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A randomized trial of low versus moderate exposure busulfan for infants with severe combined immunodeficiency (SCID) receiving TCRαβ+/CD19+ depleted transplantation: A Phase II study by the Primary Immune Deficiency Treatment Consortium (PIDTC) and Pediatric Blood and Marrow Transplant Consortium (PBMTC)

PIDTC "CSIDE" Protocol

(Conditioning SCID Infants Diagnosed Early)

PBMTC NMD 1801

Short title: Busulfan exposure-finding for SCID

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1 February 2019

This template is adapted from the ICH guidance document E6 (Good Clinical Practices), Section 6.

Statement of Compliance

This trial will be conducted in compliance with the protocol, International Conference on Harmonization Good Clinical Practice E6 (ICH-GCP), and the applicable regulatory requirements. The clinical trial associated with the protocol will follow the U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46 and 21 CFR including parts 50 and 56 concerning informed consent and IRB regulations, if under IND, 21 CFR 312). All personnel associated with administration of the trial will have appropriate Protection of Human Subjects Training.

Signature Page 1

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator: _____
Print/Type

Signed: _____ Date: _____
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Signature Page 2

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

The Investigator(s) of Record (signature(s) on IDE Investigator Agreement) from each participating clinical site should sign the Signature Page 2 as appropriate. This Signature Page 2 should be maintained at each site.

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Signed: _____ Date: _____

Additional Investigators: _____
Print/Type

Signed: _____ Date: _____

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List of Abbreviations

This list should be modified to include protocol-specific terms.

ADA	Adenosine Deaminase
AE	Adverse Event/Adverse Experience
ATG	Anti-thymocyte globulin
AUC	Area Under the Curve
BM	Bone marrow
cAUC	Cumulative Area Under the Curve
CFR	Code of Federal Regulations
CIBMTR	Center for International Blood and Marrow Transplant Research
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CSA	Cyclosporine A
DSMC	Data and Safety Monitoring Committee
FDA	Food and Drug Administration
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
GVHD	Graft Versus Host Disease
HCT	Hematopoietic Cell Transplantation
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human Leukocyte Antigen
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IL2RG	Interleukin-2 receptor gamma
IRB	Institutional Review Board
JAK3	Janus Activating Kinase 3
MOP	Manual of Procedures
N	Number (typically refers to participants)
NBS	Newborn screening
NCI	National Cancer Institute, NIH
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
PBMTC	Pediatric Bone Marrow Transplant Consortium
PBSC	Peripheral blood stem cells
PHA	Phytohemagglutinin
PHI	Protected Health Information
PI	Principal Investigator

List of Abbreviations

PID	Primary immune deficiency
PIDTC	Primary Immune Deficiency Treatment Consortium
PK	Pharmacokinetics
QA	Quality Assurance
QC	Quality Control
RAG1	Recombinase Activating Gene 1
RAG2	Recombinase Activating Gene 2
rATG	Rabbit Anti-thymocyte Globulin
SAE	Serious Adverse Event/Serious Adverse Experience
SCID	Severe Combined Immunodeficiency
SOP	Standard Operating Procedure
STR	Short tandem repeat
TCR	T cell receptor
TREC	T Cell Receptor Excision Circle
VOD	Veno-Occlusive Disease

Protocol Summary

Full Title	A randomized trial of low versus moderate exposure busulfan for infants with severe combined immunodeficiency (SCID) receiving TCRαβ+/CD19+ depleted transplantation: A Phase II study by the Primary Immune Deficiency Treatment Consortium (PIDTC) and Pediatric Blood and Marrow Transplant Consortium (PBMTC)
Short Title	**Conditioning SCID Infants Diagnosed Early (CSIDE)**
Clinical Trial Phase	II
IND Sponsor (if applicable)	IDE cross references: BB MF 12011, SN 073 (CliniMACS® CD19 reagent system), BB MF 12251, NS 054 (CliniMACS® depletion tubing set), and BB MF 15678, SN 012, CliniMACS® TCR α/β reagent system), all held by the manufacturer, Miltenyi Biotec, Inc., Cambridge, MA. IND/IDE for this project will be sponsored by Children's Hospital Los Angeles (CHLA) with Michael Pulsipher as the Sponsor-Investigator
Conducted By	Primary Immune Deficiency Treatment Consortium (PIDTC) in collaboration with the Pediatric Blood and Marrow Transplant Consortium (PBMTC) and the Center for International Blood and Marrow Transplant Research (CIBMTR)
Protocol Chair	Sung-Yun Pai MD (PI), Michael Pulsipher MD (Co-PI)
Sample Size	64
Study Population	Neonates and infants with SCID
Accrual Period	4 years
Study Design	This is a prospective phase II study comparing low dose and moderate exposure busulfan-based regimens in conjunction with TCRαβ+/CD19+ depleted allogeneic hematopoietic cell transplantation (HCT) in patients with SCID without active infection, who lack matched related donors and have either a suitable haploidentical related or unrelated donor available. Two genetically defined cohorts of SCID patients will be studied, patients with defects in cytokine signaling (*IL2RG* or *JAK3* mutations) and patients with defects in antigen receptor recombination (*RAG1* or *RAG2* mutations). Patients with IL2RG/JAK3 will receive busulfan and rabbit anti-thymocyte globulin; patients with RAG1/2 will

	receive busulfan and rabbit anti-thymocyte globulin in conjunction with fludarabine and thiotepa. The donor, whether haploidentical or unrelated, will be selected by the physician at the enrolling site. The study seeks to determine by a randomized trial whether the established dose of busulfan is necessary for high proportions of patients to achieve combined cellular and humoral reconstitution, or whether a lower exposure of busulfan would be sufficient. A total of 64 patients (32 at each exposure level) will be randomized, stratified by genetic mutation cohort (IL2RG/JAK3 and RAG1/2). Primary outcome is vaccine specific antibody response. Safety/feasibility of the novel TCR αβ+/CD19+ depleted allogeneic HCT strategy will be monitored in an ongoing manner using stopping rules for lack of neutrophil engraftment and other important short-term toxicities. Data and blood samples will be collected at baseline, during the conditioning regimen, day 0, and at 7 days, 14 days, 30 days, 42 days, 60 days, 100 days, 6 months, 9 months, 12 months, 2 years and 3 years post-HCT.
Study Duration	Target enrollment over 4 years, follow-up 3 years after intervention.
Study Agent/Intervention Description	TCRαβ+/CD19+ depleted allogeneic peripheral blood stem cell transplant following intravenous busulfan-based regimens given at 2 targeted exposure levels with pharmacokinetic monitoring and adjustment
Inclusion Criteria	1. Infants with SCID, either typical or leaky or Omenn syndrome. a. Typical SCID is defined as either of the following: • Absence or very low number of T cells (CD3+ T cells <300/microliter AND no or very low T cell function (<10% of lower limit of normal) as measured by response to phytohemagglutinin OR • Presence of maternally derived T cells b. Leaky SCID is defined as the following • Absence of maternally derived T cells • AND either one or both of the following (i,ii): *i.* <50% of lower limit of normal T cell function as measured by response to PHA OR <30% of lower limit of normal T cell function as measured by response to CD3 ii. Absent or <10% of lower limit of normal proliferative responses to candida and tetanus toxoid antigens (must document post vaccination or exposure for this criterion to apply)

- **AND** at least two of the following (i through iii):
  i. CD3 T cells < 1500/microliter
  ii. ≥ 80% of CD3+ or CD4+ T cells are CD45RO+ AND/OR ≥80% of CD3+ or CD4+ T cells are CD62L negative AND/OR >50% of CD3+ or CD4+ T cells express HLA-DR (at ≤ 4 years of age) AND/OR are oligoclonal T
  iii. Low TRECs and/or the percentage of CD4+/45RA+/CD31+ or CD4+/45RA+/CD62L+ cells is below the lower level of normal

c. Omenn syndrome:

- Generalized skin rash
- Maternal lymphocytes tested for and not detected
- ≥80% of CD3+ or CD4+ T cells are CD45RO+ AND/OR ≥80% of CD3+ or CD4+ T cells are CD62L negative AND/OR >50% of CD3+ or CD4+ T cells express HLA-DR (≤2 years of age)
- Absent or low (up to 30% lower limit of normal (LLN)) T cell proliferation to antigens (Candida, tetanus) to which the patient has been exposed

IF: Proliferation to antigen was not performed, but at least 4 of the following 8 supportive criteria, at least one of which must be among those marked with an asterisk (*) below are present, the patient is eligible as Omenn Syndrome.

- Hepatomegaly
- Splenomegaly
- Lymphadenopathy
- Elevated IgE
- Elevated absolute eosinophil count
- * Oligoclonal T cells measured by CDR3 length or flow cytometry (upload report)
- *Proliferation to PHA is reduced to < 50% of lower limit of normal (LLN) or SI <30
- *Low TRECs and/or percentage of CD4+/RA+ CD31+ or CD4+/RA+ CD62L+ cells below the lower level of normal

2. Documented mutation in one of the following SCID-related genes:
   a. Cytokine receptor defects (*IL2RG, JAK3*)
   b. T cell receptor rearrangement defects (*RAG1, RAG2*)
3. No available genotypically matched related donor (sibling)
4. Availability of a suitable donor and graft source

	a. Haploidentical related mobilized peripheral blood cells b. 9/10 or 10/10 allele matched (HLA-A, -B, -C, -DRB1, -DQB1) volunteer unrelated donor mobilized peripheral blood cells 5. Age 0 to 2 years at enrollment NOTE: To ensure appropriate hepatic metabolism, age at time of busulfan start: • For *IL2RG/JAK3*: 8 weeks • For *RAG1/RAG2*: 12 weeks 6. Adequate organ function defined as: a. Cardiac: Left ventricular ejection fraction (LVEF) at rest ≥ 40% or, shortening fraction (SF) ≥ 26% by echocardiogram b. Hepatic: Total bilirubin < 3.0 x the upper limit of normal (ULN) for age (patients who have been diagnosed with Gilbert's Disease are allowed to exceed this limit) and AST and ALT < 5.0 x ULN for age c. Renal: GFR estimated by the updated Schwartz formula ≥ 90 mL/min/1.73 m^2. If the estimated GFR is < 90 mL/min/1.73 m^2, then renal function must be measured by 24-hour creatinine clearance or nuclear GFR, and must be > 50 mL/min/1.73 m^2 d. Pulmonary: No need for supplemental oxygen *and* O2 saturation > 92% on room air at sea level (with lower levels allowed at higher elevations per established center standard of care).
Exclusion Criteria	1. Presence of any serious life-threatening or opportunistic infection at time of enrollment and prior to the initiation of the preparative regimen. Serious infections as defined below that occur after enrollment must be reported immediately to the study coordinator, and enrollment will be put on hold until the infection resolves. Ideally enrolled subjects will not have had any infection. If patients have experienced infections, these must have resolved by the following definitions: a. <u>Bacterial</u> i. <u>Positive</u> culture from a sterile site (e.g. blood, CSF, etc.): Repeat culture(s) from same site must be negative and patient has completed appropriate course of antibacterial therapy (typically at least 10 days). ii. Tissue-based clinical infection (e.g. cellulitis): Complete resolution of clinical signs (e.g. erythema, tenderness, etc.) and patient has completed appropriate course of antibacterial therapy (typically at least 10 days)

	iii. Pneumonia, organism not identified by bronchoalveolar lavage: Complete resolution of clinical signs (e.g. tachypnea, oxygen requirement, etc.) and patient has completed appropriate course of antibacterial therapy (typically at least 10 days). If possible, radiographic resolution should also be demonstrated b. <u>Fungal</u> i. Positive culture from a sterile site (e.g. blood, CSF, etc.): Repeat culture(s) from same site is negative and patient has completed appropriate course of antifungal therapy (typically at least 14 days). The patient may be continued on antifungal prophylaxis following completion of the treatment course c. <u>Pneumocystis</u> i. Complete resolution of clinical signs (e.g. tachypnea, oxygen requirement, etc.) and patient has completed appropriate course of anti-PCP therapy (typically at least 21 days). If possible, radiographic resolution should also be demonstrated. The patient may be continued on anti-PCP prophylaxis following completion of the treatment course d. <u>Viral</u> i. Viral PCRs from previously documented sites (blood, nasopharynx, CSF) must be re-tested and are negative ii. If re-sampling a site is not clinically feasible (i.e. BAL fluid): Complete resolution of clinical signs (e.g. tachypnea, oxygen requirement, etc.). If possible, radiographic resolution should also be demonstrated 2. Patients with HIV or HTLV I/II infection will be excluded
Primary Objective	To determine the incidence of humoral immune reconstitution by 2 years post-transplant in 2 SCID cohorts (*IL2RG*/*JAK3*, *RAG1*/*RAG2*) undergoing alternative donor HCT by randomized assignment to a busulfan preparative regimen targeted at cumulative area-under-the-curve (cAUC) exposure of 25-35 mg*h/L vs 55-65 mg*h/ L.
Secondary Objectives	Secondary objectives are to assess immune reconstitution, cell type specific engraftment, survival and event-free survival, and transplant-

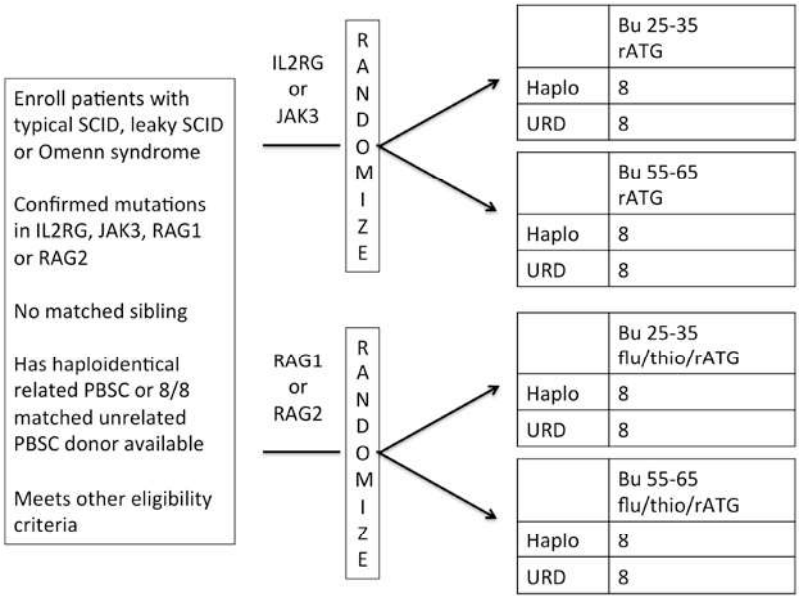
	related complications. We will also assess the accuracy of busulfan targeting and graft characteristics. We will assess: 1. T cell number and function, naïve T cell generation, kinetics of humoral immune response, response to live viral vaccine. 2. Donor cell engraftment in whole blood and peripheral blood sorted for CD3, CD19, CD56, CD15 post-HCT. 3. Event-free and overall survival. 4. Incidence of transplant-related toxicity, acute and chronic GVHD, autoimmunity. 5. Observed cumulative exposure of busulfan. 6. The relationship of graft characteristics (CD34+ cell, TCRαβ+, TCRγδ+ cell and CD19+ cell counts/kg) to rates of engraftment, acute and chronic GVHD, and immune reconstitution.
Exploratory Objectives	1. To study the relationship between busulfan exposure and lineage specific donor engraftment from sorted peripheral blood ($CD3^+$, $CD19^+$, $CD3^-$ $CD56^+$, $CD15^+$) and between busulfan exposure and in vitro expanded $CD34^+$ peripheral blood cells as a surrogate for bone marrow HSC chimerism. 2. To analyze B cell phenotype (percentage of memory B cells), B cell function (in vitro generation of plasmablasts in response to CD40L and IL-21) and *IGH* repertoire, examine correction of abnormalities seen within each genotype cohort, and correlate with donor B cell engraftment level and vaccine response. 3. To examine the correlation between split chimerism (T donor, B and/or myeloid host) or mixed chimerism in the B and myeloid lineages with markers of T cell reconstitution and exhaustion. 4. To explore the mechanisms of tolerance post-HCT (clonal deletion versus peripheral regulation) and the correlation between the dominant mechanism of tolerance and HLA-mismatch (haploidentical versus matched unrelated donor). 5. Relationship of pre- and post-transplant active ATG area-under-the-curve and IL-7 levels with outcomes including survival, T cell recovery and acute GVHD. 6. To explore the pharmacokinetics of fludarabine and thiotepa in this population and the relationship between these and graft rejection, lineage specific donor cell engraftment.

Primary Endpoint	Humoral immune reconstitution by 2 years post HCT as defined by specific antibody response to tetanus toxoid. Criteria for evaluation of humoral immune response are the following: • Donor T cell chimerism ≥50% • B cell count ≥50 cells/microliter • IVIG independent for ≥12 weeks Subjects meeting the criteria receive 3 doses of tetanus toxoid at least 4 weeks apart, followed by measurement of tetanus titer at least 4-6 weeks after the 3rd dose. Those who achieve tetanus titer of ≥0.15 IU/ml after vaccination will meet the primary endpoint. Patients who have documented humoral immune response at a time prior to 2 years will be considered a success for the primary endpoint, while patients who do not have humoral immune response evaluated by 2 years will be considered failures for the primary endpoint.
Secondary Endpoints	Immune reconstitution o T cell immune reconstitution at 30 days, 60 days, 100 days, 6 months, 12 months, and 2 years post-HCT. o Naïve T cell generation and thymic output at 100 days, 6 months, 12 months and 2 years post-HCT. o Freedom from immunoglobulin substitution will be assessed on all patients at 6 months, 9 months, 12 months, 2 years and 3 years post-HCT. Patients who have not received IVIG for at least 12 weeks at the time of assessment will be considered free from immunoglobulin substitution. o Tetanus responses on all patients who complete a trial of vaccination by additional timepoints of 12 months, 18 months and 3 years post-HCT. o Live vaccine responses on all patients who undergo trial of vaccination by 3 years post-HCT Engraftment o Neutrophil engraftment will be assessed on all patients and defined as achieving an absolute neutrophil count of ≥500 cells/microliter for 3 consecutive lab values by day 42 post-HCT o Donor cell chimerism (whole blood, sorted CD3 (T-cell), CD19 (B-cell) and CD56 (NK cell) and granulocyte (CD15)) at 42 days, 100 days, 6 months, 12 months, and 2 years post-HCT. Absolute B cell, NK cell and granulocyte counts will be measured. Survival o Overall survival o Event free survival

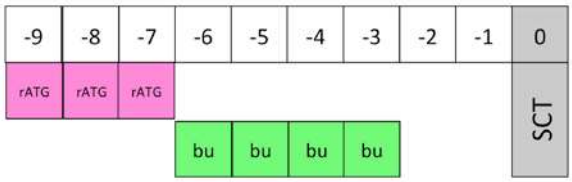
	Events will be defined as 1) death from any cause, 2) rejection of the graft (T-cell and/or whole blood chimerism <5% donor), 3) graft failure necessitating a second HCT procedure from the same donor or a different donor, with or without conditioning, 4) DLI given for treatment of falling chimerism Graft-versus-host disease o Occurrence of acute (grade II-IV and grade III-IV) GVHD by 100 days and 6 months post-HCT. o Occurrence of chronic GVHD by 6 months, 12 months and 2 years post-HCT. Post-HCT complications o Infections o Targeted regimen related toxicity (severe veno-occlusive disease of the liver, idiopathic pneumonitis syndrome) o Autoimmunity Busulfan PK o Comparison of desired target exposure with observed target exposure Graft composition o Frequency of achieving the target CD34+ cell/kg and TCRαβ+/CD19+ depletion goals and the relationship of infused cells to key outcomes (engraftment, immune reconstitution, GVHD).
Exploratory endpoints	**1.** Relationship of busulfan exposure with lineage specific donor chimerism **2.** Correlation between level of donor B cell chimerism and vaccine response to correction of abnormalities of B cell phenotype, B cell function, *IGH* repertoire **3.** Correlation between mixed or split chimerism in B and myeloid lineages with markers of T cell reconstitution and exhaustion **4.** Examination of T cell tolerance and correlation with HLA-mismatch **5.** Relationship between pre- and post-transplant active ATG area-under-the-curve and outcomes including survival, T cell recovery and acute GVHD **6.** To explore the pharmacokinetics of fludarabine and thiotepa in this population and the relationship between these and graft rejection, lineage specific donor cell engraftment.

