Phase I/II Feasibility Study of Busulfan Fludarabine and Thiotepa Conditioning Regimen for Allogeneic Hematopoietic Stem-Cell Transplantation (HSCT) for Children and Young Adults with Non-Malignant Disorders

NCT03513328

Protocol Version 19JUL2020

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Protocol Number: **PEDS024**

IRB #: **201800798**

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Clinical Phase:	Phase I/II
IND Status:	Not applicable
Protocol Agent(s):	busulfan, fludarabine, thiotepa, anti-thymocyte globulin, tacrolimus, methotrexate – IV only, mycophenolate mofetil
Date of Original Protocol:	**April 4, 2018**
Date of Version 1.1 Protocol :	September 1, 2018
Date of Current Protocol	**July 15, 2020**
Version of Current Protocol:	1.2

PROTOCOL REVISION RECORD

Keywords:	allogeneic, hematopoietic stem cell transplantation, pediatric, non-malignant disorders

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1. ABBREVIATIONS

ABO	blood group system
ADL	activities of daily living
AE	adverse event
ANC	absolute neutrophil count
araA	L-arabinose isomerase
ARDS	acute respiratory distress syndrome
ATN	acute nephrotic toxicity
AUC	area under curve
BM	bone marrow
BMT	bone marrow transplant
Bu	busulfan
BUN	blood urea nitrogen
C	centigrade
CBC	complete blood count
cGVHD	chronic graft vs host disease
CI	confidence interval
CIBMTR	Center for International Blood & Marrow Transplant Research
CMV	cytomegalovirus
CNS	central nervous system
CRF/eCRF	case report form/electronic case report form
CSF	cerebrospinal fluid
CSTD	closed-system transfer device
CTEP-AERS	Cancer Therapy Evaluation Program – Adverse Event Reporting System
CTCAE	Common Terminology Criteria for Adverse Events
CTMS	clinical trials management system
CTO	Clinical Trials Office
D5W	dextrose 5% in water
DFS	disease-free survival
DL	deciliter
DLCO	diffusing capacity of the lungs for carbon monoxide
DLT	dose-limiting toxicity
DMA	dynamic mechanical analysis
DNA	deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
EBV	Epstein-Barr virus
EDTA	ethylenediaminetetraacetic acid
e.g.	example
F	fahrenheit
FACT	Foundation for Accreditation of Cellular Therapy

FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act
FEV1	forced expiratory volume in one second
FLU	fludarabine
FSH	follicle stimulating hormone
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor
GI	gastrointestinal
GVHD	Graft vs Host Disease
HBV	hepatitis B virus
HCO-60	polyoxyethylene castor oil derivative
HCV	hepatitis C virus
HHV-6	human herpes virus 6
HIV	Human immunodeficiency virus
HLA	human leukocyte antigen
HRT	hormone replacement therapy
HSCT	hematopoietic stem cell transplant
IATA	International Air Transport Association
IBW	ideal body weight
ICAO	International Civil Aviation Organization
ICF	informed consent form
ICH	International Conference on Harmonization
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	Immunoglobin M
IMPDH	Inosine-5′-monophosphate dehydrogenase
IND	investigational new drug application
IRB	Institutional Review Board
ITP	idiopathic thrombocytopenic
IU	international unit
IV	intravenous
JC	John Cunningham
kg	kilogram(s)
KM	Kaplan-Meier
L	liter
LFT	liver function test
m	meter
MED	minimum effective dose
MF1	Mean fluorescence intensity
mg	milligram

MMF	mycophenolate mofetil
MPA	mycophenolic acid
MPAG	7-O-MPA-Beta-Glucuronide (metabolized mycophenolic acid)
MRD	minimal residual disease
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
MTX	methotrexate
NaCL	sodium chloride
NK-SCID	natural killer cell-positive severe combined immunodeficiency disease
NSAIDS	non-steroidal anti-inflammatory drugs
NS	normal saline
NSAE	non-serious adverse event
NSC	identifying number assigned an agent or product
NTPR	National Transplant Pregnancy Registry
OS	overall survival
PB	peripheral blood
PBSC	peripheral blood stem cell
PCR	polymerase chain reaction
PFT	pulmonary function test
pH	potential of hydrogen
PHI	protected health information
PI	principal investigator
PK	pharmacokinetics
PML	progressive multifocal leukoencephalopathy
PO	by mouth
rATG	active rabbit antithymocyte globulin
SAE/SE	serious adverse event
SD	standard deviation
SOC	standard of care
SOS	sinusoidal obstruction syndrome
SNP	single nucleotide polymorphisms
TBI	total body irradiation
TID	three times a day
TNC	total nucleated cell
UF	University of Florida
ULN	upper limit of normal
µ	micron
µm	micrometer
US	United States
USP	United States Pharmacopeia

