				X[10]
Thymic Function (T-cell recovery and flow cytometry)	X				X[10]
Purified CD4+, CD8+, and CD19+ B Cells for Expression Profiling	X				X[10]
Tolerance Assays (Blood and BAL (if available)	X		If there is excess BAL during a surveillance bronchoscopy, a sample may be sent to the lab at the discretion of the Investigator based on the clinical profile of the subject.		X[10]
Pathogen Specific Humoral Immunity (Blood, for CGD subjects or those not receiving IVIG)[12]	X				
McDyer Lab (optional, if a bronchoscopy is clinically indicated prior to BOLT and if sample is feasible to obtain)					
Pathogen Specific T-cell responses (BAL, Blood)	X		Blood collected within 14 days of each bronchoscopy. BAL collected during the bronchoscopy, if available.		X
RNA Sequencing (bronchial brushing; explanted lung at baseline[11])	X		If collected during the bronchoscopy.		X

[1] Blood chemistries: sodium, potassium, chloride, carbon dioxide, anion gap, BUN, creatinine, glucose, calcium, magnesium, phosphorus, albumin, total protein, GFR (by cystatin c or nuclear only), total bilirubin, direct bilirubin, ALT(SGPT), AST(SGOT), GGTP

[2] HIV by serology and PCR, HTLV by serology, HBsAg, HCV RNA by PCR.

[3] Females >10 years old or who have reached menarche, unless surgically sterilized. May be serum or urine.

[4] PHA, CD3/28 proliferation, flow cytometry, TCRab, TCRgd, BCR spectratyping

[5] If the subject in an inpatient and unable to complete spirometry due to a tracheostomy, this will not be considered a protocol deviation.

[6] CD3+, CD3+/CD4+, CD3+/CD45RA+, CD4+/CD45RA+/CD62L.

[7] T-cells (CD3), Helper cells (CD4), Suppressor cells (CD8), B-cells (CD19), NK cells (CD16/CD56), Helper/Suppressor Ratio.

[8] If a bronchial wash (BW) is obtained in lieu of BAL, the results from the BW will be collected and this will not be considered a protocol deviation.

[9]DSA may be repeated as clinically indicated and results will be collected as a part of the study.

[10]These central laboratory assessments may be collected at for cause visits at the discretion of the Investigator.

[11]Collected if the explanted lung is processed.

[12]Anytime prior to transplant.

Appendix 2. Schedule of Events (Recipient: Relative to BMT)

		Days				Months								For Cause
Time points	**Pre-BMT[1]**	**0**	**7**	**14**	**21**	**1**	**2**	**3**	**6**	**9**	**12**	**18**	**24**	
Visit #	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	
Visit Window (Days)	**See Footnote 1**		±3	±3	±3	±7	±7	±7	±21	±21	±28	±28	±28	
General Assessments														
Once the subject undergoes the BMT, all attempts should be made to align visits in this schedule with the Post-Transplant Lung Schedule in Appendix 1														
Informed consent	X													
Physical Exam	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pulmonary Function Testing[2]	X										X		X	
Chest x-ray									X		X		X	
Imaging of Abdomen, Chest and Pelvis (CT, MRI or Ultrasound)	X													
CT or MRI Head	X													
Echocardiogram and EKG	X										X		X	
Bone Density											X		X	
Adverse Events	X	→	→	→	→	→	→	→	→	→	→	→	→	X
Concomitant Medications	X	→	→	→	→	→	→	→	→	→	→	→	→	X
GVHD Assessment			→	→	→	→	→	→	→	→	→	→	→	X
Engraftment		Data regarding engraftment will be chart abstracted and entered into the clinical database.												
Laboratory Assessments														
CBC w/ Differential	X		X	X	X	X	X	X	X	X	X	X	X	X
Chemistry Panel[3]	X		X	X	X	X	X	X	X	X	X	X	X	X
Thyroid Function Testing[4]											X		X	
Pregnancy test[5]	X													
EBV/CMV/Adenovirus PCR Testing[12]	X		Most recent test results, along with date tested, will be collected at the time-points above.											
Toxoplasmosis PCR (prior to conditioning)	X													
QUIGs (IgA, IgE, IgM, IgG)[10]	X					X		X	X	X	X	X	X	
DiGeorge Panel[14]	X					X		X	X	X	X	X	X	
Chimerism (whole blood STR assay, CD3+T-cell STR assay, CD33+ myeloid)	X					X		X	X	X	X	X	X	