CSIDE Protocol v2.0

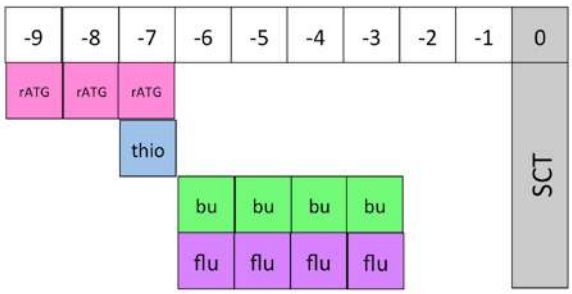
Schematic of Study Design:



Schema for IL2RG/JAK3 cohort



Schema for RAG1/RAG2 cohort



1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Severe combined immunodeficiency disorder (SCID) is a group of congenital diseases caused by mutations in genes critical for T cell and often B cell development, resulting in a profound deficiency of functional T and B lymphocytes (Buckley 2004). SCID is fatal due to opportunistic infection before the age of 2 years, unless curative therapy is given, with standard treatment being allogeneic hematopoietic cell therapy (HCT). HCT using a variety of methods (with or without pre-transplant chemotherapy conditioning) and donor sources (matched sibling, haploidentical parent, matched unrelated donor, umbilical cord blood) has been shown to be successful in reconstitution of T cells and preventing death from opportunistic infection, but reconstitution of humoral immunity i.e. B cell engraftment and function, have been more variable (Buckley, Schiff et al. 1999, Haddad, Le Deist et al. 1999, Neven, Leroy et al. 2009, Railey, Lokhnygina et al. 2009, Haddad, Leroy et al. 2013, Pai, Logan et al. 2014). As a result, many SCID patients post-HCT remain dependent on intravenous immunoglobulin substitution, and other long-term complications including failure to thrive, autoimmunity, and non-opportunistic infections may occur (Neven, Leroy et al. 2009). While survival after matched sibling donor HCT is excellent (Gennery, Slatter et al. 2010, Pai, Logan et al. 2014), survival after alternative donor transplants remains inferior (60%-80% versus >90%) for SCID patients who receive HCT from alternative donors (Buckley 2004, Grunebaum, Mazzolari et al. 2006, Railey, Lokhnygina et al. 2009, Gennery, Slatter et al. 2010, Buckley 2011, Pai, Logan et al. 2014). Thus outcomes for HCT for SCID patients, particularly those who lack a matched sibling donor, remain non-optimal.

Current HCT approaches for SCID thus result in tradeoffs in efficacy and toxicity. The optimal approach would use effective agents to engraft donor HSC with minimum toxicity and result in sufficient multi-lineage engraftment to support full immune reconstitution and donor-host tolerance. Now that universal newborn screening (NBS) for SCID is active in many states with a recommendation from the Secretary of Health and Human Services to extend SCID NBS to all states (Howell 2011), the need to identify minimally toxic approaches for young infants with SCID is essential. Progress in the field of HCT for SCID is hampered by the lack of well designed, cooperative, multi-institutional, prospective treatment studies. Barriers in the past include the rarity of SCID and the lack of a functioning multi-institutional consortium. In North America, we have formed a multi-institutional consortium of 33 institutions, including participating centers from the Pediatric Bone Marrow Transplant Consortium, called the Primary Immune Deficiency Treatment Consortium (PIDTC) with the ultimate goal of designing intervention trials for SCID and other PIDs (Griffith, Cowan et al. 2008, Griffith, Cowan et al. 2009, Griffith, Cowan et al. 2013). PIDTC is funded by a U54 cooperative agreement from NIAID/ORDR, and is currently carrying out natural history and retrospective studies in HCT for SCID and other PIDs. Results from these studies in SCID have been recently published (Dvorak, Cowan et al. 2013, Shearer, Dunn et al. 2013, Pai, Logan et al. 2014), and support the design of this trial.

Genetic subtypes of SCID

At least 14 genetic subtypes of SCID have been described (Al-Herz, Bousfiha et al. 2014), and are generally divided into those that preserve or abrogate B cell development (T- B+ SCID versus T- B- SCID). Table 1 shows the genetic causes of SCID divided in this fashion, and highlights those forms of B+ SCID, which have intrinsic defects of B cell function. The most common form of SCID is the X-linked form due to defects in *IL2RG*, which encodes the common gamma cytokine receptor (γc) (Noguchi, Yi et al. 1993, Puck, Deschênes et al. 1993). The γc receptor is shared between the IL-2, -4, -7, -9, -15 and -21 receptors, which all signal through *JAK3* (Kovanen and Leonard 2004). Mutations in either *IL2RG* or *JAK3* (Macchi, Villa et al. 1995) result in T- B+ SCID, and B cells deficient in γc function are unable to differentiate into plasmablasts or secrete antibody in response to IL-21 (Recher, Berglund et al. 2011). In contrast defects in *IL7R, CD3D, CD3G, CD3Z* and *CD45* result in lack of T cell development, but spare B cell development and B cell function. Defects in genes encoding for components of the recombination machinery such as the recombinase activating genes 1 (*RAG1*, *RAG2*) are the next most common genetic cause of SCID (Buckley 2004). The RAG1/RAG2 complex catalyzes recombination of the T cell receptor (TCR) and immunoglobulin loci; mutations in either *RAG1* or *RAG2* thus result in a failure to rearrange immune receptors and therefore a failure in T and B cell development (Schwarz, Gauss et al. 1996). Certain genes resulting in T- B- SCID are also associated with radiosensitivity, due to their roles in DNA repair, and are also associated with toxicities when exposed to alkylating agents (Schuetz, Neven et al. 2014). Adenosine deaminase (ADA) deficiency results in SCID due to the accumulation of toxic metabolites, and can be restored by enzyme replacement therapy, or by HCT (Parkman, Gelfand et al. 1975, Hershfield, Buckley et al. 1987). Patients with mutations in adenylate kinase 2 (*AK2*) have a subform of SCID, reticular dysgenesis, which is also associated with severe neutropenia and hearing deficit (Pannicke, Hönig et al. 2009).

Table 1: Genetic defects resulting in B+ or B- SCID. * Results in intrinsic B cell dysfunction. # Results in radiosensitivity.

T- B+	T- B-
*IL2RG**	*RAG1*
*JAK3**	*RAG2*
IL7R	*ADA*
CD3D	*DCLRE1C*[#]
CD3G	*PRKDC*[#]
CD3Z	*AK2*
CD45	

Historical and recent outcomes of HCT for SCID

Unlike HCT for most other malignant and non-malignant conditions, the severe impairment of adaptive immunity in SCID patients allows HCT to be performed without pre-transplant immunosuppressive conditioning. The purpose of conditioning in other diseases is to prevent rejection, to reduce/ablate the number of host hematopoietic stem cells (HSC) and give donor HSC a survival advantage, and to eliminate abnormal (i.e. malignant) cells. SCID patients, who by definition lack functional T cells, and often lack NK cells, generally lack the capacity to reject allogeneic cells. Infusion of bone marrow from a matched sibling donor contains mature HLA-matched T cells that can provide immediate T cell immunity over the first 1-3 months post HCT, and committed progenitors and HSC that generate thymically derived host-tolerant T cells starting around 4 months post HCT (Gatti, Meuwissen et al. 1968, Buckley, Schiff et al. 1999). Because the T cells are fully HLA matched, no prophylaxis of graft versus host disease (GVHD) is needed. Because pre-HCT conditioning is not given, the percentage of donor HSC that engraft is assumed to be very low, at most 5%, and usually less (Tjønnfjord, Steen et al. 1994,

Stiehm, Roberts et al. 1996). This typically leads to a state of split chimerism, in which T cells are donor-derived and B cells and other cells are recipient-derived. These split chimeric SCID recipients of HLA matched sibling grafts have T cell function but only a portion of them develop B cell function. In a recently published retrospective study of outcomes of HCT for SCID, examining 240 patients treated at PIDTC institutions from 2000 through 2009, 21 of 26 survivors of matched sibling donor HCT were no longer receiving immunoglobulin (Ig) infusions (Pai, Logan et al. 2014).

Haploidentical related donor HCT, unlike matched sibling donor HCT, requires manipulation of the graft to remove mature HLA-haploidentical T cells that would otherwise cause lethal GVHD (Reisner, Kapoor et al. 1983, Cowan, Wara et al. 1985, Buckley, Schiff et al. 1986). Rigorous T cell depletion is effective GVHD prophylaxis and these recipients generally do not require GVHD medications. Because mature T cells are excluded from the graft, T cell reconstitution relies on intrathymic development of the engrafted donor HSC and progenitors and thus typically takes at least 4-6 months to be detectable (Reisner, Kapoor et al. 1983, Cowan, Wara et al. 1985, Buckley, Schiff et al. 1986, Buckley, Schiff et al. 1999, Patel, Gooding et al. 2000). At many institutions pre-HCT conditioning is not given, and therefore the donor HSC engraftment percentage, in the few cases examined, is very low (Tjønnfjord, Steen et al. 1994, Stiehm, Roberts et al. 1996). Few patients achieve donor B cell engraftment, and while some SCID patients without donor B cell engraftment become free of Ig substitution, the majority (~60%) do not, including in our retrospective study where 46 of 73 survivors of haploidentical HCT remained on Ig substitution at 2-5 years post-HCT (Railey, Lokhnygina et al. 2009, Buckley 2011, Pai, Logan et al. 2014).

Unrelated donor HCT using adult volunteers or umbilical cord blood is performed without T cell depletion and in published experience commonly was performed with full myeloablative conditioning, with busulfan-containing regimens such as 16 doses of busulfan and 200 mg/kg of cyclophosphamide (Antoine, Müller et al. 2003, Grunebaum, Mazzolari et al. 2006, Gennery, Slatter et al. 2010, Pai, Logan et al. 2014). The use of conditioning increases the likelihood of donor HSC engraftment, as indicated by myeloid (granulocyte, monocyte) donor percentage, as a surrogate marker (Cavazzana-Calvo, Carlier et al. 2007, Pai, Logan et al. 2014). In studies where donor chimerism in myeloid cells has been reported, many patients have full or >95% donor chimerism. Donor B cell engraftment and hence B cell function are also more frequent than after unconditioned MSD or haploidentical HCT (Haddad, Landais et al. 1998, Haddad, Le Deist et al. 1999, Grunebaum, Mazzolari et al. 2006, Mazzolari, Forino et al. 2007, Slatter, Brigham et al. 2008, Pai, Logan et al. 2014).

GVHD causes significant morbidity and mortality in SCID patients, with rates of ~10-30% for grade 2-4 acute GVHD and 5-15% chronic GVHD reported in the literature (Grunebaum, Mazzolari et al. 2006, Neven, Leroy et al. 2009, Railey, Lokhnygina et al. 2009, Pai, Logan et al. 2014). In SCID patients, GVHD prevention relies almost exclusively on T cell depletion for haploidentical related donors and post-transplant immunosuppression for unmanipulated unrelated donor volunteer or cord blood donors. The use of post-HCT immunosuppression, typically with calcineurin inhibitors such as cyclosporine A or tacrolimus, delays

immunocompetence by interfering with T cell function for up to a year post-HCT and has significant side effects including nephrotoxicity, hypertension, electrolyte derangement and neurotoxicity. In contrast, T cell depletion using soybean agglutination and E-rosetting (SBA/E) or using CD34+ cell selection with the CliniMACS® system (Miltenyi Biotec) are highly effective and avoid the unwanted effects of calcineurin inhibitors, but have other drawbacks. SBA/E has a long track record, having been used for decades in centers specializing in HCT for SCID (Reisner, Kapoor et al. 1983, Cowan, Wara et al. 1985, Buckley, Schiff et al. 1986) and has good efficacy with ~3-log depletion of CD3+ T cells. However, SBA/E is not licensed, the reagents are highly unstandardized, and the technology has been largely abandoned. CD34+ selection is unlicensed for use in SCID, though is approved for use in adults with acute myelogenous leukemia, is commercially available and results in a more rigorous T cell depletion (>4-log depletion of CD3+ T cells). Because of the critical role of mature CD3+ T cells in both inducing GVHD and in facilitating engraftment, T cell depletion and CD34+ selection in particular increases the risk of graft rejection and results in prolonged immunoincompetence, (Aversa, Terenzi et al, 2005) as neogenesis of donor-origin T cells in the thymus takes at least 3-6 months.

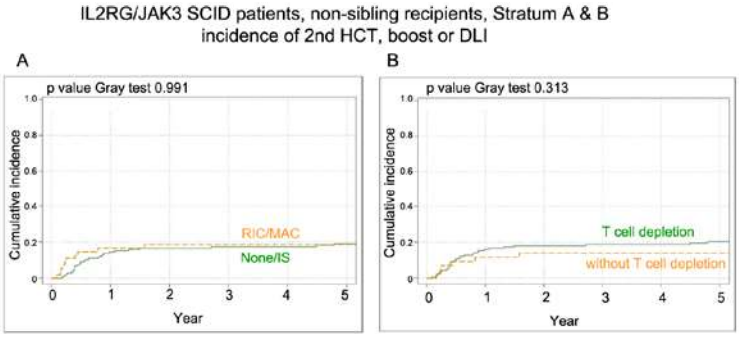
There is thus a critical need for more effective techniques that prevent GVHD while minimizing graft rejection and facilitating rapid post-HCT T cell immunocompetence. One such promising technique is the use of the CliniMACS® system to deplete only CD3+ T cells expressing αβ T cell receptors (TCRαβ+ T cells) along with depletion of CD19+ B cells to minimize risks of post-HCT EBV-LPD (Li Pira, Malaspina et al. 2016). While the vast majority of allogeneic T cells are removed by this technique, T cells expressing γδ T cell receptors are intact, and infusion of TCR γδ+ T cells appears to facilitate engraftment and provide post-HCT adoptive immunity (Airoldi, Bertaina, et al 2015, Bertaina, Merli et al, 2014). In addition, retention of neutrophils, monocytes, macrophages, NK-cells, and other WBC lineages within the graft provide other immune benefits to the recipient.

Engraftment and immune reconstitution in children with SCID varies according to genotype and HCT approach. Most studies to date have analyzed outcomes of SCID patients as a whole, or when possible have examined outcomes of B+ versus B- phenotypes; analysis of specific genetic subtypes has been limited to date. Preliminary analysis of our retrospective cohort undergoing HCT at PIDTC centers from 1968-2010 (RDCRN PIDTC Protocol 6902) combined with patients enrolled on the prospective natural history study of PIDTC (RDCRN PIDTC Protocol 6901) reveals significant differences in the need for second HCT and in immune reconstitution outcome depending on genetic subtype and HCT approach.

The cytokine receptor defects, *IL2RG* and *JAK3*, account for 211 of 662 patients with typical SCID (Stratum A) and leaky or Omenn syndrome (Stratum B), overall 32%. Survival of this group is excellent, even with donors other than matched siblings, >80% at 2 years. While infection remains an important determinant of survival in IL2RG/JAK3 patients, the rate of needing second HCT, boost or donor lymphocyte infusion (DLI) is low, and the use of conditioning does not affect the need for subsequent treatment (Fig. 1). Recipients of both T replete and T cell depleted grafts generally need only one intervention and there is no difference

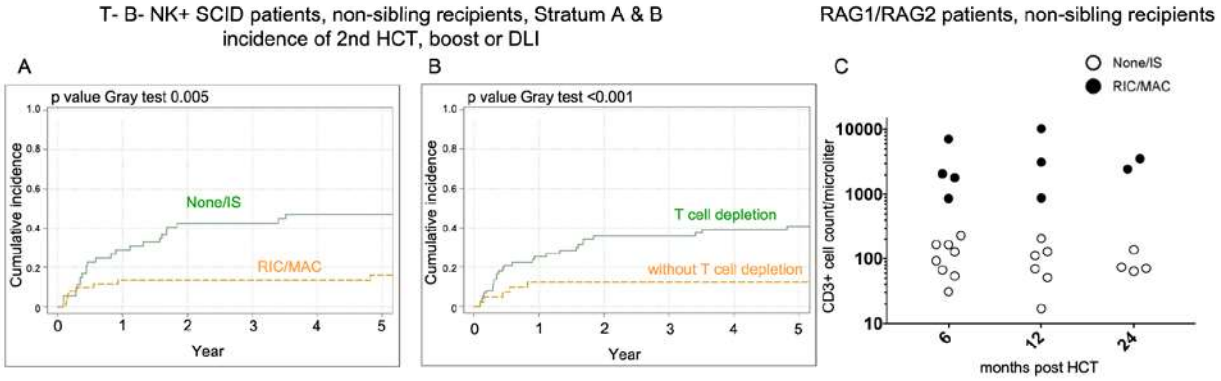
between different T cell depletion methodologies. T cell reconstitution and function as measured by CD3 counts and proliferation to phytohemagglutinin (PHA) are also equivalent regardless of conditioning or T cell depletion (data not shown, courtesy E. Haddad, B.R. Logan, R.J. O'Reilly).

Figure 1: Outcomes of non-matched sibling donor HCT for IL2RG/JAK3 SCID from the PIDTC retrospective registry, including both typical and leaky/Omenn SCID (Stratum A & B). Incidence of 2nd HCT, boost, or donor lymphocyte infusion (DLI) depending on conditioning (A) or T cell depletion (B).



Patients with *RAG1/RAG2* defects are expected to have a higher resistance to engraftment for several reasons. Unlike *IL2RG/JAK3* deficiency, *RAG1/RAG2* deficiency does not impact NK cell development and there is evidence in murine models that *RAG1/RAG2* deficient NK cells are hyperactivated, potentially increasing rejection risk (Karo, Schatz, Sun 2014). RAG deficiency results in accumulation of thymocyte precursors at the stage prior TCR rearrangement, and occupancy of the thymic niche compromises the ability of donor-derived HSC to engraft in the absence of conditioning in murine models (Prockop and Petrie, 2004). Historically hypomorphic or partially functional *RAG1/RAG2* mutations are responsible for the majority of cases of Omenn syndrome and leaky SCID; of 45 RAG-deficient patients in the PIDTC retrospective cohort, 28 are typical (Stratum A) and 17 are leaky or Omenn syndrome (Stratum B). The proportion of patients with RAG deficiency with atypical features is even higher currently, due to early identification through newborn screening; of 25 RAG deficient patients in the prospective PIDTC cohort, 7 were typical/Stratum A and 18 were leaky or Omenn/Stratum B (personal communication, C.C. Dvorak). Indeed T- B- NK+ patients in the retrospective cohort have a much higher rate of needing second HCT, boost or DLI when no conditioning or immunosuppression is used versus reduced intensity or myeloablative conditioning (Figure 2a). T- B- NK+ recipients of T cell depleted grafts are also at high risk of needing another intervention (Figure 2b). Combining 56 recipients of non-matched sibling donor HCT in the two cohorts, second HCT was much more frequent when conditioning was not used (8/15 versus 4/41). Among survivors, unlike *IL2RG/JAK3* patients, conditioning clearly was associated with higher T cell counts at 6 months, 12 months and 24 months post-HCT (Figure 2c). The vast majority of RAG deficient recipients undergoing transplant with conditioning received busulfan-based regimens, typically high dose busulfan (16 mg/kg total) and cyclophosphamide (200 mg/kg total).

Figure 2: Outcomes of non-matched sibling donor HCT for T-B-NK+ or RAG1/RAG2 SCID from the PIDTC retrospective registry including both typical and leaky/Omenn SCID (Stratum A & B). Incidence of 2nd HCT, boost or donor lymphocyte infusion (DLI) depending on conditioning (A) or T cell depletion (B). CD3 counts post-HCT in RAG1/RAG2 patients undergoing HCT without (open symbols, none/IS) or with conditioning (closed symbols, RIC/MAC)



It is important to reiterate that recipients who have successfully engrafted T cells have variable reconstitution of B cell function. Certain genetic variants, particularly patients with B+ SCID forms that affect intrinsic B cell function (*IL2G/JAK3*) (Recher, Berglund et al. Blood) and B- SCID forms that severely compromise B cell development (*RAG1/RAG2*) would be expected to have poor humoral outcome in the absence of conditioning and the absence of donor-derived B cell engraftment. We examined our retrospective cohort undergoing HCT between 2000-2009 (Pai, Logan et al, 2014), and found that only 5 of 39 patients with these 4 genetic subtypes undergoing HCT with donors other than matched sibling without chemotherapy conditioning were off of Ig substitution (13%). This finding was confirmed when analyzing the entire retrospective cohort of SCID patients (1968-2010); only 16 of 144 IL2RG/JAK3 and 0 of 15 RAG1/RAG2 patients at 2 years were off of Ig substitution (11-19%, 0-13% accounting for patients with unknown status). Conversely, conditioning, even when given at myeloablative doses, does not guarantee humoral immune function as reviewed recently (Haddad, Leroy et al, 2013). Examining the subset of the PIDTC retrospective cohort transplanted between 2000-2009 (Pai, Logan et al. 2014), 23 of 40 *ILRG/JAK3* and 6 of 14 *RAG1/RAG2* patients 2 years surviving at 2 years post undergoing alternative donor HCT with reduced intensity or myeloablative conditioning were off of Ig substitution (57-80%, 43-71%, accounting for patients with unknown status). Exposure to myeloablative conditioning leads to short and long-term toxicity. Regimen related toxicities include infection during neutropenia, veno-occlusive disease, pulmonary toxicity such as idiopathic pneumonitis syndrome and others. Long-term effects may include short stature, infertility, learning disabilities, secondary malignancies and others (Allewelt, El-Khorazaty et al 2016). Furthermore, conditioning would be contraindicated in patients with SCID who have active or treatment-resistant infections at the time of diagnosis (Pai, Logan et al. 2014). Historically many patients treated with myeloablative busulfan/cyclophosphamide received oral busulfan, without pharmacokinetic adjustment. It is unknown whether lower doses of busulfan, administered IV with uniform pharmacokinetic adjustment might be sufficient to promote B cell reconstitution and humoral immune responses.