VOD	veno-occlusive disease
V_{ss}	volume steady state
WOCBP	women of childbearing potential

2. PROTOCOL SIGNATURE PAGE

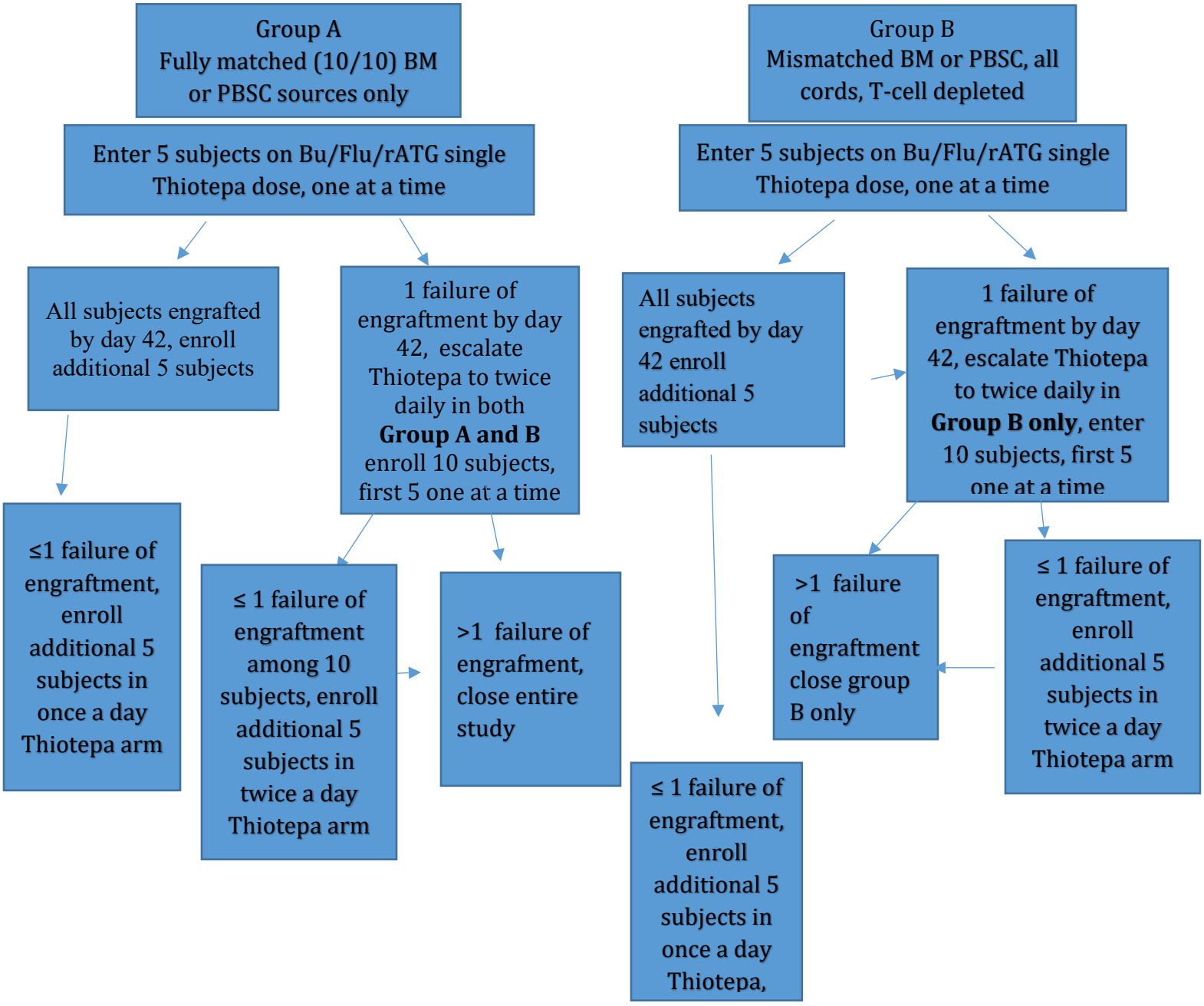
Feasibility Phase I/II Radiation-Free Allogeneic Hematopoietic Stem-Cell Transplantation (HSCT) for Children with High-Risk Hematologic Malignancies

Principal Investigator:

Signature of Investigator	Date
Biljana Horn, MD Printed Name of Investigator	
University of Florida Name of Facility	
Gainesville, Florida Location of Facility (City/State)	

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.