		Days				Months								For Cause
Time points	**Pre-BMT[1]**	**0**	**7**	**14**	**21**	**1**	**2**	**3**	**6**	**9**	**12**	**18**	**24**	
Visit #	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	
Visit Window (Days)	**See Footnote 1**		±3	±3	±3	±7	±7	±7	±21	±21	±28	±28	±28	
Lymphocyte Subset[15]	X					X		X	X	X	X	X	X	
Gonadal Function[6]											X			
Bone Marrow Aspirate														X[13]
Study Intervention														
CD3+/CD19+ Depleted BMT Infusion		X												
Mechanistic Assays														
Szabolcs Lab														
Chimerism[7,]	X					X		X	X	X	X	X	X	X[16]
Global Cellular Immunity[8,10]	X					X	X[8]	X	X	X	X	All of these studies performed prior to IS w/d and >2m following IS w/d		X[16]
Thymic Function (T-cell recovery and flow cytometry)[17]	X							X	X	X	X			X[16]
Tolerance Assays (BAL[9], Blood[11,17])	X							X	X	X	X			X[16]
Purified CD4+, CD8+, and CD19+ B Cells for Expression Profiling[10]	X									X[10]				X[16]
Bone Marrow Aspirate (Host Derived Dendritic Cells, Pre-BMT)	X													
Pathogen Specific Humoral Immunity (Blood)[10]		Samples to be collected 1) subject is off IVIG for at least 3 months and not yet vaccinated and 2) vaccinated with at least two administrations												
McDyer Lab														
Pathogen Specific T-cell responses (BAL, Blood)[17]	Blood collected within 14 days of each bronchoscopy. BAL collected during the bronchoscopy, if available.													X
RNA Sequencing (bronchial brushing)[17]	Collected with each bronchoscopy, if feasible.													X

[1]Occurs within 60 days prior to BMT with the exception of the bone marrow aspirate which may occur closer to the BMT. May align with the Month 2 or 3 bronchoscopy and mechanistic studies outlined in Appendix 1.

[2]Pulmonary Function Tests will also follow the Schedule of Events in Appendix 1.

[3]Blood chemistries: sodium, potassium, chloride, carbon dioxide, anion gap, BUN, creatinine, glucose, calcium, magnesium, phosphorus, albumin, total protein, total bilirubin, direct bilirubin, ALT(SGPT), AST(SGOT), GGTP. GFR (by cystatin c or nuclear only) will be performed at baseline and 3 months post BMT.

[4]Thyroid Function Tests: TSH, free T4.

[5]Females >10 years old or who have reached menarche, unless surgically sterilized.

[6]Gonadal Function: Females: FSH, LH, Total Estrogen; Males: FSH, LH, Testosterone.

[7]Chimerism (optional at Month 1 post BMT).

[8]PHA, CD3/28 proliferation, flow cytometry, TCRab, TCRgd, BCR spectratyping. Not all tests will be feasible at two months post-BMT.

[9] Performed on BAL only if a clinical bronchoscopy is done and BAL is available, even if it falls out of window.

[10] Samples to be collected 1) subject is off IVIG for at least 3 months and not yet vaccinated and 2) vaccinated with at least two administrations

[11] If host T-cells are ≥10% and includes anti-donor TCR VB immunosequencing, anti-donor proliferation, anti-donor cytokine profile and anti-donor cytotoxicity.

[12] EBV, CMV and Adenovirus testing is performed according to UPMC guidelines and as clinically indicated.

[13] Following BMT, data will be collected for any clinically indicated bone marrow aspirates.

[14] CD3+, CD3+/CD4+, CD3+/CD45RA+, CD4+/CD45RA+/CD62L.

[15] T-cells (CD3), Helper cells (CD4), Suppressor cells (CD8), B-cells (CD19), NK cells (CD16/CD56), Helper/Suppressor Ratio.

[16] These central lab specimens may be repeated during any surveillance or for cause visit (at the discretion of the Investigator) provided the site does not exceed the local institutional blood volume guidelines.

[17] These samples should be collected prior to the initiation of immunosuppression withdrawal (estimated between 9-12 months post BMT) and > 2 months after completion of immunosuppression taper (estimated between 14-24 months post BMT).