Building from the observations made above, the PIDTC has designed a cooperative multi-

institutional trial of HCT for SCID with the goal of recruiting subjects from PIDTC centers and capitalizing on the success of the consortium to date. The proposed study would aim to address directly the minimal dose of conditioning needed to engraft sufficient donor HSC and support both T cell and B cell immune reconstitution with minimal rates of GVHD in patients with SCID. Patients with *IL2RG/JAK3* SCID or *RAG1/RAG2* SCID who lack matched sibling donors would undergo either haploidentical or matched unrelated donor HCT with busulfan-based regimens tailored to each genotype. Given our data above, *RAG1/RAG2* patients will receive additional agents to ensure high rates of T cell engraftment. To facilitate comparison of different donor sources and prevent GVHD without the need for post-transplant immunosuppressive medications, all grafts will be TCRαβ+/CD19+ cell depleted. If successful, this trial would be the basis for future prospective clinical trials of other novel conditioning approaches as they become available for clinical evaluation. In addition, the optimized busulfan approaches could be considered for other types of SCID or non-malignant disorders.

2.1.1 **Description of the Study Agent(s)/Intervention(s)**

To test the hypothesis that a reduced intensity conditioning regimen based on submyeloablative exposure of busulfan will be sufficient to effect engraftment of donor-derived B cells in patients with SCID affecting B cell development and/or intrinsic function, we will enroll patients with SCID of these genotypes lacking a matched sibling donor to undergo TCRαβ+/CD19+ depleted HCT using either a low dose or moderate busulfan exposure-based regimen.

2.1.2 **Summary of Relevant Clinical Studies**

Reduced intensity regimens with busulfan in patients with SCID

That busulfan-containing regimens are associated with a higher proportion of patients having donor B cell engraftment and humoral immune reconstitution, often defined as freedom from immunoglobulin substitution, is well established (summarized in (Haddad, Leroy et al. 2013)) However, the variability of humoral outcome, even among patients undergoing a myeloablative regimen, remains unexplained, and studies to date are inadequate to address the question. Studies published to date are retrospective, spanning many years, with different regimens, combining all genetic subtypes of SCID, and generally lack correlation of busulfan pharmacokinetics with outcome.

Busulfan has been used to promote engraftment for patients with nonmalignant disease for decades, and was first demonstrated to be efficacious in PID for treatment of Wiskott-Aldrich syndrome in combination with cyclophosphamide (Kapoor, Kirkpatrick et al. 1981). This approach has been extended to treatment of SCID due to the concern that certain genotypes, particularly those with functional NK cells such as defects in *RAG1* or *RAG2*, may be at risk of rejection despite the lack of T cells (Haddad, Landais et al. 1998, Antoine, Müller et al. 2003, Gennery, Slatter et al. 2010, Schuetz, Neven et al. 2014). The traditional dose and schedule of busulfan in these studies was 16 mg/kg, typically given as 1 mg/kg per dose every 6 hours for 16 doses or 4 days, with dose limiting toxicities of mucositis and veno-occlusive disease. In

more recent years, the anti-rejection alkylating agent cyclophosphamide has been replaced with fludarabine, a purine analog that induces profound lymphopenia. A 4-day regimen of busulfan and fludarabine has been used for treatment of malignancy (Russell, Tran et al. 2002, Bornhauser, Storer et al. 2003, de Lima, Couriel et al. 2004, Jenke, Freiberg-Richter et al. 2005, Andersson, de Lima et al. 2008, O'Donnell, Artz et al. 2010) and also for non-malignant disorders, including SCID and other PID (Classen, Schulz et al. 2001, Cancrini, Ferrua et al. 2010, Law, Cowan et al. 2012, Triplett, Wang et al. 2012). Excluding the second alkylating agent appears to be associated with a lower incidence of veno-occlusive disease, an important regimen related toxicity associated with busulfan-containing and other myeloablative regimens.

The high variability of busulfan exposure when dosed orally or in patients of different ages previously hampered attempts to control toxicity or correlate busulfan dose with therapeutic effect. The PK and pharmacodynamics of drugs in infants can differ widely between children and adults (Kearns, Abdel-Rahman et al. 2003, Bartelink, Rademaker et al. 2006, van den Anker, Schwab et al. 2011). Within the first year of life, age-related developmental changes in physiologic and metabolic processes can significantly lead to altered drug disposition (Kearns, Abdel-Rahman et al. 2003, Hines 2008). The group of Long-Boyle, in collaboration of members of PIDTC, has published several reports characterizing the variability of busulfan PK using advance population PK methodologies in a variety of pediatric HCT populations ranging from infants to children/adolescents and young adults (Savic, Cowan et al. 2013, Long-Boyle, Savic et al. 2015). This work demonstrates that individualized (e.g. personalized) model-based algorithms for busulfan clearance that incorporate body size and/or age provide improved targeted therapy when compared to stratified weight or age-based regimens alone (Bleyzac, Souillet et al. 2001, Tse, Duerst et al. 2009, Trame, Bergstrand et al. 2011, Bartelink, van Kesteren et al. 2012, Paci, Vassal et al. 2012).

Busulfan pharmacokinetics is typically measured around a single dose at local or commercial laboratories, and results used to adjust subsequent doses. The single dose pharmacokinetics can be expressed in several different units to achieve a myeloablative or submyeloablative/reduced intensity range (see Table 2). Total busulfan exposure of 14,400-19,200 umol*min (900-1200 umol*min single dose every 6 hours for 16 doses) or 59-79 mg*h/L is tolerated in combination with cyclophosphamide. Exposures up to 23,200 umol*min (1450 umol*min single dose every 6 hours for 16 doses) or 95 mg*h/L given in combination with fludarabine are associated with similar rates of veno-occlusive disease (O'Donnell, Artz et al. 2010). These ranges of exposures correlate with suppression of myeloid and erythroid colony-forming unit capacity of normal adult bone marrow in in vitro studies (see Figure 3, reproduced from (Hassan, Hellström-Lindberg et al. 2002)).

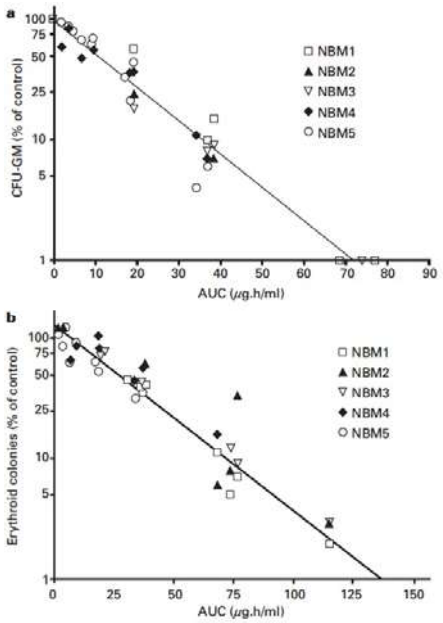
As busulfan pharmacokinetic monitoring and individual adjustment has become more widely available, efforts have been made to define submyeloablative busulfan exposures that are safe and efficacious for treatment of particular conditions. Two groups have published regimens showing efficacy of moderate exposure busulfan with fludarabine showing safety and efficacy in primary immunodeficiency patients.

Table 2: Ranges of target busulfan exposure with RIC and MAC regimens, and also those proposed in this trial. Css (continuous steady state), area under the curve (AUC) and cumulative AUC (cAUC) are shown.

		$Css_{(0\text{-}24h)}$ ng/ml	$AUC_{(0\text{-}6h)}$ single dose	$AUC_{(0\text{-}24h)}$ single dose	Cumulative AUC (cAUC)
		ng/ml	uM*min	uM*min	mg*h/L
Proposed in trial	**Low**	313	380-535	1830	**30 (25-35)**
	Moderate	625	840-990	3660	**60 (55-65)**
RIC/MAC regimens	RIC bu/flu	469-677	680-980	2720-3920	45-65
	MAC (bu/cy)	616-821	900-1200	3600-4800	59-79
	MAC (bu/flu)	833-989	1200-1450	4800-5800	80-95

Gungor et al published the outcomes of reduced intensity busulfan and fludarabine and allogeneic HCT in a multi-institutional study of 56 high-risk patients with chronic granulomatous disease (Gungor, Teira et al. 2014). The target busulfan exposure was moderate (45-65 mg*h/L), similar to the range used when busulfan is combined with cyclophosphamide (59-79 mg*h/L). Serotherapy of anti-thymocyte globulin, thymoglobulin or alemtuzumab was given according to donor type. The regimen was very well tolerated with rapid engraftment, no veno-occlusive disease, no grade 3-4 mucositis, 96% survival, and >90% donor chimerism in the myeloid lineage except for 1 early and 2 late graft failures.

Figure 3: Normal adult bone marrow was incubated with busulfan and plated in methylcellulose for 14 days. The remaining fraction of colonies is depicted. AUC was measured in the culture medium (from Hassan et al).



Ward et al studied the outcome of 33 patients with nonmalignant disease including 17 with SCID undergoing HCT with a regimen that included 2 daily doses of busulfan (8000-10,000 umol*min or 33-41 mg*h/L, fludarabine, rabbit anti-thymocyte globulin (rATG) (Ward, Kletzel et al. 2015. Some patients at higher risk of rejection (umbilical cord blood recipients) additionally got 5 mg/kg of thiotepa. Engraftment rates were generally high at 85%, and within the cohort who received the higher range of busulfan, 90%. Among 9 patients with IL2RG/JAK3 and 3 patients with RAG1/RAG2 treated with this regimen, with busulfan exposures 23.9-46.5 mg*h/L, there were no graft failures, all but one who died of EBV PTLD are alive, and 10 evaluable patients are off of Ig replacement (manuscript in preparation, unpublished data courtesy of W. Tse). Thus busulfan-based regimens with exposures of 25-65 mg*h/L, also containing fludarabine and serotherapy such as rATG, have been effective in achieving engraftment in patients with SCID and other immunodeficiencies.

That low exposure busulfan may be sufficient to promote B cell function is further supported by data from the PIDTC prospective natural history study (RDCRN PIDTC Protocol 6901). Ten patients (5 with IL2RG/JAK3 and 5 with RAG1/RAG2) underwent alternative donor transplant

with IV busulfan-containing regimens, and had documented exposure between 24.1-46.2 mg*h/L. We found that low to moderate exposures to busulfan did indeed result in 7 of 10 patients reconstituting humoral immunity, measured by freedom from Ig or vaccine response. The chance of humoral immune reconstitution seemed higher in RAG1/2 patients (4 of 5) and donor B chimerism data suggests dose dependence. In contrast neither B cell chimerism nor humoral immune reconstitution in IL2RG patients was related to level of exposure, but a low exposure of 25-45 mg*h/L nevertheless resulted in humoral immune reconstitution and high level B chimerism in 3 patients. There was no correlation with donor type, with other agents received during conditioning, or with age at HCT.

In the setting of autologous gene therapy using retroviral or lentiviral transduction of CD34+ hematopoietic progenitor cells, engraftment can be achieved without the need for agents to counter rejection. Indeed low dose busulfan has been used successfully as a single agent in gene therapy trials for ADA-deficient SCID, to promote engraftment of manipulated HSC and generation of multi-lineage gene-marked cells (Gaspar, Bjorkegren et al. 2006, Aiuti, Cattaneo et al. 2009, Gaspar, Cooray et al. 2011a, Candotti, Shaw et al. 2012). For X-linked SCID (IL2RG), gene therapy has been successful in restoring T cell development when performed without conditioning (Hacein-Bey-Abina, Le Deist et al. 2002, Gaspar, Parsley et al. 2004, Hacein-Bey-Abina, Pai et al. 2014), but B cell marking and function remains variable and poor, similar to the outcome after HCT (Hacein-Bey-Abina, Hauer et al. 2010, Gaspar, Cooray et al. 2011b, Hacein-Bey-Abina, Pai et al. 2014). Recently it was shown that older patients with IL2RG SCID undergoing gene therapy after low dose busulfan (5039.2-9057.8 umol*min or 20.7-37.1 mg*h/L) not only had T cell recovery but also multi-lineage gene marking and restoration of B cell function (De Ravin, Wu et al. 2016). Thus, busulfan exposure of ~30 mg*h/L is sufficient in patients with IL2RG SCID getting gene therapy to engraft gene-marked HSC.

Overall these data show that regimens that include low to moderate busulfan exposure (25-65 mg*h/L) with fludarabine and serotherapy have efficacy in patients with SCID and other non-malignant disorders. In the case of IL2RG SCID patients undergoing gene therapy, low dose busulfan alone is sufficient.

<u>TCRαβ+/CD19+ depleted HCT in nonmalignant and malignant disorders in children</u>
Approximately 2 decades ago, Miltenyi Biotec introduced a commercially available platform for T-cell depletion using an automated antibody/magnetic beads column system. Humanitarian Exemption IDE approval has been obtained for CD34+ cell selection as a method of T-cell depletion by this company. With very slow immune recovery and high rates of infection and rejection, newer antibody combinations using this platform have been introduced. Most recently, investigators throughout the world have shown promising outcomes using a TCRαβ+/CD19+ depletion approach. This approach removes the cells most closely linked with GVHD (TCRαβ cells) while preserving γδ-T cells and other key white cells in the product. In addition, the approach removes CD19+ cells to minimize risk of EBV-LPD. Table 3 shows the differences in graft composition compared to previous methods (Chaleff, Otto et al. 2007, Keever-Taylor, Devine et al. 2012, Li Pira, Malaspina et al. 2016, O'Reilly, Koehne et al. 2015, Rossi, Bernasconi et al. 2003). Using this approach, investigators have shown improved rates of

immune reconstitution compared to CD34+ depletion (Lang, Feuchtinger et al. 2015), low rates of GVHD, and high rates of survival (Bertaina, Merli et al. 2014, Airoldi, Bertaina et al. 2015, Lang, Feuchtinger et al. 2015, Balashov, Shcherbina et al. 2015, Li Pira, Malaspina et al. 2016). Bertaina et al published EFS in excess of 90% using no post-HSCT immune suppression after this T-cell depletion approach in a series of children undergoing HSCT for non-malignant disorders (including 8 patients with SCID) (Bertaina, Merli et al. 2014). A recent publication regarding efficiency of the procedure over time showed that with more than 200 procedures performed over several years, using procedures outlined by the manufacturer, efficiency of depletion was consistently high and products were high quality (Li Pira, Malaspina et al. 2016). These publications and others overall show rates of graft failure and GVHD that are comparable or lower than those seen using other methods. For comparison, in the PIDTC retrospective dataset, acute grade 2-4, grade 3-4 and chronic GVHD rates with either of these methods of TCD was 21-23%, 11-12% and 11-13% respectively; a 2nd treatment (2nd HCT, boost or donor lymphocyte infusion) was needed in 21% of SBA/E and 30% of CD34+ selected graft recipients. Regimens cited above and using CD34+ selection generally include serotherapy, most typically rATG. The use of serotherapy to prevent GVHD in SCID patients is further supported by a study of SCID patients undergoing HCT without alkylating agents (Dvorak, Hassan et al. 2014), and a recent study suggesting that the increased risk of GVHD seen with transplacental maternal engraftment can be mitigated by using serotherapy (Wahlstrom, Patel et al. 2016).

Table 3 (right): Comparison of graft characteristics from the literature for soybean lectin agglutination/sheep erythrocyte depletion (SBA- E-), CD34+ selection and TCRαβ/CD19 depletion.

	SBA- E-	CD34+ selection CliniMACS	TCRαβ/CD19 depletion
CD3 $\log_{10}$ depletion	3	4-5	4.1-4.2
CD3/kg (x 10^4)	5	1	1000
TCRαβ/kg (x 10^4)	2.5	n.r.	<10
TCRγδ/kg (x 10^4)	2.5	n.r.	1000
B cell/kg (x 10^4)	5	5	50
NK cell/kg (x 10^4)	40	1	3900

Table 4 (below): Results in the literature for pediatric patients undergoing TCRαβ/CD19 depleted mismatched unrelated donor (MMRD, haploidentical) or unrelated donor (URD) HCT.

	Disease		Donor							
Ref	Nonmalignant (SCID)	Malignant	MMRD	URD	Conditioning	Serotherapy	Graft failure	aGVHD 2-4	aGVHD 3-4	cGVHD
Maschan 2016		33	13	20	Treo/mel/flu	hATG	0/33	0.390	0.160	0.30
Im 2016	8 (0)	34	42		Flu/Cy/TBI	rATG	0/42	0.310	0.120	0.17
Lang 2015	5	36	41		flu/clo/mel/TT	OKT3 or ATG-F	5/41	0.244	0.146	0.28
Lang 2016	6 (1)	24	30		Flu/mel/TT	rATG or 7Gy	5/30	0.033	0.000	n.r.
Total malignant	**19**	**127**	**126**	**20**			0.07	0.25	0.11	0.20
Bertaina 2014	23 (8)		23		Bu/flu/TT	rATG	2/23	0.131	0.000	0.00
Balashov 2015	37 (5)		10	27	Treo/flu	rATG/Campath	10/37	0.215	0.028	0.05
Kapoor 2016	23 (11)		23		Treo or bu based	rATG	0/23	0.040	0.000	0.04
Total nonmalignant	**83**	**0**	**56**	**27**			**0.14**	**0.14**	**0.01**	**0.04**

Dr. Pulsipher is PI and holds an IDE for a large multicenter trial looking at the role of KIR favorable haploidentical transplantation using TCRαβ+/CD19+ haploidentical donors (NCT026468390). The trial is open and enrolling at 12 PIDTC centers and has enrolled 25 patients. Another large, multicenter trial sponsored by Bellicum Pharmaceuticals (NCT02065869) is running at 10 centers in the US (all PIDTC centers), with CHLA being the highest accruing center. This group presented data at the American Society of Hematology

meeting 2016 on 23 PID patients having undergone this procedure (11 SCID patients). All patients engrafted and rates of GVHD are low (Kapoor, Bertaina et. al., ASH 2016). Centers participating in this protocol will either have TCRαβ+/CD19+ depletion validated for performance by their local stem cell labs, or will use one of 5 central processing labs to perform the selection followed by shipment to the local center.

2.1.3 **Summary of Epidemiological Data**

SCID is rare, and patients with SCID are typically asymptomatic at birth. Universal newborn screening for SCID by the absence of T cell receptor excision circles (TREC) in dried blood spots has been implemented in 42 states and 3 other regions in the United States, accounting for 86.8% of the births. Of 8 states not currently screening, 5 have plans for pilots in the next 2 years. Diagnosis of SCID in asymptomatic newborns has led to a revision of estimates of the incidence of SCID. Based on data from 11 screening programs and 3,030,083 births, 52 cases of SCID (42 typical, 10 leaky or Omenn syndrome) were found, in other words 1 in 58,000 infants (Kwan, Abraham et al. 2014). Additionally, the distribution of genotypes appeared to be different than previously reported. While previous series found 36-50% of SCID in the United States were due to mutations in *IL2RG*, and only 3-7% due to mutations in *JAK3* or *RAG1/RAG2*, among 52 infants with typical or leaky SCID found by newborn screening, 10/52 (19%), 3/52 (6%), and 9/52 (17%) had mutations in *IL2RG*, *JAK3*, and *RAG1/RAG2* respectively.

Based on the 2012 US birth data with 3,952,841 births, we thus project that 59 newborns/year will be diagnosed with SCID in states currently screening, or 64/year in 2018 once states planning pilot programs have begun screening.