3. STUDY SCHEMA I: PHASE I/II THIOTEPA DOSE ESCALATION



4. PROTOCOL SYNOPSIS

Title:	***Phase I/II Feasibility Study of Busulfan Fludarabine and Thiotepa Conditioning Regimen for Allogeneic Hematopoietic Stem-Cell Transplantation (HSCT) for Children with Non-Malignant Disorders***
Funding Organization:	***TBD***
Rationale:	Hematopoietic stem cell transplantation is the only curative choice for a number of inherited bone marrow failure syndromes, hemoglobinopathies, metabolic disorders and primary immune deficiencies. While survival of these patients is typically better than survival of patients with malignancies, toxicities of conditioning regimens and failure of engraftment remain challenges. Most children with non-malignant disorders present with normocellular or even hypercellular bone marrow, posing a barrier to engraftment and requiring intensive conditioning. Commonly used backbone of busulfan and fludarabine, although well tolerated, results in variable engraftment, in particular with mismatched unrelated donors and cord blood recipients. In this study we test 2 dose levels of thiotepa (5 mg/kg and 10 mg/kg) added to the backbone of targeted reduced dose IV busulfan, fludarabine and rabbit anti-thymocyte globulin (rATG) in order to determine the minimum effective dose required for reliable engraftment. Subjects are stratified in groups A and B based the risk of graft failure.
Objectives:	**Primary:** • Determine the minimum effective dose (MED) of thiotepa in combination with reduced-dose busulfan, fludarabine and rATG required to achieve engraftment in >90% subjects undergoing hematopoietic stem cell transplantation for non-malignant disorders. The MED will be determined for subjects stratified in group A (lower risk of graft failure) and group B (high risk of graft failure). The 2 groups will be merged if group A requires escalation to the higher dose level. **Secondary:** • Evaluate the risk of early (by day 42) and late (by day 365) graft failure/rejection. • Evaluate 12 and 24 month disease free survival (DFS) and overall survival (OS) of children undergoing HSCT using busulfan/fludarabine/rATG/thiotepa conditioning regimen.

	• Evaluate transplant-related mortality at 100 days and 12 months post-transplant. • Evaluate rate of acute (Grade II-IV) graft-versus-host disease (GVHD) by 12 months post-transplant • Evaluate rate of chronic GVHD by 24 months post-transplant • Evaluate transplant-related complications at 12 and 24 months post-transplant **Exploratory objectives:** • Describe changes in neurocognitive functioning compared to pre-transplant • Describe association between pharmacogenomics of genes involved in metabolism of chemotherapy agents and toxicity • Describe immune reconstitution following this conditioning regimen
Study Design:	This is a Phase I/II feasibility study of allogeneic stem cell transplantation using a conditioning regimen which tests 2 different doses of thiotepa added to the backbone of reduced-dose busulfan, fludarabine and rATG for children with non-malignant disorders in order to achieve reliable engraftment. Following donor identification and standard of care pre-transplant evaluation, subjects will start conditioning regimen. The study builds in rules for escalation of thiotepa dose based on graft failure by day 42. Patients are stratified in two groups A (lower risk of graft failure) and B (higher risk of graft failure). Patients undergoing 10/10 HLA matched bone marrow and peripheral blood transplants are assigned to group A, and patients receiving <10/10 matched bone marrow or peripheral blood, or receiving cord blood, even if fully matched, as well as T-cell depleted transplants, are assigned to group B. If criteria, based on graft failure rate, for thiotepa dose escalation are met first in group A, which has a lower risk of graft failure, thiotepa dose will be escalated for all subjects (group A and B). If criteria for thiotepa dose escalation are met first in group B, dose will be escalated only in group B, and group A will continue enrolling patients at a lower dose level. Treatment will start in each group with addition of a single dose of thiotepa (5mg/kg/day) for the first 5 subjects. If failure of engraftment has not been observed by day 42, 5 additional subjects will be enrolled in the same dose level. If 0 or 1 failure of engraftment is observed among the first 10 subjects of each

	dose level, 5 additional subjects will be enrolled at the same dose level. If a failure of engraftment is observed among the first 5 subjects, the dose of thiotepa will be increased to twice a day and 10 subjects enrolled at that dose level. If there is 0 or 1 graft failure among the first 10 subjects, additional 5 subjects will be enrolled in twice a day thiotepa arm. If there are 2 or more graft failures among the 10 subjects at twice a day thiotepa dose level in group A, the entire study will be closed to further accrual. If there are 2 or more graft failures in group B, only that group will be closed to further accrual. With this design, a minimum of 15 subjects will be enrolled at each effective dose level. The study may confirm a different MED of thiotepa for group A and group B. Exploratory objectives include evaluating neurocognitive functioning following this conditioning regimen and assessment of immune reconstitution. We will also gather additional information on pharmacogenomics and possible associations between genes involved in metabolism of busulfan, fludarabine and thiotepa and toxicity and efficacy.
Accrual Goal:	The study will enroll a minimum of 15 patients at each effective thiotepa dose level. The maximum number of patients will be 35.