Appendix 3. Conditioning Regimen

Conditioning Regimen for BMT																					
Treatment	Day -28	Days -27 to -15	Day -21	Day -20	Day -14	Day -13	Day -12	Day -11	Day -10	Day -9	Day -8	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day +1	Day +35
Hydroxyurea	X	X			X	X	X	X	X	X	X	X	X	X	X	X					
Rituximab	X																				X[1]
Alemtuzumab (prior ACR)			X[2]	X[2]																	
Alemtuzumab (no prior ACR)					X[2]																
ATGAM																X	X	X			
Thiotepa																	X				
TBI[3] (250 cGy)																		X			

[1]Administration window of +/- 2 days for Rituximab dose on Day +35.

[2] Administration window of +/- 1 day for Alemtuzumab.

[3]Subjects with a radiation sensitive condition will receive 200cGy.

Appendix 4. Donor Assessments

		Visit 1
GENERAL ASSESSMENTS		
Demographics	Age, Race, Gender	X
Donor Information	Basic demographics	X
LABORATORY ASSESSMENTS		
Blood Type [1]	A, B, O	X
HLA Typing [1]	I (A, B, C), II (DR, DP, DQ)	X
Viral Panel [1]	(Hepatitis B & C, HIV, CMV, EBV)	X
SPECIMEN COLLECTION OF DONOR FOR MECHANISTIC ASSAYS OF SUBJECT		
Lung Tissue, optional, if available)	Sample banking (Biorepository)	X
Filter Set from Vertebral Bodies for MSC prep, Szabolcs Lab[2]	Sample banking (Biorepository)	X
Dendritic Cells/CD34 prep, Szabolcs Lab[2]	Expanded from BM CD34+ Cells	X
Purified CD4+, CD8+, CD19+ (B Cells and T-cells[3]) Szabolcs Lab[2]	Sample banking (Biorepository)	X

[1] Retrospective data collection based on pre-transplant evaluation.

[2] Donor unmodified, bone marrow sample (approximately 10-20mL) and if available, donor blood (ideally 10-20mL) will be collected for the above mechanistic assays.

[3]CD3+/CD19+ positively selected (nontarget) cells utilized for mechanistic studies, as applicable.

Appendix 5. Disease Specific Post-BMT Outcomes

Diagnosis	Disease Specific Post-BMT Outcomes
SCID	Lymphocyte subsets, CD3 number, naïve T cells number, proliferation to mitogens ≥30% lower limit of controls, TREC, TCR Vβ spectratyping, B cell subsets, immunoglobulin levels off supplementation, freedom from IVIG, vaccine response (≥3---fold increase in anti---tetanus antibody or other vaccine response), correction of molecular defect (e.g. pSTAT5 activity in JAK deficient SCID, CD127/132 expression in γc SCID)
CID	Lymphocyte subsets, CD3 number, naïve T cells number, proliferation to mitogens ≥30% lower limit of controls, TREC, TCR Vβ spectratyping, B cell subsets, immunoglobulin levels off supplementation, freedom from IVIG, vaccine response (≥3---fold increase in anti---tetanus antibody or other vaccine response)
SCN	Absolute Neutrophil Count >1000 without G---CSF support
CGD	Presence of oxidative burst activity as measured by DHR or NBT testing, resolution of autoinflammatory conditions present pre---transplant (e.g. colitis)
Hyper IgE Syndrome	Th17 number and activity (STAT3 deficiency), B cell subsets, immunoglobulin levels off supplementation, freedom from IVIG, vaccine response (≥3---fold increase in anti---tetanus antibody or other vaccine response, significant improvement of IgE levels, eosinophil numbers, significant improvement in allergic disease, lymphocyte subsets, CD3 number, naïve T cells number, proliferation to mitogens ≥30% lower limit of controls
Hyper IgM Syndrome	Presence of CD40 or CD40L (based on original diagnosis) by flow cytometry, B cell subsets, Significant improvement of IgM levels, immunoglobulin levels off supplementation, freedom from IVIG, vaccine response (≥3-fold increase in anti-tetanus antibody or other vaccine response), T cell subsets (some reports of decreased TH1 in HIGM)
WAS	Significant improvement of platelet count and size, Lymphocyte subsets, CD3 number, naïve T cells number, proliferation to mitogens ≥30% lower limit of controls, isohemagglutinins, immunoglobulin levels off supplementation, freedom from IVIG, vaccine response (≥3---fold increase in anti---tetanus antibody or other vaccine response), improvement in autoimmune disease (if present)
MSMD	Significant improvement of IFN production and/or response (depending on genetic etiology)