2.2 Rationale

This study targets a high-risk population of patients with SCID and aims to improve immune reconstitution outcome while minimizing toxicity and GVHD. Our data and others amply demonstrate that SCID patients undergoing haploidentical or unrelated donor transplant have lower survival, higher rates of GVHD, and poorer immune reconstitution compared to matched sibling donor recipients (Antoine, Müller et al. 2003, Gennery, Slatter et al. 2010, Haddad, Leroy et al. 2013, Pai, Logan et al. 2014). We will thus enroll patients with SCID, as PIDTC has defined, either typical, leaky or Omenn syndrome (Shearer, Dunn et al. 2013) who lack matched sibling donors.

To test the effect of submyeloablative busulfan exposure on B cell reconstitution post-HCT, we must ensure T cell reconstitution in all patients. The rate of T cell reconstitution is high after unconditioned haploidentical HCT in SCID patients in general, (Buckley, Schiff et al. 1999, Buckley 2011). In a recently reported retrospective review of 37 patients with SCID undergoing unconditioned HCT using unrelated adult volunteer or umbilical cord blood donors, the rate of T cell engraftment was high, 92% (Dvorak, Hassan et al. 2014). We detailed above that T cell reconstitution varies by genotype, in part because of the higher percentage of patients with

leaky SCID and Omenn syndrome among *RAG1/RAG2* patients. To ensure T cell engraftment therefore, agents typically used in standard reduced intensity regimens described above (fludarabine, thiotepa and rATG) will be included for *RAG1/RAG2* patients.

To test our hypothesis in well-defined populations most in need of improvement in humoral reconstitution, we will limit enrollment to common genotypes of SCID that have intrinsic defects in B cell function (*IL2RG*, *JAK3*) or lack B cells (*RAG1, RAG2*). We will exclude patients who are otherwise undefined and patients with genetic subtypes associated with increased toxicity to busulfan.

GVHD is an undesirable complication in SCID patients. We will use TCRαβ+/CD19+ depletion as GVHD prevention, which avoids the need for post-transplant immunosuppressive medications and also retains immune cells in the graft anticipated to promote immune reconstitution and earlier immunocompetence than other methods.

We hypothesize that the majority of patients undergoing alternative donor HCT after low (25-35 mg*h/L) or moderate (55-65 mg*h/L) busulfan exposure will achieve humoral immune reconstitution and high level donor B cell chimerism. Because our goal is to identify the least toxic regimen that results in full T and B cell function, if both regimens are equally efficacious, the low exposure regimen would be favored for future clinical use. Comparison of the primary endpoint between arms will ensure that we detect whether moderate busulfan exposure is more efficacious than low busulfan exposure.

2.3 Potential Risks and Benefits

As reviewed in section 2.1, SCID is fatal in early childhood unless treated with HCT. Approaches to HCT for SCID have varied from simple infusion of bone marrow or peripheral blood stem cells without a preparative regimen to fully myeloablative regimens. Full engraftment and consistent B and T-cell immune reconstitution using donors other than matched relatives (unrelated or haploidentical related) rarely occurs unless preparative chemotherapy is given. Inadequate immune reconstitution can either result in a second transplant with more exposure to potentially toxic chemotherapy or severe infections due to poor host immunity. In this context, this protocol treats patients with genetic potential for engraftment with low or moderate dose exposure to busulfan chemotherapy who are in a state where success is most likely (newborn screened and/or non-infected at the time of transplant) and compares a busulfan exposure achieved with traditional busulfan/cyclophosphamide regimen (55-65 mg*h/L) to a reduced busulfan exposure (25-35 mg*h/L) in an attempt to define a minimally toxic approach that results in T- and B-cell immunity in the majority of patients. In one of our genotypic cohorts (IL2RG/JAK3) there is little to no T-cell function, and therefore we are further reducing risk of chemotherapy exposures in these patients by using busulfan without any other chemotherapeutic agents. Many patients in the RAG1/2 cohort, however, are at risk of failure of T cell reconstitution, and successful engraftment at the lower busulfan exposure will be facilitated by the addition of fludarabine and thiotepa. Based on the effectiveness of thiotepa to improve engraftment of T cell depleted grafts in mice (Terenzi, Lubin et al. 1990), thiotepa has been used extensively in transplantation for thalassemia at 8-10 mg/kg (Bernardo, Zecca et al.

2008, Bernardo, Piras et al. 2012, Choudhary, Sharma et al. 2013, Angelucci, Matthes-Martin et al. 2014); for this protocol we will choose the lower dose of 8 mg/kg. All patients will receive rATG for prevention of GVHD. Although the risks of specific agents will be reviewed in section 2.3.1, it should be understood that the intent of the protocol is to decrease the toxicity patients often receive from higher dose, standard preparative regimens.

2.3.1 **Potential Risks**

Major risks associated with HCT for SCID disorders include:

1. Death due to infection while the patient is recovering immunity
2. Organ damage caused by infection or by chemotherapy given during the preparative regimen prior to transplantation
3. Rejection of the infused hematopoietic cells necessitating subsequent infusions of cells or a second transplant with another preparative regimen,
4. Graft vs. host disease which can cause short or long-term organ damage, require long-term immune suppression, and increase risk of mortality before it resolves,
5. Inadequate immune reconstitution leading to long-term need of IVIG and impairment of quality of life due to a chronic immune deficiency state, and
6. Late effects due chemotherapy associated with the preparative regimen, graft vs. host disease or infections that occur prior to immune recovery.

Some centers give full myeloablative preparative regimens as a standard of care, resulting in full B- and T-cell immune reconstitution in many of their patients, but with an increased risk of short-term mortality and late effects. Other centers recommend minimal or no preparative regimens resulting in inadequate immune reconstitution and long-term chronic immune deficiency in many patients. **This protocol seeks to define whether one or both of two busulfan-based preparative regimens (low or moderate dose) are adequate to establish T- and B-cell immune reconstitution in a defined genotypic population.** There is a chance that the doses chosen may be too low, resulting in inadequate engraftment with resultant long-term immunodeficiency or a need for a second procedure. If doses are too high, there may not be the desired decrease in toxicity compared to standard myeloablative approaches.

The risks of specific agents associated with this approach are described below. The exact risks of infertility, organ damage, or other risks listed below using the approach taken in this protocol is unknown, but it is anticipated that the risks will be decreased compared to high dose standard busulfan and cyclophosphamide regimens.

Busulfan (Bu)

Toxicities:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, fever, electrolyte changes (hypokalemia, hypomagnesemia, hypocalcemia, hypophosphatemia, and hyponatremia), hyperglycemia, dizziness, rash, pruritus, urticaria, injection site pain and inflammation, back pain, tachycardia, chest pain, edema, insomnia, anxiety, depression, headache, abdominal pain, diarrhea (L) or constipation, anorexia, rectal discomfort, dyspnea, epistaxis	Weight gain, confusion	Seizures (rare with phenytoin prophylaxis), hematemesis, hyperuricemia, arrhythmias other than tachycardia, pleural effusion, alveolar hemorrhage
Prompt: Within 2-3 weeks	Myelosuppression, asthenia, immunosuppression (L), mucositis, hyperbilirubinemia	Hepatotoxicity, sinusoidal obstruction syndrome (SOS, formerly VOD) (L), mild alopecia (L), arthralgia, myalgia, hemorrhagic cystitis, hyperpigmentation (L), elevated creatinine and BUN	Reduced adrenal function (L), esophagitis, radiation recall reactions
Late: Any time after completion of treatment	Infertility, testicular atrophy and azoospermia, amenorrhea, ovarian failure		Secondary malignancy, breast enlargement, cataracts, idiopathic pulmonary syndrome (cough, dyspnea, pleural effusion, infiltrates, and hypoxemia), bronchopulmonary dysplasia with interstitial pulmonary fibrosis and pneumonitis, myocardial fibrosis, osteonecrosis

Anti-thymocyte globulin (ATG)

Common side effects of ATG include nausea, fever, chills, diarrhea, rash, dizziness, headache and tiredness. More serious side effects can include severe allergic reaction, serum sickness, easy bleeding/bruising, fast/irregular heartbeat, joint/muscle pain, stomach/abdominal pain, and weakness. Because this drug works by weakening the immune system, it lowers the ability to fight infections. No dose adjustments are required.

Fludarabine

The major dose-limiting toxicity of fludarabine is myelosuppression. Nausea and vomiting are usually mild. Side effects reported commonly include anorexia, fever and chills, alopecia and rash. Neurotoxicity can be manifested by somnolence, fatigue, peripheral neuropathy, mental status changes, cortical blindness and coma and is more common at high doses. Neurotoxicity is usually delayed, occurring 21-60 days after the completion of a course of therapy and may be irreversible. Side effects reported less commonly include diarrhea, stomatitis, increased liver function tests, liver failure, chest pain, arrhythmias and seizures. Pulmonary toxicity includes allergic pneumonitis characterized by cough, dyspnea, hypoxia and pulmonary infiltrates. Drug induced pneumonitis is a delayed effect, occurring 3-28 days after the administration of the third or later course of therapy. Administration of corticosteroids usually results in resolution of these symptoms.

Thiotepa

Dose limiting toxicity is myelosuppression. The leukocyte nadir may occur at any time from 10 to >30 days. Other toxicities include pain at the injection site, nausea and vomiting, anorexia, mucositis, dizziness, headache, amenorrhea, interference with spermatogenesis, and depigmentation with topical use. Allergic reactions, including skin rash and hives, have been reported rarely. Rare cases of apnea, hemorrhagic cystitis, and renal failure have occurred. Thiotepa is mutagenic, carcinogenic, and teratogenic in animals. Pregnancy category D.

2.3.2 **Potential Benefits**

As outlined in section 2.1, it is highly desirable to optimize the amount of chemotherapy needed in a preparative regimen, giving the lowest amount that will result in the desired target of an immune system with both T- and B-cell function. Increasing the number of SCID children achieving productive immune responses without having to undergo a myeloablative regimen and thus likely have less toxicity (both short and long term) is a major potential benefit. By using TCRαβ+/CD19+ depletion we also aim to reduce the risk of acute and chronic GVHD as much as possible while avoiding the need for post-HCT immunosuppression and the attendant side effects of those medications.

3 STUDY OBJECTIVES

To evaluate the safety and efficacy of TCRαβ+/CD19+ depleted allogeneic HCT following either a low dose or moderate dose busulfan-based reduced intensity conditioning regimen in the treatment of 2 genotype-driven cohorts of infants diagnosed at birth with SCID.

3.1 Primary Objective

To determine the incidence of humoral immune reconstitution by 2 years post-transplant in 2 SCID cohorts (*IL2RG/JAK3*, *RAG1/RAG2*) undergoing TCRαβ+/CD19+ depleted haploidentical related and unrelated donor HCT by randomized assignment to a busulfan-based preparative regimen targeted at cumulative area-under-the-curve (cAUC) exposure of 25-35 mg*h/L vs. 55-65 mg*h/L.

3.2 Secondary Objectives

Secondary objectives are to assess immune reconstitution, cell type specific engraftment, survival, event-free survival, and transplant-related complications. We will also assess the accuracy of busulfan targeting and graft characteristics. We will assess:

1. T cell number and function, naïve T cell generation, kinetics of humoral immune response, response to live viral vaccine.

2. Donor cell engraftment in whole blood and blood sorted for CD3, CD19, CD56, CD15 post-HCT.

3. Event-free and overall survival.

4. Incidence of transplant-related toxicity, acute and chronic GVHD, autoimmunity.

5. Observed cumulative exposure of busulfan.

6. The relationship of graft characteristics (CD34+ cell, TCRαβ+ cell, TCRγδ+ and CD19+ cell counts/kg) to rates of engraftment, acute and chronic GVHD, and immune reconstitution.

3.3 Exploratory Objectives

1. To study the relationship between busulfan exposure and lineage specific donor engraftment from sorted peripheral blood ($CD3^+$, $CD19^+$, $CD3^-$ $CD56^+$, $CD15^+$) and between busulfan exposure and in vitro expanded $CD34^+$ peripheral blood cells as a surrogate for bone marrow HSC chimerism.

2. To analyze B cell phenotype (percentage of memory B cells), B cell function (in vitro generation of plasmablasts in response to CD40L and IL-21) and *IGH* repertoire, examine correction of abnormalities seen within each genotype cohort, and correlate with donor B cell engraftment level and vaccine response.

3. To examine the correlation between split chimerism (T donor, B and/or myeloid host) or mixed chimerism in the B and myeloid lineages with markers of T cell reconstitution and exhaustion.

4. To explore the mechanisms of tolerance post-HCT (clonal deletion versus peripheral regulation) and the correlation between the dominant mechanism of tolerance and HLA-mismatch (haploidentical versus closely matched unrelated donor).

5. To examine the relationship between pre- and post-transplant active ATG area-under-the-curve, IL-7 levels and outcomes including survival, T cell recovery and acute GVHD.

6. To explore the pharmacokinetics of fludarabine and thiotepa in this population and the relationship between these and graft rejection, lineage specific donor cell engraftment.

4 STUDY DESIGN

4.1 Description of the Study Design

This is a prospective, multicenter, phase II, open-label study of two reduced busulfan dose levels in newborns identified at birth with SCID of appropriate genotype/phenotype and clinical status, undergoing either haploidentical related or well-matched unrelated donor TCRαβ+/CD19+ depleted HCT. Subjects will be enrolled on either of 2 strata according to genotype (defects of cytokine receptor function i.e. *IL2RG* or *JAK3* and defects of receptor recombination i.e. *RAG1* or *RAG2)*. Thus up to 32 subjects on each of 2 strata or 64 subjects total would be enrolled over 4 years with 3 years follow-up.

Patients with *IL2RG/JAK3* would be randomized to receive busulfan targeted either to cumulative exposure of 25-35 mg*h/L or 55-65 mg*h/L with Thymoglobulin. Patients with *RAG1/2* would be randomized to receive busulfan targeted to cumulative exposure of 25-35 mg*h/L or 55-65 mg*h/L, in conjunction with fludarabine, thiotepa and Thymoglobulin. Safety/feasibility of the novel TCR αβ+/CD19+ depleted allogeneic HCT strategy will be monitored on an ongoing basis using stopping rules for lack of neutrophil engraftment and other important short-term toxicities.

Donor selection would be determined clinically at the discretion of the treating clinicians at each site. Pharmacokinetic monitoring of busulfan exposure will be performed per local practices at CLIA-certified laboratories. Patients will receive busulfan and pharmacokinetic measurement to individualize dosing. Time-concentration data of the initial dose and subsequent doses will be reviewed centrally (Dr. Janel Long-Boyle) using a cloud-based application (InsightRx) to guide dose adjustment in real-time (Long-Boyle, Chan, Keizer, 2017, ASBMT Tandem abstract accepted). Clinical and laboratory data will be collected at defined time points over 3 years and entered in an electronic data capture system using study-specific case report forms. These data will be used to measure the outcomes including the primary outcome (cAUC of busulfan that promotes humoral immune reconstitution at 2 years post HCT with acceptable regimen-related toxicity at 42 days post HCT) and secondary outcomes (the quality of donor cell engraftment and immune function achieved in B and T cell compartments and survival). Mechanistic studies supporting the exploratory endpoints will be conducted centrally in designated laboratories.

4.2 Study Endpoints

4.2.1 Primary Endpoints

Humoral immune reconstitution by 2 years post HCT, defined by specific antibody response to tetanus toxoid.

Criteria for evaluation of humoral immune response are the following:

- Donor T cell chimerism ≥50%
- B cell count ≥50 cells/microliter
- IVIG independent for ≥12 weeks (see Section 6.3)

Subjects meeting the criteria receive 3 doses of tetanus toxoid at least 4 weeks apart, followed by measurement of tetanus titer at least 4-6 weeks after the 3rd dose (see Section 6.3). Those who achieve tetanus titer of ≥0.15 IU/ml after vaccination will meet the primary endpoint. Patients who have documented humoral immune response at a time prior to 2 years will be considered a success for the primary endpoint, while patients who do not have humoral immune response evaluated by 2 years will be considered failures for the primary endpoint.

4.2.2 Secondary Endpoints

1. Immune reconstitution
    - T cell immune reconstitution will be assessed on all patients at 30 days, 60 days, 100 days, 6 months, 12 months, and 2 years post-HCT. We will assess immune reconstitution based on the following 4 criteria:
        - Sorted donor T cell chimerism (CD3) measured by STR >80%
        - Absolute CD3 count >1000/microliter
        - Absolute CD4 count >500/microliter
        - Lymphocyte proliferation to PHA >30% of the lower limit of normal control
    - Naïve T cell generation and thymic output will be assessed on all patients at 100 days, 6 months, 12 months and 2 years post-HCT. We will measure naïve CD4 and CD8 T cell percentages and recent thymic emigrants using flow cytometry (CD45RA, CCR7, CD31) as part of extended lymphocyte phenotyping. We will measure TREC levels at 1 year and 2 years.

- Freedom from immunoglobulin substitution will be assessed on all patients at 6 months, 9 months, 12 months, 2 years and 3 years post-HCT. Patients who have not received IVIG for at least 12 weeks at the time of assessment will be considered free from immunoglobulin substitution.

- Tetanus responses will be assessed on all patients who complete a trial of vaccination by additional timepoints of 12 months, 18 months and 3 years post-HCT.

  Subjects who do not meet the primary endpoint but undergo vaccination trial before 3 years will be assessed for tetanus toxoid response as detailed for the primary endpoint.

- Live vaccine responses will be assessed on all patients who undergo trial of vaccination by 3 years post-HCT

  Patients who meet the CDC guidelines for live vaccination post-HCT (1-5 years old CD4+ T cell count ≥1000/microliter, ≥6 years old CD4+ T cell count ≥500/microliter), without evidence of chronic GVHD, and documented response to tetanus will receive MMR and Varicella vaccine with pre- and post-vaccination (>4 weeks) titer measurement.

2. Engraftment

   - Neutrophil engraftment will be assessed on all patients and defined as achieving an absolute neutrophil count of >500 cells/microliter for 3 consecutive days by day 42 post-HCT

   - Donor cell chimerism will be assessed on all patients at 42 days, 3 months100 days, 6 months, 12 months, and 2 years post-HCT. Absolute B cell, NK cell and granulocyte counts will be measured. Whole blood, sorted CD3, CD19, CD56 and CD15 chimerism will be measured and scored as follows:

     - <5% donor = autologous reconstitution

     - 5-50% donor = low mixed chimerism

     - 51-95% donor = high mixed chimerism

     - >95% donor = full donor chimerism

3. Survival

   - Overall survival at 1 year, 2 years and 3 years post-HCT

     Events will be defined as death from any cause.

- Event free survival at 1 year, 2 years and 3 years post-HCT

  Events will be defined as 1) death from any cause, 2) rejection of the graft (T-cell and/or whole blood chimerism <5% donor), 3) graft failure necessitating a second HCT procedure from the same donor or a different donor, with or without conditioning, 4) DLI given for treatment of falling chimerism.

4. Graft-versus-host disease

   - Occurrence of acute (grade II-IV and grade III-IV) GVHD by 100 days and 6 months post-HCT. Any skin, gastrointestinal or liver abnormalities fulfilling the consensus criteria of grade II-IV acute GVHD or grades III-IV acute GVHD are considered events. Death is a competing risk, and patients alive without acute GVHD will be censored at the time of last follow-up.

   - Occurrence of chronic GVHD by 6 months, 12 months and 2 years post-HCT. Occurrence of symptoms in any organ system fulfilling the criteria of limited or extensive chronic GVHD will be recorded along with a second classification of mild, moderate, and severe according to NIH criteria. Death is a competing risk, and patients alive without chronic GVHD will be censored at time of last follow-up.

5. Post-HCT complications

   - Infections

   The following significant infections will be reported the first year post-HCT: 1) bacteremia, not including cultures thought clinically to be contaminants, 2) viremia with CMV, HSV, VZV, or EBV and/or pathological confirmation of tissue invasion with these viruses, 3) fungemia or suspected and/or confirmed invasive fungal infection, and 4) serious documented or suspected bacterial or viral respiratory infections resulting in hospitalization for pneumonia, prolonged hospitalization, or intubation. These infections will be reported by site of disease, organism, date of onset, and resolution.

   - Targeted regimen related toxicity

   The proportion of subjects experiencing regimen related toxicity in the first 42 days post HCT will be measured. Regimen related toxicity includes:

     - Severe veno-occlusive disease of the liver

     - Idiopathic pneumonitis syndrome

- Autoimmunity

  Occurrence of autoimmunity requiring treatment with immunosuppression or other therapy, including autoimmune cytopenias.