Inclusion Criteria:	Individuals eligible for Phase I/II study participation must meet the following criteria:
	• Subjects with any non-malignant disorder known to benefit from allogeneic transplantation for cure or amelioration of symptoms of the underlying disease. The following groups of diagnoses are eligible:
	  • Hemoglobinopathies (e.g. thalassemia or sickle cell disease),
	  • Cytopenias (e.g.Diamond-Blackfan anemia, congenital or acquired neutropenia, congenital or acquired thrombocytopenia, congenital or acquired anemia, and others, regardless clonality),
	  • Hemophagocytic lymphohystiocytosis,
	  • Primary immunodeficiencies (e.g. Wiscott Aldrich Syndrome, chronic granulomatous disease, common variable immune deficiency, X-linked lymphoproliferative disease, NK+ severe combined immune deficiencies),

	• Metabolic disorders (Hurler's syndrome, mannosidosis, adrenal leuko-dystrophy)
	• Other non-malignant disorders for which there is published evidence that HSCT is a curative therapy.
	• Donor Requirements
	  • Related or unrelated donor who is suitable and willing to donate bone marrow or peripheral blood stem cells. HLA typing should be done by high-resolution typing at A, B, C, DrB1 and DQ loci and the donor should be a ≥8/10 match (with one antigen/allele mismatch allowed at A, B, or C-loci and other at DQ loci).
	  • Cord blood units must be matched at a minimum of 6/8 antigens/alleles at A, B, C and DrB1 loci. High resolution typing at all loci is required. The minimum TNC dose pre-cryopreservation must be ≥3.7 x10^7/kg of recipient's weight, if a single cord blood unit is used, or at least 2x10^7/kg per unit, if two cord blood units are used. The mismatches cannot be at the same loci (e.g. double A mismatch).
	  • Haploidentical related stem cell donor who is suitable and willing to donate peripheral blood stem cells. T-cell depletion is required if haploidentical donors are used.
	
	• Adequate organ function defined as:
	  • Cardiac: ejection fraction ≥55% or shortening fraction ≥30%
	  • creatinine clearance ≥70 ml/min/1.73m^2
	  • Pulse oximetry >95% on room air or FEV1/DLCO >60%
	  • LFTs < 3 x ULN, Total bilirubin <3 mg/dl (unless due to non-hepatic cause (e.g. Gilbert's syndrome or hemolysis)
	• Lansky/Karnofsky score ≥60%
	• Age: ≥3 months and ≤39 years of age at the time of signing informed consent
	• Written informed consent obtained from the subject or parental/guardian permission ± child's assent per institutional guidelines

	• Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy for at least 1 month after completion of conditioning.
	WOCBP include any woman who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or who is not post-menopausal. Post-menopause is defined as:
	  • Amenorrhea that has lasted for ≥ 12 consecutive months without another cause, or
	  • For women with irregular menstrual periods who are taking hormone replacement therapy (HRT), a documented serum follicle-stimulating hormone (FSH) level of greater than 35 mIU/mL.
	• Males with female partners of childbearing potential must agree to use physician-approved contraceptive methods (*e.g.*, abstinence, condoms, or vasectomy) for at least one month after completion of conditioning.

Exclusion Criteria:	Subjects with any of the following will not be eligible for study participation: • Diagnoses that do not require myeloablative transplant for cure (e.g. NK- SCID patients), unless they previously did not engraft with non-myeloablative or reduced intensity conditioning transplant. • Known or suspected sensitivity to chemotherapy or radiation (e.g Fanconi's anemia, Dyskeratosis congenita, Ligase IV deficiency, etc). • Cytopenias with increased blasts (>5%) • Patients with fast-progressing neurodegenerative disorders (e.g. Krabbe disease or adrenal leukodystrophy with Loes score of ≥10) • Presence of anti-donor HLA antibodies (positive anti-donor HLA antibody is defined as a 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 with mean fluorescence intensity (MFI)>3000 by solid phase immunoassay. This will be measured before the final donor selection.

	• Prior allogeneic stem cell transplant, except for patients with immune deficiencies who underwent previous non-myeloablative or reduced intensity transplants. • Haploidentical donor using in vivo T-cell depletion (e.g. post transplant cyclophosphamide). • Uncontrolled bacterial, viral, or fungal infection at the time of enrollment. Uncontrolled is defined as currently taking medication and with progression or no clinical improvement on adequate medical treatment. • Seropositive for HIV • Active Hepatitis B or C determined by a detectable viral load of HBV or HCV by PCR • Bridging fibrosis or liver cirrhosis • Females or males of childbearing potential who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and for at least 1 month after the end of conditioning • Females who are pregnant or breastfeeding • History of any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of protocol therapy or that might affect the interpretation of the results of the study or that puts the subject at high risk for treatment complications, in the opinion of the treating physician. • Subjects demonstrating an inability to understand the study and comply with the study and/or follow-up procedures.
Efficacy Assessments:	**<u>Primary Endpoint</u>:** • Determine the minimum effective dose (MED) of thiotepa in combination with reduced-dose busulfan, fludarabine and rATG required to achieve engraftment in >90% subjects undergoing hematopoietic stem cell transplantation for non-malignant disorders. The MED will be determined for subjects stratified in group A (lower risk of graft failure) and group B (high risk of graft failure). The two groups will be merged if the group A requires escalation to the higher dose level.