6. Busulfan PK

   Comparison of desired target exposure with observed target exposure

7. Graft composition

   Frequency of achieving the target CD34+ cell/kg and TCRαβ+/CD19+ depletion goals and the relationship of infused cells to key outcomes (engraftment, immune reconstitution, etc.).

4.2.3 **Exploratory endpoints**

1. Relationship of busulfan exposure with lineage specific donor chimerism

   - Measurement of total area-under-the-curve exposure to busulfan in patients randomized to 30 mg*h/L versus 60 mg*h/L

   - Comparison of chimerism in sorted T, B, NK and myeloid cells and in expanded peripheral blood CD34+ cells in patients randomized to busulfan exposure of 30 mg*h/L versus 60 mg*h/L

2. Correlation between level of donor B cell chimerism and vaccine response to correction of abnormalities of B cell phenotype, B cell function, *IGH* repertoire

   - Measure markers of B cell function (percentage of CD27+ IgD- switched memory B cells, plasmablast generation, somatic hypermutation rates) pre-transplant and serially post-transplant in subjects with different levels of B cell chimerism (>95%, 51-95%, 5-50%, <5%)

3. Correlation between mixed or split chimerism in B and myeloid lineages with markers of T cell exhaustion

   - Measure markers of T cell exhaustion (percentage of T cells expressing inhibitory receptors, percentage of T cells expressing more than 2 inhibitory receptors, percentage of CD8+ T cells that are CD45RA+ CCR7-) in subjects with full donor (>95%) versus mixed/split B cell and/or myeloid chimerism (0-94%)

4. Examination of T cell tolerance and correlate with HLA-mismatch

    - Determine the degree to which subjects exhibit peripheral regulation versus clonal deletion as the dominant mechanism of tolerance after haploidentical versus unrelated donor transplant

5. Relationship of pre- and post-transplant active ATG area-under-the-curve and IL-7 levels with outcomes including survival, T cell recovery and acute GVHD

    - Measure pre- and post-transplant active ATG area-under-the-curve

    - Measurement of lymphocyte subsets at day 0 and IL-7 levels pre- and post-transplant to assess the relationship between pre-transplant active ATG area-under-the-curve, IL-7 levels and lymphocyte counts.

    - Compare survival, T cell count at 100 days and incidence of acute GVHD in patients with different pre- or post-transplant ATG levels

6. To explore the pharmacokinetics of fludarabine and thiotepa in this population and the relationship between these and graft rejection, lineage specific donor cell engraftment.

5 STUDY POPULATION

SCID is a disorder that affects infants, and this study seeks to enroll infants diagnosed with SCID at birth, through universal newborn screening or clinical screening due to positive family history. Because the majority of children evaluated for inclusion will be identified by universal newborn screening, the proportion of minorities included in the study is expected to be comparable to that of the general North American population. Both the X-linked form and certain autosomal recessive forms of SCID will be included and thus both boys and girls will be enrolled.

To avoid enrolling subjects with a known increased risk of toxicity after exposure to busulfan, an alkylating agent, potential subjects who do not have a genetic diagnosis will undergo rapid genotyping for the eligible genotypes. Potential subjects found to have mutations in genes known to be associated with radiation sensitivity as listed below will be excluded.

Because different genotypes may have different outcomes, subjects will be enrolled in separate cohorts based on genotype. The proposed sample size is a total of up to 64 subjects, with 16 subjects randomized to each busulfan dose intensity for each of the 2 genotype categories.

Subjects will be enrolled at PIDTC and PBMTC centers, the majority of which will be drawn from states conducting statewide universal newborn screening for SCID.

5.1 Description of the Study Population

5.1.1 Participant Inclusion Criteria

Inclusion Criteria

1. Infants with SCID, either typical or leaky or Omenn syndrome.

   a. Typical SCID is defined as either of the following

   - Absence or very low number of T cells (CD3+ T cells <300/microliter AND no or very low T cell function (<10% of lower limit of normal) as measured by response to phytohemagglutinin

   OR

   - Presence of maternally derived T cells

   b. Leaky SCID is defined as the following
   - Absence of maternally derived T cells
   - **AND** either one or both of the following (i, ii):

     i) <50% of lower limit of normal T cell function as measured by response to PHA OR <30% of lower limit of normal T cell function as measured by response to CD3

     ii) Absent or <10% of lower limit of normal proliferative responses to candida and tetanus toxoid antigens (must document post vaccination or exposure for this criterion to apply)

   - **AND** at least two of the following (i through iii):

     i) CD3 T cells < 1500/microliter

     ii) ≥80% of CD3+ or CD4+ T cells are CD45RO+ AND/OR ≥80% of CD3+ or CD4+ T cells are CD62L negative AND/OR >50% of CD3+ or CD4+ T cells express HLA-DR (at ≤ 4 years of age) AND/OR are oligoclonal T

     iii) Low TRECs and/or the percentage of CD4+/45RA+/CD31+ or CD4+/45RA+/CD62L+ cells is below the lower level of normal.

   c. Omenn syndrome

   - Generalized skin rash
   - Maternal lymphocytes tested for and not detected.

- ≥80% of CD3+ or CD4+ T cells are CD45RO+ AND/OR ≥80% of CD3+ or CD4+ T cells are CD62L negative AND/OR >50% of CD3+ or CD4+ T cells express HLA-DR (≤2 years of age)

- Absent or low (up to 30% lower limit of normal (LLN)) T cell proliferation to antigens (Candida, tetanus) to which the patient has been exposed

IF: Proliferation to antigen was not performed, but at least 4 of the following 8 supportive criteria, at least one of which must be among those marked with an asterisk (*) below are present, the patient is eligible as Omenn Syndrome.

1. Hepatomegaly
2. Splenomegaly
3. Lymphadenopathy
4. Elevated IgE
5. Elevated absolute eosinophil count
6. *Oligoclonal T cells measured by CDR3 length or flow cytometry (upload report)
7. *Proliferation to PHA is reduced to < 50% of lower limit of normal (LLN) or SI < 30
8. *Low TRECs and/or percentage of CD4+/RA+ CD31+ or CD4+/RA+ CD62L+ cells below the lower level of normal

2. Documented mutation in one of the following SCID-related genes

   a. Cytokine receptor defects (*IL2RG, JAK3*)

   b. T cell receptor rearrangement defects (*RAG1, RAG2*)

3. No available genotypically matched related donor (sibling)

4. Availability of a suitable donor and graft source

   a. Haploidentical related mobilized peripheral blood cells

   b. 9/10 or 10/10 allele matched (HLA-A, -B, -C, -DRB1, -DQB1) volunteer unrelated donor mobilized peripheral blood cells

5. Age 0 to 2 years at enrollment

   Note: to ensure appropriate hepatic metabolism, age at time of busulfan start:
   For *IL2RG/JAK3*: 8 weeks
   For *RAG1/RAG2*: 12 weeks

6. Adequate organ function defined as:

   a. Cardiac:
   Left ventricular ejection fraction (LVEF) at rest ≥ 40% or, shortening fraction (SF) ≥ 26% by echocardiogram.

   b. Hepatic:
   Total bilirubin < 3.0 x the upper limit of normal (ULN) for age (patients who have been diagnosed with Gilbert's Disease are allowed to exceed this limit) and AST and ALT < 5.0 x ULN for age.

   c. Renal:
   GFR estimated by the updated Schwartz formula ≥ 90 mL/min/1.73 m^2. If the estimated GFR is < 90 mL/min/1.73 m^2, then renal function must be measured by 24-hour creatinine clearance or nuclear GFR, and must be > 50 mL/min/1.73 m^2.

   d. Pulmonary
   No need for supplemental oxygen *and* O_2 saturation > 92% on room air at sea level (with lower levels allowed at higher elevations per established center standard of care).

5.1.2 **Participant Exclusion Criteria**

Exclusion criteria

1. Presence of any serious life-threatening or opportunistic infection at time of enrollment and prior to the initiation of the preparative regimen. Serious infections as defined below that occur after enrollment must be reported immediately to the Study Coordinating Center, and enrollment will be put on hold until the infection resolves. Ideally enrolled subjects will not have had any infection. If patients have experienced infections, these must have resolved by the following definitions:

   a. <u>Bacterial</u>

      i. Positive culture from a sterile site (e.g. blood, CSF, etc.): Repeat culture(s) from same site must be negative and patient has completed appropriate course of antibacterial therapy (typically at least 10 days).

      ii. Tissue-based clinical infection (e.g. cellulitis): Complete resolution of clinical signs (e.g. erythema, tenderness, etc.) and patient has completed appropriate course of antibacterial therapy (typically at least 10 days).

      iii. Pneumonia, organism not identified by bronchoalveolar lavage: Complete resolution of clinical signs (e.g. tachypnea, oxygen requirement, etc.) and patient has completed appropriate course of antibacterial therapy (typically at least 10 days). If possible, radiographic resolution should also be demonstrated.

b. Fungal

i. Positive culture from a sterile site (e.g. blood, CSF, etc.): Repeat culture(s) from same site is negative and patient has completed appropriate course of antifungal therapy (typically at least 14 days). The patient may be continued on antifungal prophylaxis following completion of the treatment course.

c. Pneumocystis

i. Complete resolution of clinical signs (e.g. tachypnea, oxygen requirement, etc.) and patient has completed appropriate course of therapy (typically at least 21 days). If possible, radiographic resolution should also be demonstrated. The patient may be continued on prophylaxis following completion of the treatment course.

d. Viral

i. Viral PCRs from previously documented sites (blood, nasopharynx, CSF) must be re-tested and are negative.

ii. If re-sampling a site is not clinically feasible (i.e. BAL fluid): Complete resolution of clinical signs (e.g. tachypnea, oxygen requirement, etc.). If possible, radiographic resolution should also be demonstrated.

2. Patients with HIV or HTLV I/II infection will be excluded.

5.1.2.1 Co-enrollment Guidelines

Subjects on this study may not enroll on any other interventional transplant studies that proscribe conditioning regimen, GVHD prophylaxis, or manipulation of the donor graft. When possible, subjects will be co-enrolled on PIDTC 6901 (A Prospective Natural History Study of Diagnosis, Treatment and Outcomes of Children with SCID Disorders) and have comprehensive data submitted to CIBMTR.

5.2 Donor selection

5.2.1 **Allowed Donor Sources**

1. Fully matched sibling donors are not allowed. Patients with these donors available should use other approaches.

2. Unrelated donors. HLA typing of at least 10 alleles is required. Donor must be matched at 9/10 or 10/10 alleles (HLA-A, -B, -C, -DRB1, -DQB1).

3. Haploidentical matched family members. Minimum match level full haploidentical (at least 5/10; HLA-A, -B, -C, -DRB1, -DQB1 alleles), but use of haploidentical donors with extra matches (e.g. 6, 7, or 8/10) encouraged.

4. Cord blood is not allowed as a stem cell source on this trial.

5.2.2 Donor Eligibility

1. Matching as described above.

2. Size and vascular access appropriate by center standard for PBSC collection

3. Must meet appropriate screening/eligibility requirements

    a. Haploidentical matched family members (age 18 years or older): screened by center health screens and found to be eligible

    b. Unrelated donors: meet eligibility criteria as defined by the NMDP

4. HIV negative.

5. Not pregnant or lactating.

6. Recipient must not have high-level donor specific anti-HLA antibodies according to institutional practices. High level donor specific antibodies may be defined as positive cross-match test of any titer by complement-dependent cytotoxicity or flow cytometric testing, or the presence of anti-donor HLA antibody to the high expression loci HLA-A, B, C, DRB1, or DPB1 with mean fluorescence intensity (MFI) >3000 by solid phase immunoassay.

7. Must agree to donate PBSC

8. Must give informed consent

    a. Haploidentical matched family members: *CSIDE Related Donor Consent*

    b. Unrelated donors: CSIDE *Unrelated Donor Consent*

5.3 Strategies for Recruitment and Retention

Potential subjects will be identified at participating PIDTC centers in the course of clinical care of patients with SCID. States with active statewide universal newborn screening programs are expected to enroll the most subjects. As of 2017, all states except for AL, AZ, IN, KS, LA, MO, NC, NV are screening. Screening is also active in the District of Columbia, Puerto Rico and the Navajo nation. Based on the birth rates of these states in comparison to the United States birthrate and the current incidence of SCID identified in newborn screening programs, it is estimated that approximately 86.8% of the births are subject to screening in 2017 and 94% will be subject to screening by 2018, with 64 babies diagnosed with SCID per year. Because PIDTC/PBMTC centers see a large proportion of SCID patients requiring HCT, it is anticipated

that a high proportion of these infants will be referred for clinical care to a PIDTC or PBMTC center.

In addition, IRB approved advertisements will be distributed to major transplant centers in the US and the trial will be advertised on websites including the PIDTC website, clinicaltrials.gov, the PBMTC website, and SCID family advocacy groups.

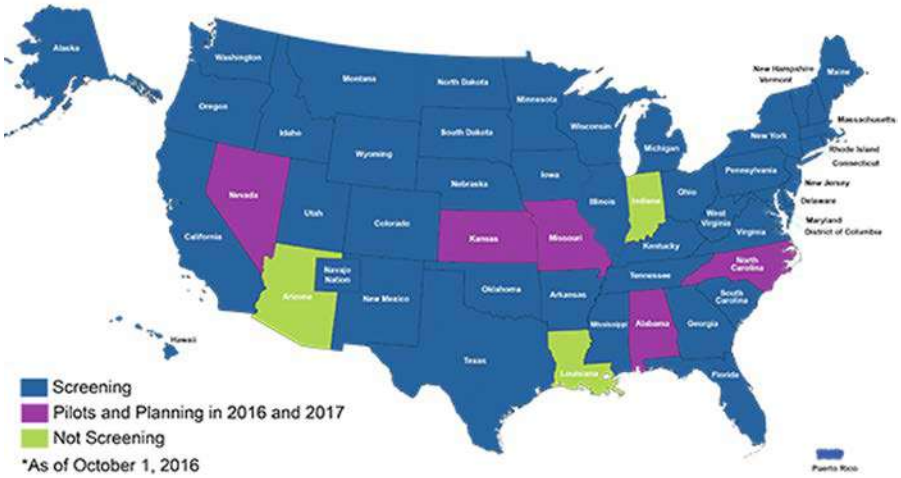


Figure 4: Current status of universal newborn screening in the United States and territories. Blue states are currently screening. Purple states have pilots or plan to conduct pilots in 2016 and 2017. Green states are not screening. Data courtesy of the Immune Deficiency Foundation, current as of October 1, 2016.

6 TREATMENT PROGRAM/INTERVENTIONS

6.1 Conditioning Regimen

All patients will receive the conditioning regimen as shown in Table 5. Busulfan will be targeted to the dose level at which the subject is enrolled. Any deviation from the conditioning regimen considered to accommodate donor cell collection or processing must be discussed first with the protocol chair/co-chair.

Table 5: Conditioning regimen

Day	IL2RG/JAK3	RAG1/RAG2
-9	Thymoglobulin	Thymoglobulin
-8	Thymoglobulin	Thymoglobulin
-7	Thymoglobulin	Thymoglobulin, Thiotepa
-6	Busulfan	Busulfan, Fludarabine
-5	Busulfan	Busulfan, Fludarabine
-4	Busulfan	Busulfan, Fludarabine
-3	Busulfan	Busulfan, Fludarabine
-2	Rest	Rest
-1	Rest	Rest
0	TCRαβ+/CD19+ depleted PBSC	TCRαβ+/CD19+ depleted PBSC
+1	Rituximab (depending on B cell depletion efficiency)	Rituximab (depending on B cell depletion efficiency)

Thymoglobulin: 3 mg/kg IV daily

Fludarabine IV daily, model-based dosing per individual

Thiotepa 4 mg/kg/dose IV every 12 hours x 2 doses
Busulfan dose to be randomized, IV daily, model-based dosing per individual
Rituximab 375 mg/m^2 x 1 IV on day +1 if B cell depletion incomplete

6.1.1 **Thymoglobulin**

All patients will receive Thymoglobulin®, based on a formula that incorporates actual body weight and baseline absolute lymphocyte count, derived from simulated models (Admiraal, van Kesteren et al, 2014). Due to the observed rapid metabolism of infants in the weight range anticipated for this study, patients will receive a fixed dose of 3 mg/kg daily of ATG. Administration of ATG should be over 8 hours IV daily for 3 days from day -9 to day -7. It is important that the Thymoglobulin® be administered at a constant rate divided over the full 8 hour period in order for the pharmacokinetic analysis to be performed. Premedication should follow local institutional practice but should include a minimum of 1 mg/kg methylprednisolone prior to the infusion (equivalent steroid allowed), preferably repeated in 3-4 hours during the infusion. Note: Thymoglobulin® is the required preparation of ATG for this study. Patients will not be eligible if the treating center plans to use other preparations of ATG.

6.1.2 **Busulfan**

Determination of the First Dose: Busulfan will be administered IV daily for a total of 4 doses beginning on day -6 through day -3. Pharmacy will provide busulfan pre-filtered and diluted in normal saline to a final concentration of 0.5mg/ml. The proposed targeted cumulative busulfan exposure over the duration of therapy for the two dose level cohorts is a cAUC of 30 (range 25-35) and 60 (55-65) mg*hr/L, respectively. This is equivalent to $Css_{0\text{-}24h}$ target for every 12 hour dosing of 313 and 625 ng/mL, respectively (Table 6). Initial doses for busulfan will be individualized for each subject by age and weight based on our groups validated models for busulfan clearance in infants and young children using the freely available web-based software, Insight-rx (www.insight-rx.com). (Savic, Cowan et al. 2013, Long-Boyle, Savic et al. 2015) Briefly, the software will ask for patient-specific covariates including date of birth, weight, height, sex, and serum creatinine of the individual child required to perform the dose estimation function. Additionally, the software will prompt the user for the assigned randomization for cAUC. Based on the specified covariates and exposure target an initial dose will be estimated by the model. All initial doses will be verified through the Insight-rx platform by the study pharmacologist (Janel Long-Boyle, PharmD, PhD) remotely prior to the certification of chemotherapy as outlined in the MOP. Each subject's profile and the required covariates and exposure target should be entered into the Insight-rx platform no later than 24 hours prior to the first dose of busulfan to ensure timely verification.

Table 6

Cumulative AUC (mg*hr/L)	Dosing interval (hours)	Number of Doses	AUC_{0-24} Target (mg*hour/L)	Css_{0-24} Target (ng/mL)
30	24	4	7.5	313
60	24	4	15	625

PK Sampling: Busulfan will be infused at a continuous rate over 3 hours. Blood collections for PK sampling will be performed with dose 1 and used to determine dose modifications for subsequent doses, if needed. Recommended times for blood collections are based on an individualized optimal sampling strategy with blood collection times at 3.25, 4, 6 and 8 hours post start of busulfan infusion. Blood collection times for subsequent doses are 3.25, 4, 6, and 8 hours post the start of busulfan infusion. Irrespective of if a dose modification is needed guided by Dose 1 data, PK sampling will be repeated in all patients following dose 3 and used to calculate cumulative busulfan exposure (cAUC). Plasma samples will be analyzed by institution-specific, CLIA approved, core facilities using validated assays for the quantification of busulfan in plasma.

Calculation of Busulfan AUC Exposure: The estimation of individual exposure (AUC and cAUC) will also be determined by the web-based software, Insight-rx (www.insight-rx.com). Insight-rx is therapeutic drug monitoring tool recently developed by the UCSF group, which allows for Bayesian dose-individualization of busulfan using an individual's time-concentration data. This web-based, HIPAA compliant software program performs Bayesian dose predictions based on published PK models, patient-specific characteristics, and measured drug concentrations and allows for dose optimization irrespective of the target.(Savic, Cowan et al. 2013, Long-Boyle, Savic et al. 2015, Chan, Ivaturi, Long-Boyle, 2017) Briefly, once PK data is available from institution-specific laboratories the raw time-concentration data should be emailed to the study pharmacologist (Janel Long-Boyle). Users may then input the PK data corresponding to the correct dose PK that was collected into the Insight-rx platform. The PK model will then estimate a new dose based on the patient's individual PK data, covariates, and therapeutic target. As with initial doses, any dose modifications will be verified through the Insight-rx platform by the study pharmacologist (Janel Long-Boyle, PharmD, PhD) remotely prior to the certification of chemotherapy by the clinical team as outlined in the MOP.

Anti-seizure prophylaxis is recommended with Levetiracetam at 10mg/kg/dose intravenously from day -6 to day 0, for at least 48 hours after the last dose of busulfan.

6.1.3 **Fludarabine**

Determination of the First Dose: Patients with RAG1/RAG2 will receive fludarabine. Fludarabine will be administered IV over 1 hour daily for a total of 4 doses beginning on day -6 through day -3. The proposed targeted cumulative fludarabine exposure over the duration of therapy is a cAUC of 18mg*hr/L (Ivaturi et al. 2017). Estimated doses for fludarabine aim to achieve an equivalent $AUC_{(0\text{-}inf)}$ of f-ara-a to a standard dose of 40 mg/m^2/day in children x 4

doses (4.5 mg*h/L x 4 doses = 18mg*hr/L). Initial doses will be individualized for each subject by weight and creatinine clearance based on a validated model for fludarabine clearance in infants and young children using the software, Insight-rx (www.insight-rx.com), similar to the busulfan calculation described above. (Ivaturi at al. 2017, Chan, Ivaturi, Long-Boyle, 2017) Each subject's profile and the required covariates and exposure target should be entered into the Insight-rx platform no later than 24 hours prior to the first dose of fludarabine to ensure timely verification. No dose adjustments of fludarabine will be made over the four days of therapy.

PK Sampling:
All patients will undergo PK sampling following a single dose of fludarabine. The dose for which PK sampling occurs with each individual drug is purposely flexible and supported by population PK methods to ensure staff availability for sample processing. Additionally, intervals of collection times are provided maximize overlap with timing of other required labs and thus minimize blood loss.

Drug	1st PK collection interval	2nd PK collection interval	Volume of whole blood collected per sample	Collection tube
Fludarabine	0-2 hours post end of infusion	4-8 hours post end of infusion	1.0mL	2mL EDTA (purple top)

6.1.4 **Thiotepa**
Patients with RAG1/RAG2 will receive thiotepa 4 mg/kg IV every 12 hours for 2 doses on day -7.

PK Sampling:
All patients will undergo PK sampling following a single dose of thiotepa. Similar to fludarabine, the dose for which PK sampling occurs with each individual drug is flexible and intervals of collection times are provided maximize overlap with timing of other required labs and thus minimize blood loss.

Drug	1st PK collection interval	2nd PK collection interval	Volume of whole blood collected per sample	Collection tube
Thiotepa	0-2 hours post end of infusion	2-6 hours post end of infusion	0.5mL	2mL EDTA (purple top)

6.2 Selection, processing and infusion of donor cell product

Individual site clinical teams will select haploidentical related or unrelated donor according to site preference and donor availability. Sites with the appropriate capabilities will perform processing of donor cell product for their own patients. For the remaining sites, apheresis

products will be shipped to a validated centralized processing site for selection and shipped back to the patient's site for infusion.

6.2.1 Mobilization for donors

A mobilization regimen with granulocyte colony stimulating factor (G-CSF) will be used to obtain a PBSC product from donors who have given consent. Apheresis will be performed according to institutional standards aiming for 3-4 times the total blood volume as tolerated by the donor. The target infusion goal will be at least 10 x 10^6 $CD34^+$ cells/kg. The donor mobilization guidelines are as follows:

Days	Mobilization regimen
1, 2, 3, and 4	G-CSF 10 mcg/kg/day subcutaneous.
5 and 6	G-CSF 10 mcg/kg/day subcutaneous. Apheresis begins.

Apheresis will typically begin on the 5^{th} day of mobilization. Apheresis may need to be performed over a two-day period, though most donors are likely to be able to provide the stem cell doses required for transplant after one day of apheresis. If required, additional days of apheresis will be done at the transplant or apheresis physician's discretion. Every effort will be made to infuse a fresh stem cell product; however, a frozen product may be infused when necessary.

For unrelated donors, mobilization and apheresis will be performed according to standard NMDP practice. The target infusion goal will be at least 10 x 10^6 $CD34^+$ cells/kg.

All donors will be monitored and PBSC products will be collected according to FACT-JACIE international standards for cell therapy, product collections, processing and administration.

6.2.2 Graft processing: CliniMACS® TCRαβ+/CD19+ cell depletion with optional CD34+ selection

All cell processing will take place at participating center stem cell labs using validated Standard Operating Procedures. If more than 1 apheresis event is performed to collect a sufficient cell dose, HPC-A products may be combined prior to T- & B-cell depletion. HPC-A products will be T- & B-cell depleted using the CliniMACS® device according to the manufacturer's instructions. Briefly, this will first involve performing an initial assessment of the product by performing a cell count and viability. Prior to immunomagnetically labeling the HPC-A product, the HPC-A product will be washed to remove platelets and the cell concentration will be adjusted in preparation for antibody labeling. The PBSC product will be labeled using the CliniMACS® TCRαβ Biotin kit and CD19+ Microbeads. After immunomagnetically labeling cells with TCRαβ and CD19 antibodies, the cells will be washed to remove unbound microbeads. The HPC-A product will be loaded onto the CliniMACS® device and the negative cell fraction will be collected. The cells from the negative fraction will then be centrifuged and reconstituted to obtain the final product. Cell viability, cell counts, sterility, gram stain and endotoxin testing will

be performed on the final product prior to infusion. In addition, the final product will be enumerated and assessed for viable stem cell (CD34+) T-cell (TCR αβ+CD3+, TCR γδ+CD3+) and B-cell content using anti-CD20 (due to blockage of the CD19 antigen by the reagent used for depletion) by flow cytometry. The HPC-A graft product will be infused fresh after completion of release testing. If necessary the final product may be stored overnight at 4°C prior to infusion. Every effort will be made to infuse a fresh stem cell product, however, a frozen product may be infused when necessary,

Cell dose parameters for the *primary HSC* infusion donor graft are as follows. The cell doses noted are defined as the total αβ+CD3+, CD34+, and B-cell counts contained in the final product.

- The target HSC dose is at least 10 x 10^6 CD34+ cells/kg recipient weight. The minimum cell dose will be 2-5 x 10^6 CD34+ cells/kg with no maximum dose (centers may choose a max doses, e.g. 60-80 x 10^6 CD34+ cells/kg. but the CD34+ dose is usually limited by the need to not exceed the αβ+CD3+ dose threshold below).
- The target αβ+ CD3+ cell dose content in the primary infusion donor product will be ≦1.0 x 10^5 αβ+CD3+ cells/kg recipient weight. If projected CD34+ content of a graft will be < 5.0 x 10^6 /kg, centers may increase the αβ+CD3+ to a maximum of 5.0 x 10^5 αβ+CD3+ cells/kg to increase CD34+ content in the graft. Alternatively, the cell processing lab may perform a CD34 selection on a portion of the collection or a second collection in order to optimize the CD34+ numbers for infusion.
- The total number of CD19+ B-cells will be monitored. If >1.0 x 10^5 CD20+ cells/kg recipient weight are infused in order to achieve the cell dose goals noted above, the patient will receive rituximab 375 mg/m^2 x 1 IV on day +1 .

Optional CD34+ selection to boost CD34+ dose. If at the end of the negative depletion procedure, the residual number of TCRαβ T cells would be greater than 1x10^5/kg recipient weight, a residual part of the selected graft may undergo further CD34 selection and both allograft products returned to the subject. Alternatively, a saved portion of the first collection or a second leukapheresis collection may be selected for CD34 after the target residual αβ-T cells has been reached. CD34+ selection will be performed following the standardized protocol in the User's Manual for the CliniMACS or Prodigy (Miltenyi Biotech), operating under the cell processing lab's standard operating laboratory procedures. The total graft will consist of one or two sequential T & B-cell depleted HPC-A cell infusions plus CD34+ selected cells if the center chooses to perform this optional procedure to increase the CD34+ dose.

6.2.3 **Procedures for centralized processing**

Detailed procedures and SOPs are contained within the MOP.

6.2.4 **Infusion**

Infusion of the final TCRαβ and CD19 depleted product will be performed intravenously without cryopreservation whenever possible, or thawed if cryopreserved, according to institutional standards. The day of first cell infusion will be designated as day 0.

6.2.5 **GVHD prophylaxis**

This protocol does not use GVHD prophylaxis because $\leq$1.0 x 10^5 αβ+CD3+ cells/kg recipient weight will generally be administered. Although we suspect that few if any patients will require an increased dose of TCRαβ+CD3+ cells in order to achieve goal levels of CD34+ cells, studies using this approach have shown an increase in risk of GVHD at higher TCRαβ+CD3+ cell doses/kg. To avoid increased risk of GVHD if higher doses of TCRαβ+CD3+ cell doses/kg are given, we recommend the following: 1. Physicians contemplating giving higher cell doses due to cell processing issues should discuss the cell dosing approach with the protocol chair/co-chair to find ways to avoid giving higher TCRαβ+CD3+ cells doses/kg if possible. 2. If doses exceeding 1.0-2.0 x 10^5 TCRαβ+CD3+ cells/kg are given, we recommend that patients be placed on the calcineurin inhibitor used routinely at their institution (cyclosporine or tacrolimus) for 3 months with a taper over 3 months after the HCT procedure.

If patients develop aGVHD, centers will treat aGVHD according to local standard of care. If patients require stem cell boosts, or undergo a second BMT procedure due to poor graft function, GVHD prophylaxis should be administered according to local center standards associated with a second infusion protocol.

6.2.6 **EBV PTLD prophylaxis and follow up monitoring**

A dose of rituximab (375mg/m^2) will be administered within approximately 24 hours of the stem cell transplant infusion if the B-cell count exceeds 1x10^5/kg recipient weight. If centers choose, they may also give this dose if the recipient is EBV+, but this is optional. This additional safeguard, along with CD19 depletion, is provided as part of the pre-transplant preparative regimen to prevent EBV PTLD. This intervention has been chosen because of studies indicating that the EBV DNA level in the peripheral blood is suggestive of PTLD. Rituximab has been demonstrated to be an effective therapy in PTLD. The current standard clinical practice will be to administer rituximab when peripheral blood EBV DNA levels exceed 2000 copies/microgram. This is done regardless of whether the patient has concurrent clinical signs and symptoms in order to prevent onset and/or progression of PTLD. The medication will continue at the discretion of the treating physician as indicated based on ongoing EBV copy levels and clinical assessment.

6.3 Evaluation for humoral immune reconstitution

6.3.1 **Intravenous immunoglobulin replacement and cessation plan**

In order to measure the primary endpoint (vaccination response to tetanus toxoid vaccination), subjects must first achieve the criteria stated below (see also Section 4.2.1):

- Donor T cell chimerism ≥50%
- B cell count ≥50 cells/microliter
- IVIG independent for ≥12 weeks

The definition of IVIG independence is freedom from IVIG replacement for 12 weeks. We recommend that subjects receive IVIG every 3-4 weeks to keep levels above the 5th percentile for age, which is ≥300 mg/dL for children under 2 years of age (Appendix). If a subject maintains IgG without replacement ≥300 mg/dL for 12 weeks, vaccination trial can begin when the patient has met criteria.

IgG levels should be monitored monthly. Prophylactic antibiotics may be administered at the discretion of the treating physician. If IgG levels are <300 mg/dL or if the patient received IVIG for treatment of infection or exposure to infection (e.g. to Varicella or measles), vaccination trial will be deferred until IVIG independence is documented.

Some subjects may meet criteria who are on immunosuppressive treatment, particularly calcineurin inhibitors such as cyclosporine A or tacrolimus, due to acute GVHD or other circumstances. For these patients, vaccination trial can begin whenever the above criteria are met and IVIG independence has been documented.

Subjects who develop chronic GVHD may remain on calcineurin inhibitors beyond 1 year post-HCT. Because of the deleterious effects of GVHD on immune reconstitution, we anticipate most subjects with chronic GVHD will not meet lymphocyte, chimerism, and/or IVIG independence criteria. Testing of vaccine response in such subjects should be discussed with the protocol chairs.

6.3.2 **Tetanus vaccination plan**

Vaccination with tetanus toxoid will be given as 3 doses 4-8 weeks apart, as recommended for catch-up vaccination guidelines (Centers for Disease Control and Prevention 2015). Pre-vaccine tetanus titer will be measured within 1 month of the 1st dose. Post-vaccine tetanus titer will be measured no sooner than 4-6 weeks after the 3rd dose.

Subjects who are on corticosteroids may receive killed vaccines, and may mount a response that is transient. Subjects who undergo the vaccination series within 2 weeks of starting steroids

or while on steroids, will undergo a trial of boosting at least 3 months after immunosuppression has been discontinued.

6.4 Supportive Care Guidelines

The following supportive care guidelines describe consensus recommendations for this trial of PIDTC investigators, and address pre-transplant and peri-transplant care specifically for SCID patients identified as newborns. The below guidelines are recommended for this protocol but lack of adherence does not constitute a protocol deviation.

6.4.1 **Pre-transplant care**

In general, protective and prophylactic measures detailed by experts in SCID diagnosis and care should be followed. Babies identified to be at risk for SCID by universal screening or by family history should be kept in an isolated environment until the diagnosis is made or ruled out. Live vaccines must be avoided.

Once a diagnosis is made, the center will perform a full immunologic work-up, rapid diagnostic testing and evaluation of potential donors. Other standard guidelines for pre-transplant care of the SCID patient include:

- Maintenance of infectious isolation
- Immunoglobulin substitution every 3-4 weeks to maintain levels >300 mg/dL
- Initiation of prophylaxis against *Pneumocystis jiroveci* (trimethoprim-sulfamethoxazole 5 mg/kg/day three days a week recommended) at 6 weeks of age
- If mother is breastfeeding, at minimum, temporary cessation of nursing while mother is screened for CMV seropositivity is recommended. If seronegative, some institutions would allow breastfeeding to resume.
- If blood product transfusion is required, products **must** be irradiated.
- Aggressive work-up of any symptoms suggestive of infection.

6.4.2 **Peri-transplant care**

Institutional standard care practice guidelines will in general be followed after transplantation for nutritional support, treatment and work-up of fever or infections and blood product support. Supportive care recommendations are detailed below.

6.4.2.1 Venous access

Recipients will undergo placement of an appropriate long-term central venous catheter per institutional practice prior to beginning the conditioning regimen. A double lumen tunneled catheter is recommended, particularly for the higher dose level of busulfan.

6.4.2.2 Growth factor

Growth factor will not be used routinely in the protocol. Recipients may receive at the discretion of the treating physician if graft failure is suspected or otherwise clinically indicated.

6.4.2.3 Treatment of fever/infections

Patients should be monitored closely for clinical manifestations of infection and treated per institutional guidelines with broad spectrum antibacterial, antiviral and antifungal agents. Early and aggressive treatment should be instituted given the underlying immunocompromise of these patients particularly in light of administration of T cell depleted grafts or Thymoglobulin.

6.4.2.4 Infectious Surveillance and Prophylaxis

6.4.2.4.1 Pneumocystis

Pneumocystis prophylaxis with TMP-SMX that was initiated at the time of SCID diagnosis should be held during conditioning, then restarted after ANC recovery (>500 for 3 days consecutively) at a dose of 5 mg/kg/day in two divided doses and administered 3 days/week on consecutive or alternate days. If ANC recovery has not occurred by day +28 or in the event of TMP-SMX intolerance, administration of an alternate agent (pentamidine or atovaquone) is permitted. Pneumocystis prophylaxis should continue until evidence of functional T cell recovery.

6.4.2.4.2 Fungus

If prophylaxis against Candida with fluconazole was initiated before admission for transplant, it should be held 7 days prior to the start of busulfan and restarted at day 0 at a dose of 6 mg/kg/day intravenously or orally once daily. If substitution is required administration of an echinocandin or amphotericin is permitted. Antifungal prophylaxis should continue until evidence of functional T cell recovery.

6.4.2.4.3 Herpes-family viruses

While administration of acyclovir is recommended for those with exposure to herpes simplex virus and in some institutions for CMV seropositivity, subjects on this protocol should not have had any exposure to herpes-family viruses, and therefore acyclovir administration is discouraged. PCR based viral monitoring for CMV, EBV, and adenovirus should be done weekly through at least day 100 and then longer depending upon T cell recovery.

6.4.2.4.4 Respiratory viruses

Prophylactic use of pavilizumab to prevent RSV acquisition is optional.

6.4.2.4.5 Bacteria

Administration of systemic anti-bacterial agents or non-absorbable antibiotics for gut decontamination is not recommended, but is permitted per institutional practice.

6.4.2.5 Prevention and monitoring for veno-occlusive disease (VOD)

All patients should receive ursodiol (15-30 mg/kg/day in 2-3 divided doses) starting at the time of busulfan initiation at the latest, and continued through at least day +21.

7 STUDY PROCEDURES/EVALUATIONS

When possible, subjects will be co-enrolled in an ongoing prospective natural history study (PIDTC Protocol 6901). Centers must register pre- and post-transplant outcomes on all hematopoietic stem cell transplants done at their institution during their time of participation to the CIBMTR. Registration is done using procedures and forms of the Stem Cell Therapeutics Outcome Database. In addition, protocol-specific data will be collected within a separate Electronic Data Capture (EDC) database. CIBMTR forms will be submitted directly to the CIBMTR at the times specific for this trial. Relevant data from these studies will be extracted and transferred for analysis. Study procedures and evaluations performed specifically for this study are denoted by the asterisk. See also Table 7 in Section 8.

7.1 Pre-transplant Clinical and Laboratory Evaluations

The following must be performed prior to initiation of the conditioning regimen and data will be gathered as baseline. Other testing should be done according to institutional standards.

- History, physical examination (within 30 days prior to initiation of conditioning)
- Lansky performance status (within 30 days prior to initiation of conditioning)
- HLA by allele-level typing of both recipient and donor at HLA-A, -B, -C, –DRB1 and –DQB1 for unrelated donor grafts and at antigen or allele-level for haploidentical related grafts
- HLA alloantibody testing to evaluate for donor-specific anti-HLA antibodies
- Baseline buccal swab sample from patient and blood sample from donor for chimerism analysis by molecular methods (STR/VNTR)
- Baseline CBC with differential and platelet count (within 30 days prior to initiation of conditioning)
- Baseline immunologic studies including: lymphocyte subsets (CD3, CD4, CD8, CD19, CD16 or CD56), proliferation to phytohemagglutinin (incorporation of ^{3}H-thymidine preferred), IgG, IgA, IgM, IgE
- SCID diagnostic testing including SCID genotyping confirming mutation in IL2RG, JAK3, RAG1 or RAG2, maternal engraftment testing
- Baseline research study samples drawn as noted in Table 7.

7.2 Post-transplant Clinical and Laboratory Evaluations

The following are considered standard evaluations for SCID patients undergoing transplant.

- History and physical examination to assess GVHD and other morbidity weekly until day 100 post-transplant, then at 6 months, 1 year and 2 years post-transplant.
- History and physical examination to assess for regimen related toxicity weekly until day +42 post-transplant
- CBC and platelets at least 3 times a week from day 0 until neutrophil and platelet engraftment, then at least weekly until day 100, then at 6 months, 9 months, 1 year and 2 years post-transplant
- Cell type specific chimerism (T cell, B cell and granulocyte) post-transplant at day 42, day 100, 6 months, 1 year and 2 years post-transplant
- Immune reconstitution studies standard of care: CD3, CD4, CD8, CD19 and CD16+56 subsets; immunoglobulin levels (IgG, IgA, IgM, IgE); pre and post vaccine titers to tetanus
- Optional and/or recommended BM assessments: Some centers routinely perform an assessment of BM cellularity along with other tests such as cytogenetic analysis prior to BMT, and either at day 100 or 1 year after HCT or both to assess cellularity, chimerism, and cytogenetics. This type of testing is encouraged but is optional, and could be considered at the time of anesthesia for a clinically indicated procedure such as central line removal. If patients have a fall in chimerism or a decrease in counts, we recommend obtaining a BM assessment along with cytogenetics to ensure that the engrafted marrow is healthy.
- Research studies samples as noted in Table 7

7.2.1 Specimen Preparation, Handling and Shipping

Research studies on this protocol will be centralized. All research studies listed in Table 7 will be processed and distributed from TransLab (Director, Myriam Armant PhD) in Boston Children's Hospital.

Samples collected will be processed by TransLab (located at Boston Children's Hospital) and banked to ship in batches to the scientific laboratories. Material that is banked (cells and serum) will also be processed and stored in TransLab. The purpose of the repository is to retain samples at key timepoints during the study for future investigations or follow-up studies of the current scientific plan.

TransLab shipping address:

ATTN Myriam Armant
TransLab
Boston Children's Hospital
61 Binney Street
Enders 208
Boston MA 02115

Please note that shipment of blood for lineage specific chimerism at 42 days, 100 days, 6 months, 12 months and 2 years must be scheduled 3 weeks in advance. See MOP for further details.