	Effective dose of Thiotepa in combination with busulfan/fludarabine and rATG is defined as a dose that results in engraftment of >90% study subjects by day 42 with acceptable toxicity, defined as ≤20% grade 5 toxicities and ≤40% combined grade 4 and 5 toxicities across all subjects treated with the same dose level. Definition of engraftment failure used for dose escalation in this study includes: • Failure to achieve sustained ANC recovery of >0.5x10^9/L with evidence of donor chimerism by day 42, in light of absence of myelosupressive medications (e.g. ganciclovir), or • Engraftment with subsequent loss of donor chimerism to <5% by day 42, or • Need for the 2nd conditioned transplant by day 42 to correct manifestations of disease or cytopenia. **<u>Secondary Endpoints</u>:** **Evaluate the risk of early (by day 42) and late (by day 365) graft rejection/failure** Proportion and associated confidence interval (CI) of all subjects who initiated conditioning regimen and have sustained engraftment failure (as outlined above) at 42 days and at 1 year post transplant. . Proportion and associated CI of subjects who initiated conditioning regimen and who die due to a cause unrelated to the underlying disease. Kaplan-Meier plots will also be generated. • **Evaluate 12 and 24 month disease free survival (DFS)/event-free-survival and overall survival (OS) of children undergoing HSCT using busulfan/fludarabine/rATG/thiotepa conditioning regimen** Proportion and associated confidence interval (CI) of all subjects who initiated conditioning regimen and are alive (OS) and without evidence of underlying disease (DFS) at 12 months post-transplant will be calculated. KM estimates of OS and DFS will also be used. OS is defined as time from

	transplant (day 0) until date of death or date of last contact for those who have not died. DFS/event-free survival is defined as time from transplant (day 0) until disease recurrence/progression, or date of death from any cause, or date of last contact for those who are disease-free. Disease progression will follow-standard of care definitions for each disorder. In general, disease-free-survival implies improvement of reversible manifestations of a disorder and arrest in further progression of symptoms in light of presence of donor engraftment and immune reconstitution. • **Evaluate transplant-related mortality at 100 days and 12 months post-transplant** Proportion and associated CI of subjects who initiated conditioning regimen and who die due to a cause unrelated to the underlying disease. Time to transplant-related mortality is defined as time from transplant (day 0) until date of transplant related mortality. Those who die from other causes will be censored at date of death; those who were alive at last contact will be censored at their date of last contact. **Rate of acute (grade II-IV) graft-versus-host disease by 12 months post-transplant:** Proportion and associated CI of subjects who reached engraftment (sustained ANC>500 x 3 days by day 42) and developed acute GVHD grade II-IV (by modified Glucksberg criteria) (Appendix B) **Rate of chronic graft-versus host disease by 24 months post-transplant:** Proportion and associated CI of subjects who reached engraftment and developed moderate or severe GVHD by 2014 NIH Consensus Criteria (Appendix B) **Transplant-related complications at 12 and 24 months post-transplant:** Complications gathered via CIBMTR post-transplant form will be tabulated and described by treatment received **Exploratory endpoints:**

	Analyses of exploratory objectives (neurocognitive testing, pharmacogenomics, and immune reconstitution) will utilize descriptive statistics, calculations of predictive values, homogeneity testing, regression analyses and others.
Statistical Considerations:	This is a Phase I/II study feasibility study of a new HSCT conditioning regimen for pediatric high-risk non-hematologic malignancies. The study is designed so that the minimum informative number of subjects is enrolled at each dose level. See statistical section for details.
Estimated Enrollment Period:	3 years
Estimated Study Duration:	4 years