8 STUDY SCHEDULE

The study schedule for recipients is shown in Table 7 below. Research blood samples in general should still be collected if outside the time window. Time windows for post-transplant visits are: 7 days ±2 days, 14 days ±2 days, 30 days ±3 days, 42 days ±3 days, 60 days ±7 days, 100 days ±10 days, 6 months ±14 days, 9 months ±14 days, 12 months ±14 days, 2 years ±42 days, 3 years ±60 days.

Table 7: Table of studies to be performed on recipient

		Baseline	Conditioning(days -9 to day -1)	Day 0 (infusion of cells)	7 Days	14 Days	30 days	42 days	60 days	100 days	6 months	9 months	12 months	2 years	3 years
	History and physical	X								X	X	X	X	X	X
	Adverse events review	X	X	X						X	X	X	X	X	
	Concomitant medications review	X													
	SCID diagnostic testing[9]	X													
	Transplant evaluation[10]	X													
Standard clinical care	CBC and differential	X					X	X	X	X	X	X	X	X	
	Busulfan pharmacokinetics[1]		X												
	Phenotypic characteristics of graft			X[3]											
	Lymphocyte subsets[2]	X		X			X		X	X	X	X	X	X	
	Proliferation to PHA	X					X		X	X	X		X	X	
	IgG	X								X	X	Monitor monthly according to 6.3.1			X
	IgA, IgM, IgE	X								X	X		X	X	
	Measles IgG													X[4]	X[4]

		Baseline	Conditioning(days -9 to day -1)	Day 0 (infusion of cells)	7 Days	14 Days	30 days	42 days	60 days	100 days	6 months	9 months	12 months	2 years	3 years
	Optional/Suggested BM Assessment	X								X[11]					
Research testing (to TransLab)	ATG pharmacokinetics and IL-7 levels	X	X	X	X	X		X							
	Thiotepa and Fludarabine levels[12]		X												
	Extended lymphocyte phenotyping	X								X	X		X	X	
	Buccal swab for patient STR genotyping	X													
	Lineage specific chimerism[5]	X						X		X	X		X	X	
	Tetanus IgG[6]											Pre- and post-vaccination according to 6.3.2			X[7]
	CD34+ cell chimerism													X	
	Plasmablast differentiation[13]	X									X		X	X	
	B cell receptor repertoire	X										X		X	
	Exhaustion panel										X		X	X	
	EBV B cell line[8]	X													
	TREC	X											X	X	
	Tolerance studies[8]												X	X	
	Bank serum	X								X	X		X	X	
	Bank peripheral blood mononuclear cells (PBMC)	X								X	X		X	X	

Notes:
[1] To be performed per clinical routine. Time-concentration data will be submitted via InsightRx platform for review by Dr. Long-Boyle.
[2] To include absolute counts of CD3+, CD4+, CD8+, CD19+ or CD20+, CD56+ or CD16+ or CD56/CD16+ cells/microliter.
[3] This will include total nucleated cell/kg, CD34+ cell/kg, CD3+ cell/kg, TCRαβ+ cell/kg, TCRγδ+ cell/kg, viability, sterility.
[4] Results of measles IgG will be gathered from the medical record if available. Only patients who meet clinical criteria for live viral vaccination will receive measles vaccination.
[5] Blood will be sorted to obtain $CD3^+$, $CD19^+$, $CD3^-$ $CD56^+$, $CD15^+$ cells for chimerism determination.
[6] Tetanus IgG will be performed centrally as a research test in TransLab.
[7] Patients who are vaccinated but do not have post-vaccination testing by 2 years will be assessed at the 3 year visit.
[8] These will be performed only on IL2RG/JAK3 cohort patients.
[9] SCID diagnostic testing will include performing or gathering the results of: SCID newborn screening, maternal engraftment testing, SCID genotyping
[10] Transplant evaluation will include performing or gathering the results of: HLA typing, HLA alloantibody screening
[11] It is optional to obtain a bone marrow specimen for cellularity and cytogenetics prior to conditioning and post-transplant between 100 days and 1 year. Results of screening or of bone marrow aspiration performed for clinical purposes due to falling counts or falling chimerism will be collected.
[12]These will be performed only on RAG1/2 cohort patients.
[13]Plasmablast differentiation will be performed on both cohorts at 6 months, 12 months, and 2 years, and only for the IL2RG/JAK3 cohort at baseline.

The study schedule for donors is shown in Table 8 below. Research blood samples should be collected prior to PBSC product collection.

For both donor types, a 10mL whole blood sample will be drawn for chimerism analysis, as well as a 10mL whole blood sample for donors to IL2RG/JAK3 patients for exploratory studies on tolerance.

Table 8: Table of studies to be performed on donor

		Baseline	Conditioning (days -12 to day -1	Day 0 (infusion of cells)	7 Days	14 Days	30 days	42 days	60 days	100 days	6 months	9 months	12 months	2 years	3 years
Research testing (to TransLab)	Tolerance studies[1]	X													
	Chimerism analysis[2] (STR/VNTR)	X													

Notes:
[1] Donor 10mL whole blood sample will be obtained to support studies on T cell tolerance for donors to IL2RG/JAK3 cohort patients (see MOP for details)
[2] Donor 10mL whole blood sample will be obtained to support chimerism analysis (see MOP for details)

8.1 Screening and Enrollment

Patients will be identified at the participating PIDTC institutions. Patients with a diagnosis of classic SCID as defined in the Eligibility criteria and in PIDTC protocol 6901 who are believed to be potentially eligible by the site investigator based on diagnosis by screening at birth and lack of a fully matched genotypically identical donor will be approached for consent.

Informed consent will include explaining to the parent/legal guardian(s) that eligibility information will be submitted to the Eligibility Review Committee for final approval. Eligibility for enrollment will be submitted in 2 steps: 1) determination of disease eligibility and 2) determination of transplant eligibility. It is not anticipated that all patients consented will be eligible for enrollment because some may be diagnosed with different types of SCID, some may not meet the definition for classic or leaky SCID, and some may not be able to clear infections. We encourage completion of disease eligibility as soon as centers are able, at least 2 weeks prior to planned transplant so that there will be time for central review and approval. Submission of transplant eligibility forms should be done within 1-2 weeks of the planned date of initiation of

the preparative regimen. Disease and transplant eligibility forms must be entered into the data capture system and patients enrolled prior to the initiation of the conditioning regimen.

The anticipated timetable is in Table 8 (this will very for patients diagnosed by non-newborn screening or who require time to complete treatment of infections). For patients enrolled with *IL2RG/JAK3* defects, treatment may begin at age 8 weeks of age. Patients with *RAG1/RAG2* defects may begin at age 12 weeks.

Table 8

Day of life/age	Event
Day of life 1-3	NBS TREC specimen sent to state lab
Age 2-4 weeks	Clinical diagnosis of SCID confirmed
Age 4-6 weeks	Genetic tests showing mutation in IL2RG/JAK3, RAG1/RAG2 resulted
Age 6-12 weeks	Study consent obtained, eligibility forms completed and patient enrollment occurs
Age 8 (*IL2RG/JAK3)* or 12 weeks (*RAG1/RAG2*)	Study treatment begins

8.2 Baseline evaluations

After informed consent is obtained, baseline data and samples for this study will be gathered, and eligibility data will be submitted for review to the Eligibility Review Committee as outlined in section 8.1 and the MOP.

8.3 Follow-up

Data from the start of conditioning through day 0 will be abstracted from the medical record or gathered on a research basis, including the doses and timing of busulfan administered, busulfan pharmacokinetics (analyzed centrally), and characteristics of the infused graft including CD34 cell count/kg, CD3 cell count/kg, TCRαβ cell count/kg, TCRγδ cell count/kg, viability and sterility.

Post-transplant follow-up will be conducted when possible at the time of regularly scheduled visits. Data will be extracted from the medical record including history, physical examination, performance score and anthropomorphic measurements; data regarding infection, GVHD, adverse events and regimen related toxicity; results of standard of care clinical laboratory tests. Research laboratory tests will be sent to a central facility for processing and distribution.

8.4 Final Study Visit

The 3-year follow-up visit will be the final study visit for this protocol. Subjects are expected to be co-enrolled in PIDTC Protocol 6901 when possible. If the subject is not enrolled in a long-term follow-up study, enrollment can be offered at this time under a different protocol.

8.5 Early Termination Visit

Participants may discontinue study participation at any time. If the participant wishes to withdraw after conditioning has started but before the graft has been infused, the local PI and treating clinicians will discuss how to complete the transplant process safely. If the participant wishes to withdraw, data collected up to that time point for inclusion in the study will be retained and permission to collect regimen related toxicity data at 42 days post-transplant and survival data at the specified time points until 3 years post-transplant will be requested. The content of the early termination discussion and whether permission was granted for either inclusion of study data collection to date and/or collection of toxicity and survival data as specified above will be documented in the medical record. Subjects who discontinue study participation will be listed according to the off-study criteria that applies.

Off-Study Criteria: The subject is no longer followed. No study-specific evaluations/procedures/tests are performed after date the subject was considered to have met off-study criteria.

Subjects will be removed from the study for the following reasons:

1. Parent/legal guardian withdrawal of consent
2. Determined ineligible by the study team after initial enrollment
3. Discontinued per medical discretion of the site study PI or Medical Monitor
4. Transplant canceled, donor reason
5. Transplant canceled, subject (recipient) reason
6. Lost to follow-up
7. Death

Off-Protocol Criteria: The subject is followed for clinical endpoint data capture only. No study-specific evaluations/procedures/tests will be performed after date the subject was considered to have met off-protocol criteria.

1. Subject received a subsequent HCT and/or donor cellular infusion (DCI)

All subjects who have received their HCT should be followed through 2 years post-HCT. However, study sites must complete the Off Study/Off Protocol form in Medidata Rave® EDC application for any subjects meeting one of these criteria. The Off Study/Off Protocol form is also completed to indicate that a subject has completed the study per protocol (i.e., 2 year visit complete).

9 ASSESSMENT OF SAFETY

Adverse Events

An adverse event (AE) is any untoward medical occurrence regardless of causality assessment. An adverse event can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome or disease associated with or occurring during the use of an investigational product whether or not considered related to the investigational product.

An AE includes, but is not limited to:

1. Any clinically significant worsening of a pre-existing condition, e.g. resulting in change of ≥1 point on the CTCAE grading scale.
2. An AE occurring from overdose (i.e., a dose higher than that prescribed by a healthcare professional for clinical reasons) of investigational product whether accidental or intentional.
3. An AE occurring from abuse (e.g., use of non-clinical reasons) of investigational product.
4. An AE that has been associated with the discontinuation of investigational product.

An AE does **not** include:

1. Medical or surgical procedures themselves (e.g., surgery, endoscopy, tooth extraction, transfusion); the condition that leads to the procedure are AEs.
2. Pre-existing diseases or conditions present or detected prior to start of investigational product administration that do not worsen.
3. Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions).
4. Death: regarding an AE, death is an **outcome** of the AE and is not an AE itself.

9.1 Adverse Events of Special Interest, Unexpected Adverse Events, and Unanticipated Problems

9.1.1 Adverse Events of Special Interest (AESI)

Reporting of expected adverse events follows guidelines as stated in the PBMTC RCI-BMT Manual of Procedures. Selected grade 3-5 expected adverse events will be collected either as specific data elements related to primary or secondary endpoints, or as adverse events of special interest.

Adverse Events of Special Interest (AESI) as defined by this protocol are:

1. **Lack of neutrophil recovery after the primary HCT infusion.** This is defined by lack of neutrophil recovery to an ANC of 500 by day +42 post HCT. Patients who recover neutrophil counts but subsequently have a decreased neutrophil count due to other events (infection, side-effect of medications, etc.) would not be considered to have this event unless the neutrophil decrease is part of a rejection process with aplasia. Because this event could trigger a stopping rule, this should be reported in an expedited fashion (See section 9.3.1.3).

2. **Failure of primary donor T-cell engraftment or rejection of donor T-cells.** Failure of T-cell engraftment or rejection of T-cells is defined as donor T-cell chimerism <5%. Because this event could trigger a stopping rule, this should be reported in an expedited fashion (See section 9.3.1.3).

3. **Grade IV acute GVHD.** Because this event could trigger a stopping rule, this should be reported in an expedited fashion (See section 9.3.1.3).

4. **Veno-occlusive Disease (VOD, Sinusoidal Obstruction Syndrome).** Investigators should report documented cases of VOD. VOD leading to the need of ICU care for organ failure (intubation, dialysis, continuous veno-venous hemofiltration, etc.) should be reported as an SAE.

9.1.2 Unexpected Adverse Events

Unexpected Adverse Events are those events the nature of which, severity, or frequency are not consistent with the known or foreseeable risk of AEs associated with the research procedures described in the protocol-related documents. Adverse events that are reflective of the patient's pre-existing condition need not be reported. If the investigator is unsure about whether an event is considered unexpected, they can either err on the side of reporting the event or consult with the study medical monitors or protocol chairs. These types of adverse events are reportable within 15 business days of discovery by the site (see Section 9.3.1.3).

9.1.3 Unanticipated Problems

Unanticipated problems include **unexpected** problems, events, or new information which are not AEs but which indicate that research participants or others are at greater risk of harm than previously believed prior to recognition of the unanticipated problem. **Unanticipated problems require expedited reporting within three business days (see Section 9.3.1.3).** Final determination of whether these problems should result in a change in the study consent form will be determined by the Medical Monitors, protocol chairs, and the DSMC.

9.2 Definition of a Serious Adverse Event (SAE)

Serious Adverse Event

An adverse event is defined as a **serious adverse event (SAE)** when the AE results in any of the following outcomes:

1. Death
2. Life-threatening (this means that the subject is at **immediate** risk of death at the time of the event without medical intervention; this does not mean that the subject was at risk for a life-threatening outcome)
3. Inpatient hospitalization or prolongation of existing hospitalization
4. Persistent or significant disability/incapacity
5. Congenital anomaly/birth defect in the offspring of a subject

6. Other: Important medical events that may not result in death, be immediately life-threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical surgical intervention to prevent one of the outcomes listed in this definition.

Clarification on SAEs:

1. All deaths, regardless of cause, must be reported for subjects on study and for deaths occurring within 30 days of last study evaluation, whichever is longer.
2. “Immediately life-threatening” means that the subject was at immediate risk of death from the event as it occurred. This does not include an event that might have led to death, if it had occurred with greater severity.
3. Complications that occur during hospitalizations are AEs. If a complication prolongs hospitalization, it is an SAE. Note that the hospital prolongation should be clearly and directly attributable to the event.
4. “Inpatient hospitalization” means the subject has been formally admitted to a hospital for medical reasons, for any length of time. This may or may not be overnight. This does not refer to evaluation in an Emergency Department without admission to the hospital. This does not include planned inpatient hospitalizations for transplant or other elective procedures.
5. Patients undergoing HCT are frequently hospitalized after the initial transplant hospitalization. Subsequent hospitalizations for reasons that meet criteria for SAE or AESI should be reported as SAE. Subsequent hospitalizations for reasons that do not meet criteria for SAE or AESI need not in and of themselves be reported as SAE.

The investigator should attempt to establish a diagnosis of the event based on signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE and not the individual signs/symptoms.

9.3 Adverse event reporting methods, timing, grading, analysis and management

9.3.1 Methodology

9.3.1.1 Categorizing and Grading Adverse Events

AEs that need to be reported will be assessed and categorized by the Site Principal Investigator using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The Site Principal Investigator will assess seriousness, expectedness, and relatedness of the event to the investigational product. After reporting, the study medical monitors and protocol chairs will assess and make a judgment regarding the seriousness, expectedness, and relatedness of a given event.

9.3.1.2 Assessing Relatedness/Attribution

Attribution of the event to the study intervention (busulfan-based preparative regimen followed by infusion of T-cell and B-cell depleted product) will be characterized as follows:

- **Definitely related:** The adverse event is clearly related to the study intervention. This is most straightforward when events previously associated with the intervention (e.g. veno-occlusive disease) occur. This is less clear for infections, as this population has inherent immune deficiency prior to the intervention and may have undetected infections or active infections when the intervention begins.
- **Probably related:** The adverse event is likely related to the study intervention. The adverse event is not likely to be caused by the subject's underlying medical condition or other concomitant therapy, and the nature of the adverse event or the temporal relationship administration leads the investigator to believe that there is a reasonable chance of causal relationship.
- **Possibly related:** The adverse event may be related to the study intervention. The adverse event could also be attributed to the subject's underlying medical condition or other concomitant therapy, but the timing of the onset of the adverse event and study intervention leads the investigator to believe that there might be a causal relationship.
- **Unlikely related:** The adverse event is probably not related to the study intervention and an alternative explanation is more likely.
- **Not related:** The adverse event is clearly NOT related to the study intervention. The adverse event is most plausibly explained by the subject's underlying medical condition or other concomitant therapy, or the adverse event has no plausible biological relationship to study intervention.

The following factors should be considered in making this assessment:

- The temporal relationship of the event to the administration of the study intervention
- Whether an alternative etiology has been identified
- Biological plausibility

9.3.1.3 What and When to Report

Events that must be reported on an expedited basis for review by the Medical Monitors are listed below. Investigators should not wait to report until full details are known; rather, an initial report should be made and the adverse event form can be updated via the study specific electronic data capture system later to report new or additional details as well as corrections to initial information submitted at the time of the initial report. Details of reporting through the Medidata system are located in the Study MOP.

Local Site Principal Investigators will submit reports of unexpected SAEs and other unanticipated problems to local IRBs in an expedited fashion as required per institutional IRB policy. These events will be reviewed by the institutional IRBs. In addition, reports must be submitted to the CIBMTR (RCI-BMT) in the following timeframes:

1. All grade 5 events (deaths) should be reported within 2 business days.

2. Other SAEs and Unanticipated Problems should be reported within 3 business days.

3. AESIs in general should be reported within 5 business days (1 week). This applies to lack of neutrophil recovery, failure of T cell development and grade IV GVHD.

4. All forms of VOD (mild or moderate or severe VOD) should be reported on data forms at required intervals. VOD leading to the need of ICU care for organ failure (intubation, dialysis, continuous veno-venous hemofiltration, etc.) should be reported as an SAE.

5. Unexpected grade 3-4 AEs should be reported within 15 business days (3 weeks).

Grade 1 and 2 AEs that do not meet the definition of SAE and grade 3 and 4 AEs that are expected and otherwise are not SAE or AESI will not be reported.

The NCI's CTCAE Version 5.0 should be used to describe events as outlined in the study MOP. The Sponsor will be responsible to submit any reportable events to the FDA per applicable timelines.

9.3.2 **Adverse Event Follow-Up**

SAE and AESI should be followed until resolution of the event, death, or until the investigator concludes that the event is stable with no further improvement anticipated.

9.3.3 **Medical Monitors**

The study has two medical monitors, one working with the coordination center at RCI-BMT and a second associated with DAIT-NIAID. The CIBMTR medical monitor will review all reported SAEs, AESIs, and unanticipated problems within two business days of becoming aware. If additional information is required, study centers will have 4 business days to respond to the request for additional information. The CIBMTR medical monitor will report SAEs, AESIs and unanticipated problems to the study Co-Chairs along with an assessment of required actions. Further reporting to the DSMC, FDA, and the central and local IRBs will occur as warranted by the judgment of the medical monitors and Co-PIs.

In addition to the reporting above, the CIBMTR medical monitor will review reports every 6 months which compile new and accumulating information on AEs, SAEs and major protocol deviations as reported within the EDC. These reports will also be reviewed by the protocol Co-PIs and shared with the DSMC and site PIs as applicable. It is the responsibility of each site PI to forward the distributed communications from the DSMC to their IRB of record.

The DAIT-NIAID medical monitor will receive copies of all reports provided to the FDA, reports of all SAE's, and all DSMC reports.

9.4 Halting Rules for the Protocol

Four stopping rules will be utilized for this protocol. These stopping rules will be applied separately for each dose level, but will utilize the combined genotype cohorts, as they are expected to be applicable for all the genotypes and combining them will improve sensitivity of the stopping rule.

- The first stopping rule is based on lack of neutrophil engraftment by day 42, which is expected to be no higher than 10%.
- The second stopping rule is based on day 100 mortality, which is also expected to be no higher than 10%.
- The third stopping rule is based on the incidence of grade 4 acute GVHD by day 100, which is similarly expected to be no higher than 10%.
- The fourth stopping rule is based on T-cell rejection at 6 months, indicated by either a 2nd tx within the first 6 months or by <5% chimerism in the T cell lineage at 6 months; this is also expected to occur no more than 10% of the time.