5. BACKGROUND

Hematopoietic Stem Cell Transplant for Pediatric Non-malignant Disorders

Hematopoietic stem cell transplant is the treatment of choice and the only curative therapy for a number of non-malignant disorders, including hemoglobinopathies, inherited bone marrow failure syndromes, primary immunodeficiency disorders and metabolic disorders. These patients present different challenges than patients with malignant disorders. They are typically younger and often with infections or organ failures related to the underlying disease (Slatter et al, 2015). Most children with non-malignant disorders have normocellular or even hypercellular bone marrow which poses a barrier to engraftment. As a result, these children may require more aggressive conditioning to establish sustained donor engraftment. Due to a variety of disorders and small number of patients in each disease category, disease-specific regimens are rare and designed for disorders such as Fanconi's anemia, or dyskeratosis congenita that require significant reduction in conditioning regimens due to known sensitivity to radiation/chemotherapy. Also, patients with severe combined immune deficiencies and those with severe aplastic anemia typically do not require myeloablative conditioning. Patients requiring specialized conditioning regimens or those for whom a disease-specific alternative protocol is available, are excluded from this study. This study will determine the dose of thiotepa (5 mg/kg/day or 10 mg/kg/day), added to the backbone of reduced dose of targeted intravenous busulfan, fludarabine and thymoglobulin, required to achieve sustained engraftment in children with non-malignant disorders.

Non-TBI Conditioning Regimens

Due to known age-related effects of total body radiation on neurocognitive functioning and growth (Willard et al, 2014; Kelly et al, 2017), children with non-malignant disorders have historically been conditioned with non-TBI regimens, originally designed for patients with myeloid malignancies.

The first well-described and widely used non-TBI regimen was oral busulfan and cyclophophamide (Santos et al, 1983; Tutschka et al, 1987). Veno-occlusive liver disease and mucositis were the major toxicities of busulfan/cyclophosphamide conditioning. This was ameliorated by introduction of intravenous busulfan which avoids hepatic first-pass effect and high busulfan concentrations in portal-hepatic venous system. Use of intravenous busulfan resulted in reduction in VOD (8% vs 33%) and 100-day mortality (Kashyap et al, 2002). As oral busulfan was changed to intravenous formulation, fludarabine was introduced as a replacement for cyclophosphamide in combination with busulfan.

In 1998 Slavin introduced the combination of fludarabine and low-dose busulfan (8mg/kg) as a non-myeloablative conditioning for malignant and non-malignant disorders (Slavin et al, 1998). Subsequently a number of studies tested IV busulfan and fludarabine combination mainly for myeloid malignancies, and confirmed that busulfan and fludarabine compare favorably to busulfan and cyclophosphamide (Bronhauser et al, 2003; Andersson et al, 2008). In 2002 Russell introduced single daily busulfan dosing of 3.2 mg/kg for 4 days infused over 3 hours on days -5 to -2, fludarabine, 50 mg/m^2 for 5 days on days -6 to -2, and rATG 4.5 mg/kg for 3 days prior to transplant (Russell et al, 2002, 2013). Daily busulfan and fludarabine has been previously studied in pediatric patients, including infants with nonmalignant disorders. In a study of 33 infants with primary immune deficiencies and metabolic disorders only 2 daily busulfan doses were given, targeting the 2 exposure levels (AUC of 4000 and 5000 μM/L*min/day), in combination with fludarabine 30 mg/sqm x 5 and rabbit ATG (2 mg/kg/day x 4). Primary or secondary graft failure occurred in 6 out of 12 (50%) of patients targeted at 4000 μM/min but only in 1/21 (5%) who had busulfan target increased to 5000 μM/min. However, 4 patients receiving umbilical cord blood in the higher busulfan AUC also received 5 mg/kg of Thiotepa a day prior to busulfan/fludarabine (Ward et al, 2015). Additional pediatric data on use of daily busulfan (targeted 5400 μM/L*min/day) fludarabine (160 mg/m^2) and serotherapy (10 mg/kg of ATG for unrelated and cord blood recipients) comes from Netherlands (Bartelink et al, 2014). They enrolled 64 pediatric patients with the median age of 4.8 years (range 0.16-18.6), 37 of whom had nonmalignant disorders (19 had primary immunodeficiencies and 16 metabolic disorders). Outcomes of these patients were compared with 50 patients receiving busulfan cyclophosphamide with or without melphalan, also receiving rATG at 10 mg/kg, if unrelated donors were used. A large number of patients received umbilical cord blood transplant in both groups (total of 75). Probability of engraftment was 98%. Busulfan/fludarabine/rATG had similar survival but improved toxicity profile when compared to busulfan cyclophosphamide ± melphalan group, in particular the incidence of lung injury, veno-occlusive liver disease, cGVHD, adenovirus infection and HHV6 reactivation was lower in busulfan/fludarabine group. Also, duration of neutropenia was reduced (11 vs 22 days) and fewer transfusions were required in busulfan/fludarabine group. The authors conclude that their excellent engraftment rate with busulfan/fludarabine was related to targeting higher AUC (~5400 μM/L*min/day) and use of ATG (Bartelink et al, 2014).