These stopping rules are outlined in the tables below, all following the same number format.

Table: Stopping rule for monitoring day 42 lack of neutrophil engraftment, 100 day mortality and grade 4 acute GVHD, and day 180 T-cell rejection. The trial will be halted for review if there are x or more events observed after ≤n patients are evaluable.

# of patients, n	≤4	≤8	≤12	≤16
# of events to trigger stopping rule, x	2	3	4	5

The likelihood of triggering these stopping rules are given in the table below, for varying event rates between 10% and 35%. This stopping rule has an 8% likelihood of being triggered when the true event rate is 10%, and an 80% likelihood of being triggered when the event rate is truly 35%. Note that these operating characteristics are the same for safety endpoints of day 42 lack of neutrophil engraftment, day 100 mortality and grade 4 acute GVHD, and day 180 T-cell rejection.

Table: Operating characteristics of stopping rules for various true event rates.

True event rate	Likelihood of triggering the stopping rule
10%	8%
25%	52%
30%	68%
35%	80%

Once a stopping rule is met, the CIBMTR, after notification from the PI or Medical Monitors, will immediately lock the EDC database for new enrollment. All centers, appropriate IRBs and DSMC contacts will be informed of enrollment closure.

10 CLINICAL MONITORING STRUCTURE

10.1 Site Monitoring Plan

The Investigator will permit study-related on-site, remote, and/or centralized monitoring visits by representatives of the CIBMTR or designees, and regulatory inspection(s) (e.g., FDA) to ensure proper conduct of the study and compliance with the protocol and all FDA safety reporting requirements. Access will be provided to the facilities where the study took place, to source documents, to data collection forms, and to all other study documents. It is important that the site Principal Investigator and relevant study personnel are available during on-site monitoring visits or audits and that sufficient time is devoted to the process.

The monitor should have access to laboratory test reports and other subject records needed to verify the entries on the CRF. The investigator [or his/her deputy] agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved. Details regarding monitoring can be found in the study monitoring plan.

10.2 Data Management

Protocol-specific subject data will be recorded in a limited access secure electronic data capture (EDC) system. All changes made to the clinical data will be captured in an electronic audit trail and available for review by the sponsor or its representative. The associated RAVE software and database have been designed to meet regulatory compliance as part of a validated system compliant with laws and regulations applicable to the conduct of clinical studies pertaining to the use of electronic records and signatures. Database backups are performed regularly.

The Investigator or delegate provides his/her electronic signature on the appropriate electronic case report forms (eCRFs) in compliance with local regulations.

10.3 Data Safety Monitoring

This study will be centrally reviewed and followed by the Data Safety and Monitoring Committee (DSMC) of the Pediatric Blood and Marrow Transplant Consortium (PBMTC). The DSMC is a standing committee, composed of a chair, patient advocate, biostatistician, nurse representative and two bone marrow transplant physicians with procedures and processes as defined in the PBMTC DSMC Charter. The DSMC will review the study protocol prior to study activation and IRB review, and will continue to review the study on a regular basis according to the committee rules.

The DSMC will meet at regular intervals to review all adverse events and deaths and determine whether any patient safety problems necessitate protocol modifications or discontinuation of the trial. The DSMC will also meet on an *ad hoc* basis if stopping guidelines are met (see Section 7.11) or if unexpected safety events occur that may necessitate study suspension or closure. The DSMC will discontinue the review of outcomes when this protocol is closed to accrual.

Before each regularly scheduled DSMC meeting, the CIBMTR will submit a report including tabular summaries of all SAEs and deaths on study to date. The report will also include a brief summary of each previously unreported SAE and death, including an assessment of whether the event was unexpected or related to the study.

If the DSMC recommends protocol or informed consent changes during the study, the recommendations will be reviewed by the Protocol Co-Chairs and incorporated into the protocol as deemed appropriate. The protocol with incorporated changes will be distributed to the participating Transplant Center after approval by the NMDP IRB. It is the responsibility of each Transplant Center PI to forward the distributed communications from the DSMC to their local IRB.

11 STATISTICAL CONSIDERATIONS

11.1 Overview and Study Objectives

This is a prospective randomized multicenter open label Phase II study of two preparative regimens with low vs. moderate doses of busulfan for TCR αβ+/CD19+ depleted haploidentical related and unrelated donor hematopoietic cell transplantation (HCT) in newborns identified at birth with specified genetic subtypes of SCID. The study seeks to determine whether the higher dose intensity is needed to obtain combined cellular and humoral reconstitution manifested by vaccine specific antibody response, or whether a lower dose intensity can obtain similar immune reconstitution with less potential toxicity burden. The dose randomization will be done separately in each of 2 strata based on genotype. Sixteen patients will be randomized to each of 2 dose levels in each of the 2 genotype strata, for a total study size of 64 patients.

11.2 Study Population

Patients with SCID without active infection with the appropriate genotype/phenotypes will be randomized to the low or moderate dose level and will undergo allogeneic HCT with the donor selected by the enrolling site.

11.3 Description of the Analyses

This study is a randomized phase II trial of two busulfan dose intensities, conducted in two strata. The analysis will be done separately in each of the strata defined by genetic abnormalities, by comparing the outcomes of the randomized dose intensity groups. Additionally, confidence intervals for each outcome will be provided, so that the outcomes of each dose group can be benchmarked against historical experience with no busulfan conditioning.

11.4 Measures to Minimize Bias

Enrollment/ Randomization/ Masking Procedures

Randomization will be performed after eligibility is confirmed. Randomization between the two dose levels will be done separately in each genotype stratum, using permuted blocks. Randomization will be stratified further by donor type (Haploidentical related vs. Matched Unrelated Donor). The study will be open label. All patients who start conditioning will be considered evaluable for all toxicity and efficacy assessments. Analysis will be conducted under a modified intention-to-treat principle among those who receive conditioning, where patients are analyzed according to the busulfan dose to which they are randomized, regardless of what dose they actually receive. All available data will be used and missing data will not be estimated or carried forward in any statistical summary or analyses. We will also collect essential data on patients who are randomized but fail to start their preparative regimen, in order to document why they did not start their preparative regimen and what their eventual outcome was. We will use this in a sensitivity analysis of all randomized patients. Patients who are randomized but do not start conditioning will be replaced from the standpoint of accrual in the sense that they will not contribute towards accrual of the target sample size for the primary analysis.

11.5 Study Hypotheses

The primary objective of this study is to compare the incidence of humoral immune reconstitution at 2 years post-transplant in each of the 2 genotype cohorts between the 2 busulfan dose levels; death and second transplant will be considered competing risks for this endpoint. The null hypothesis of the study is that there is no difference in humoral immune reconstitution between the dose levels. The alternative hypothesis of interest is one-sided, i.e. that the higher dose level has greater humoral immune reconstitution, indicating that greater exposure to busulfan is needed to improve humoral immune reconstitution despite the potential for increased toxicity risks. These null and alternative hypotheses reflect a decision-making framework where, if there is no difference in vaccine response rates, the preference should be

for the lower intensity regimen due to less toxic complications. In addition to statistical comparisons between the treatment arms, 90% confidence intervals will be determined for the primary endpoint in each genotype cohort and dose level, and these will be used to benchmark each treatment group result to historical data without busulfan conditioning, which has approximately a 15% incidence of humoral immune reconstitution.

11.6 Sample Size Considerations

Power and sample size considerations are based on the primary objective of comparing the humoral reconstitution rate between the Busulfan intensity levels, and identifying whether the higher dose is needed to obtain greater humoral reconstitution despite the increased toxicity risks. Power calculations are based on an exact unconditional test for comparing two binomial proportions using the two-sample Z test with pooled proportions (Suissa and Shuster, 1985). We power the study using a one-sided significance level of 20%; note that higher type I error rates such as this are sometimes used in randomized phase II trial settings, particularly with rare disease settings, to keep the required sample sizes down while still maintaining power (Ratain and Karrison, 2007). Power calculations were obtained using the R package 'Exact', and are shown in the table below for a range of settings for the vaccine response rates, and for differences in response rates of either 0% or 30%. The proposed sample size of 16 patients per dose level has at least 80% power when the higher dose intensity has a 30% higher vaccine response rate at 2 years. The type I error rate is typically lower than 20%, ranging from 17% to 18% depending on the setting, indicating that when there is no difference in response rates, we conclude that there is no benefit of higher busulfan intensity over 80% of the time. These operating characteristics reflect the preference in our decision making framework for the lower intensity regimen if there is no difference in vaccine response rates due to less toxic complications.

	Type I error when no difference in vaccine response rates			Power to detect 30% increase in vaccine response rates		
Response rates (Low, Moderate)	20%,20%	30%,30%	40%,40%	20%,50%	30%,60%	40%,70%
Power or type I error	17%	17%	18%	82%	81%	81%

11.7 Participant Enrollment and Follow-Up

A total of 64 patients (16 per each of two dose levels in each of 2 genotype cohorts) will be enrolled on this study. Total duration of accrual is planned to be 4 years. If accrual to one of the genotype cohorts is completed while the other cohorts are still enrolling, the cohort may be kept open to increase sample size and improve power. Patients may be followed for up to 3 years. The analysis of the primary endpoint for a particular cohort is planned to occur when 32 patients in that cohort have a minimum of 2 years of follow up.

11.8 Planned Interim Analyses (if applicable)

No interim analyses for efficacy or futility are planned for this study, because of the long duration (2 years) before the primary endpoint is available for analysis.

Interim analyses for safety are described in Section 9.4.

11.9 Final Analysis Plan

General strategy: All efficacy and safety analyses will be done on the transplanted cohort, i.e. all patients who are randomized and actually go to transplant. A modified ITT analysis will be used to analyze patients according to the busulfan dose to which they were randomized. Estimates and 90% confidence intervals will be provided for each dose level in each genotype cohort, in order to benchmark the outcomes against historical data using no conditioning. Comparisons between groups will be done using conditional exact tests for binomial proportions, and nonparametric tests for continuous outcomes, as described in more detail below. The primary endpoint analysis will use a one-sided significance level of 20%, to maintain power with modest sample sizes in this rare disease setting; all other secondary endpoint analyses will use the standard significance level of 5% due to multiple testing and the exploratory nature of the endpoints. Estimates of treatment differences along with two-sided confidence intervals will be provided with a conventional coverage probability of 95%; for the primary endpoint, we will also provide a 60% confidence interval to be consistent with the one-sided significance level used in hypothesis testing. Because there are a priori concerns that vaccine responses will be genotype dependent, primary analyses will be conducted separately in each genotype cohort. An additional secondary analysis will be conducted for all endpoints combining the genotype groups together to potentially improve power to detect differences; this will be stratified on genotype.

Primary endpoint: Exact binomial confidence intervals for the cumulative incidence of humoral immune reconstitution at two years will be constructed. Patients who die or have a second HCT will be considered failures for the primary endpoint. Busulfan dose groups will be compared using unconditional exact tests for binomial proportions based on the two-sample Z test with pooled proportions (Suissa and Shuster, 1985). Confidence intervals will be obtained by inverting these unconditional exact tests; this can be done for example using StatExact software. A secondary analysis of the incidence of humoral immune reconstitution at 1 and 3 years will also be conducted in a similar fashion.

Secondary endpoints:

T cell immune reconstitution. $CD3^+$, $CD4^+$ and $CD8^+$ T cell number, donor T cell chimerism, lymphocyte proliferation to PHA, naïve T cell percentage in $CD4^+$ and $CD8^+$ T cells and TREC will be described among survivors at each time point (100 days, 6 months, 12 months, and 2 years) quantitatively using median and range, and compared between dose groups using Mann-

Whitney tests. Proportions of patients meeting defined criteria for T cell reconstitution will also be described using frequencies, and compared between groups using unconditional exact tests for binomial proportions.

Vaccine titer response. Pre and post vaccine titers will be described in those who are vaccinated using medians and ranges. Pre and post vaccine titer responses will be compared using Wilcoxon signed rank tests. Changes in vaccine titer responses will be compared between dose groups using the Mann-Whitney test.

Freedom from immunoglobulin substitution. This is defined as freedom from IVIG for 12 weeks. The proportion of patients meeting this criteria at 6 months, 9 months, 12 months, 2 years and 3 years will be described using frequencies, and compared between groups using unconditional exact tests for binomial proportions. Additionally the proportion of patients who meet criteria for a trial of vaccination will be described.

Chimerism: Whole blood, B cell, NK cell, and granulocyte chimerism will be described among survivors at each time point (42, 100 days, 6, 12 months, and 2 years) quantitatively using median and range, as well as using frequencies according to categories described in Section 4.2.2. Quantitative chimerism values will be compared between dose intensities using Mann-Whitney tests.

Overall survival and Event-free survival: OS and EFS will be estimated at 1 year, 2 years and 3 years using the Kaplan-Meier method, with death (OS) or death, 2nd HCT, or graft failure (EFS) defined as events. OS and EFS will be compared between groups using the log-rank test.

GVHD: The incidence of acute grade 2-4, acute grade 3-4 and chronic GVHD will be described using the cumulative incidence estimator, treating death as a competing event. Acute GVHD will be summarized at 180 days, while chronic GVHD will be summarized at 2 years. GVHD will be compared between groups using Gray's test.

Infections. Infections caused by various organisms as described in Section 4.2.2 will be described using cumulative incidence for each category (bacterial, fungal, viral).

Neutrophil engraftment. Neutrophil recovery will be described using the cumulative incidence estimator, treating death as a competing event. Neutrophil recovery will be summarized at day 42, and will be compared between groups using Gray's test.

Regimen-related toxicities. VOD and IPS will be described using cumulative incidence, treating death as a competing event.

Autoimmunity: The incidence of autoimmunity will be described using cumulative incidence, treating death as a competing event.

Graft composition. Proportion of patients where the targeted CD34+ cells/kg and TCR αβ+/CD19+ depletion goals will be described separately in each dose and genotype group, as well as overall. The relationship between cell doses and key outcomes of engraftment and

immune reconstitution will be examined by comparing cell doses among patients who successfully engraft or have T cell reconstitution vs. those who did not, using a Mann-Whitney test.

12 QUALITY CONTROL AND QUALITY ASSURANCE

As outlined in 10.1, the Investigator will permit study-related on-site, remote, and/or centralized monitoring visits by representatives of the CIBMTR or designees, and regulatory inspection(s) (e.g., FDA) to ensure proper conduct of the study and compliance with all FDA safety reporting requirements. Access will be provided to the facilities where the study took place, to source documents, to data collection forms, and to all other study documents.

The monitor should have access to laboratory test reports and other subject records needed to verify the entries on the CRF. The investigator [or his/her deputy] agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

This trial will be conducted in compliance with the protocol, current Good Clinical Practice (GCP) guidelines recommended by the International Conference on Harmonization (ICH), U.S. 21 CFR Part 50 – Protection of Human Subjects, and Part 56 – Institutional Review Boards, and applicable local regulatory requirements for participating institutions. These include the tenets of the Declaration of Helsinki and review and approval by the local ethics review committee or IRBs of participating organizations.

13.1 Institutional Review Board/Ethics Committee

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate ethics review committee or Institutional Review Board (IRB) prior to the implementation of the protocol. Any amendments to the protocol or consent materials must also be approved before they are placed into use. In both the United States and in other countries, only institutions holding a current U. S. Federal-Wide Assurance issued by the Office for Human Research Protections (OHRP) may participate. Refer to: http://ohrp.cit.nih.gov/efile.

13.2 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. This study will enroll infants, and therefore consent will be sought from the parent(s) or legal guardian(s) of the research participant. Discussion of risks and possible benefits of this therapy will be provided to the parent/legal guardian(s). Consent forms describing in detail the study procedures and risks are given to the parent or guardian and written documentation of informed consent is required prior to starting study intervention. Consent forms will be approved by the local IRB and the

parent/guardian will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. The parent/guardian will sign the informed consent document prior to any procedures being done specifically for the study. The parent/guardian will have sufficient opportunity to discuss the study and process the information in the consent process prior to agreeing to participate. For this study, it is particularly important that alternative therapies including standard of care allogeneic HCT with or without conditioning and when applicable autologous transplant of gene modified stem cells (gene therapy or gene transfer) also be discussed. The parent/guardian may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the parent/guardian. The rights and welfare of the participant will be protected by emphasizing that the quality of medical care will not be adversely affected by declining to participate in this study. A copy of the consent form and documentation of the informed consent discussion will be filed in the medical record of the subject.

This study will enroll children and is anticipated to enroll non-English speakers, as the target population is newborns identified by universal newborn screening. Procedures to handle informed consent and documentation of informed consent of subjects whose parent/legal guardian do not speak or read English will be governed by the local IRB. If informed consent discussions take place with the assistance of an interpreter, the interpreter must also sign the consent form certifying his/her involvement with the consent process, per local IRB requirements.

After execution, a copy of the signed consent form will be given to the subject. The original signed consent form will be kept on file in the site's study file, available for inspection by regulatory authorities, both federal and institutional.

13.2.1 **Assent or Informed Consent Process (in Case of a Minor)**

The age of the children involved in this study is below the age of assent, and therefore assent will not be sought or required.

13.2.2 **Related Donors**

Local sites and the IRB of record for each site (whether sites are relying on the NMDP IRB or a local IRB) will be responsible for the review and continuing oversight of protocol procedures that relate to related donors consented to the study.

13.2.3 **NMDP Unrelated Donors**

The National Marrow Donor Program (NMDP) IRB will be responsible for the review and continuing oversight of protocol procedures that relate only to NMDP unrelated donors.

13.3 Exclusion of Women, Minorities, and Children (Special Populations)

This study will include females and minorities.

13.4 Participant Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

13.5 Study Discontinuation

The study maybe discontinued early by the Sponsor for valid scientific or administrative reasons and reasons related to the protection of patients. Investigators, IRBs and regulatory authorities, as applicable will be notified in writing in the event of study termination.

In the event that the study is discontinued, all patients who have begun conditioning will complete conditioning as planned and infusion of donor cells. After infusion of donor cells, subjects will no longer have study procedures or interventions.

Individual participating study center may be discontinued for reasons such as, but not limited to, lack of enrollment or repeated protocol non-compliance without justification.

14 DATA HANDLING AND RECORD KEEPING

The data collection forms for the subjects enrolled on this study include the standard CIBMTR data collection forms in the FormsNet3 Recipient module, and study-specific CIBMTR data collection forms in the study specific electronic data capture system.

Many important data elements for the study are collected on the standard reporting forms and therefore timely and accurate completion of these forms is essential.

A detailed description of each of the forms and the procedures required for forms completion and submission timelines can be found in the Forms Instructions. Forms that are not entered

into the data entry system within the specified time will be considered delinquent. A missing form will continue to be requested until either the form is entered into the data entry system, or until an exception is granted.

14.1 Data Capture Methods

14.1.1 Electronic Case Report Forms (e-CRFs)

Data will be collected in a validated system with an audit trail that is fully compliant with 21 CFR Part 11. Data will be monitored by the sponsor (or designee). For each subject enrolled, appropriate electronic case report forms (e-CRFs) will be completed. These include study-specific CRFs within the study specific electronic data capture system.

14.2 Study Records Retention

Study records will be retained for 3 years after completion of final analysis.

14.3 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with Good Clinical Practice (GCP ICH E6) Sections:

Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
Quality Assurance and Quality Control, section 5.1.1
Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations according to the guidelines of the IDE sponsor, if applicable.

Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

15 PUBLICATION POLICY

To be developed in conjunction with funding agency and PIDTC.

Following completion of the study, the investigator may publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals has adopted a trials-registration policy as a condition for publication. This policy

requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine.

Please refer to the following NIH guidelines regarding trial registration:

http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-014.html
http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-023.html

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Appendix 1

The following is a table of normal range of IgG for age, with values taken from **Jolliff CR, Cost KM, Stivrins PC, Grossman PP, Nolte CR, Franco SM, et al. Reference intervals for serum IgG, IgA, IgM, C3, and C4 as determined by rate nephelometry. Clin Chem. 1982;28(1):126–8.** These values can be used to document that the patient has maintained normal IgG level for age without substitution.

Age	N tested	Mean IgG mg/dL	95% range IgG mg/dL
Cord blood	50	1121	636-1606
1 month	50	503	251-906
2 months	50	365	206-601
3 months	50	334	176-581
4 months	50	343	196-558
5 months	50	403	172-814
6 months	50	407	215-704
7-9 months	50	475	217-904
10-12 months	50	594	294-1069
1 year	50	679	345-1213
2 years	50	685	424-1051
3 years	50	728	441-1135
4-5 years	50	780	463-1236
6-8 years	50	915	633-1280

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