Two other pediatric studies using targeted busulfan/fludarabine and rATG or alemtuzumab, targeting lower AUC in pediatric non-malignant disorders (equivalent of 3500μM/L*min/day) encountered high rejection rates in (21% in rATG and 9% in alemtuzumab group). Patients with non-malignant disorders receiving unrelated donor transplant, in particular mismatched unrelated donor transplant were at a particularly high risk of rejection (Law et al, 2012; Horn et al, 2006). Similarly, Horowitz described 10 patients with myeloid malignancies who received daily busulfan and fludarabine without ATG, followed by double cord blood transplantation. Graft failure rate was 80% and study was discontinued prematurely (Horowitz et al, 2008).

A large study of pediatric reduced-toxicity regimens using lower doses of busulfan, fludarabine and rATG or alemtuzumab also confirmed high risk of graft failure in pediatric patients receiving unrelated cord blood transplants and patients with non-malignant disorders who did not receive chemotherapy previously (Satwani et al, 2013)

Role of Thiotepa in Conditioning for Pediatric Non-Malignant Disorders

Thiotepa has been introduced in allogeneic bone marrow transplant conditioning regimens in early 1990s (Aversa et al, 1991; Przepiorka et al, 1994), in combination with busulfan and cyclophosphamide, with a goal of enhancing engraftment. Animal studies suggested that, in addition to myeloablation, thiotepa has immune suppressive properties. Thiotepa is toxic to committed progenitor population, while primitive stem cells remained relatively resistant to this drug (Down et al, 1998). In previously described study of 33 infants with primary immune deficiencies and metabolic disorders that used only 2 daily busulfan doses, fludarabine and rabbit ATG; targeting of busulfan to the AUC of 5000 μM/L*min/day and addition of a single dose of Thiotepa for umbilical cord blood recipients (5mg/kg) reduced failure of engraftment from 50% to 5% (Ward et al, 2015).

Addition of two doses of Thiotepa (5 mg/kg/day x 2) to daily Busulfan (3.2 mg/kg/day x 3), fludarabine 150 mg/m^2 over 3 days and rATG 8 mg/kg, resulted in 94% cumulative engraftment rate in a 88 adult and pediatric patients with hematologic malignancies receiving single-umbilical cord blood transplantation from unrelated donors (Sanz J et al, 2012). The study concluded that the addition of thiotepa and ATG were crucial for the improvement of engraftment.

Treosulfan in Pediatric Conditioning Regimens

Since 2004, treosulfan has increasingly replaced busulfan in pediatric transplantation for malignant and non-malignant disorders in Europe, due to its good myeloablative and immunoablative properties and toxicity profile. Treosulfan is believed to have less CNS toxicities and hepatic toxicities than busulfan; however comparative study has not been done yet. Slatter described a large cohort of 316 pediatric patients with non-malignant disorders who recieved treosulfan as a part of conditioning regimen in Europe between 2005 and 2010 (Slatter et al, 2015). Thirty percent of patients received transplant from matched related donor and others from matched or mismatched unrelated donors. The median total dose of treosulfan was 42 g/m^2 divided in 3 doses. In addition to treosulfan, 106 patients in this study received fludarabine 150 mg/m^2, 98 received cyclophosphamide 200 mg/kg, and 104 received fludarabine and thiotepa (median dose 8mg/kg). The cohort receiving fludarabine and thiotepa in addition to treosulfan had the best 3-year overall survival (88%, SE 4%), followed by patients receiving fludarabine (85%, SE 4%), and those receiving cyclophosphamide/treosulfan combination (3-year OS 79%, SE 4%). Recent US experience using treosulfan in combination with fludarabine and rabbit anti-thymocyte globulin indicated excellent engraftment among 14 patients with bone marrow failure disorders who received bone marrow or peripheral blood stem cell transplants (Burroughs et al, 2017).

Rationale for selected conditioning regimen

Since treosulfan is not an FDA approved drug and is not available for studies in the US, medications with the best track record for pediatric non-malignant disorders include carefully targeted intravenous busulfan, fludarabine and rATG. This combination provides sufficient engraftment in children receiving fully matched related or unrelated donor transplants. However, in patients receiving mismatched unrelated, and in particular mismatched cord blood transplantation, additional immune and myeloablation is needed. As previously described, thiotepa in combination with reduced dose of busulfan and fludarabine, or treosulfan and fludarabine, has a good track record of facilitating engraftment in patients receiving cord blood transplant for malignant and non-malignant disorders (Sanz J, et al, 2012, Ward et al, 2015, Slatter et al, 2015).

Literature indicates importance of optimal exposure to conditioning agents such as busulfan and fludarabine, because exposure predicts survival and toxicity. Individualized model-based algorithms for busuflan and fludarabine clearance that incorporate body size, age and creatinine level (such as Insight Rx software), provide improved targeted therapy when compared to stratified weight or age-based regimens alone (Bartelink IH, et al, 2016, Ivaturi V, et al, 2017).This protocol will be offered to infants diagnosed with metabolic disorders during newborn screening for whom there are scarce data on standard dosing of chemotherapy agents. In similar situation (CSIDE multi-institutional study for infants undergoing transplant for severe combined immune deficiencies), the Pediatric Blood and Marrow Transplant Consortium has endorsed using Insight Rx platform which provides individualized dose for fludarabine, busulfan and rATG based on desired drug exposure (area under the curve).

This study adds thiotepa to busulfan, fludarabine and rabbit ATG in order to reduce the risk of graft failure while minimizing toxicities. In addition to determining the minimum effective dose of thiotepa in combination with reduced dose of busulfan and fludarabine for groups with low and high risk of graft failure, this study will provide additional safety and efficacy data, including study of effects on neurocognitive development and immune reconstitutions in infants and children with a variety of non-malignant disorders.

Pharmacogenomics

Inter-patient variation in the pharmacokinetics of drugs can result in unpredictable variability in occurrence of adverse events and toxicity as well as variation in therapeutic efficacy. Although a multitude of factors contribute to this observed variation, inherited variation in genes involved in drug metabolism and transport has been shown to play a significant role. Thiotepa undergoes metabolism by cytochrome P450s (CYP2B6, CYP3A) and glutathione S transferases (GSTA1, GST-P1). Busulfan, the second drug in this study, undergoes significant metabolism by GSTs. Fludarabine pathway genes include deoxycytidine kinase (DCK) and ribonucleotide reductase catalytic subunit M1/2 (RRM 1/2). Genetic polymorphisms can influence the gene expression and/or activity, thereby impacting drug pharmacokinetics and causing inter-individual variation in drug levels which can alter risk of toxicity or therapeutic efficacy. One of the exploratory goals of this study is to identify pharmacogenomic biomarkers predicting drug response and toxicity. Identification of such biomarkers, followed by further validation in a larger cohort will establish clinically relevant biomarkers allowing for personalizing therapy to achieve maximum benefit and minimal side-effects in future studies.

6. OBJECTIVES

6.1 Primary

- Determine the minimum effective dose (MED) of thiotepa in combination with reduced-dose busulfan, fludarabine and rATG required to achieve engraftment in >90% subjects undergoing hematopoietic stem cell transplantation for non-malignant disorders. The MED will be determined for subjects stratified in group A (lower risk of graft failure) and group B (high risk of graft failure). The two groups will be merged if group A requires escalation to the higher dose level.

6.2 Secondary

- Evaluate the risk of early (by day 42) and late (by day 365) graft failure/rejection.
- Evaluate 12 and 24 month disease free survival (DFS) and overall survival (OS) of children undergoing HSCT using busulfan/fludarabine/rATG/thiotepa conditioning regimen.
- Evaluate transplant-related mortality at 100 days and 12 months post-transplant.
- Evaluate rate of acute (Grade II-IV) graft-versus-host disease (GVHD) by 12 months post-transplant
- Evaluate rate of chronic GVHD by 24 months post-transplant
- Evaluate transplant-related complications at 12 and 24 months post-transplant

[Comments][Comments]

6.3 Exploratory

- Describe change in neurocognitive functioning compared to pre-transplant
- Describe association between pharmacogenomics of genes involved in metabolism of chemotherapy agents and toxicity
- Describe immune reconstitution following this conditioning regimen

[Subject]

7. STUDY DESIGN

7.1 Study Overview

This is a feasibility Phase I/II multi-institutional study of allogeneic stem cell transplant using a conditioning regimen which tests 2 different doses of thiotepa added to the backbone of reduced-dose busulfan, fludarabine and rATG for children with non-malignant disorders in order to achieve reliable engraftment. Following donor identification and standard of care pre-transplant evaluation, subjects will start conditioning regimen.

The study builds in rules for escalation of thiotepa dose based on graft failure by day 42. Patients are stratified in two groups A (lower risk of graft failure) and B (higher risk of graft failure). Patients undergoing 10/10 HLA matched bone marrow and peripheral blood transplants are assigned to group A, and patients receiving <10/10 matched bone marrow or peripheral blood, or receiving cord blood, even if fully matched, or T-cell depleted transplants, are assigned to group B.

If criteria for thiotepa dose escalation are met first in group A, which has a lower risk of graft failure, thiotepa dose will be escalated for all subjects (group A and B). If criteria for thiotepa dose escalation are met first in group B, dose will be escalated only in group B and group A will continue enrolling patients at a lower dose level.

Exploratory objectives include evaluating neurocognitive functioning following this conditioning regimen and assessment of immune reconstitution. We will also gather additional information onpharmacogenomics and possible associations between genes involved in metabolism of busulfan, fludarabine and thiotepa and toxicity and efficacy.

If failure of engraftment has not been observed by day 42, 5 additional subjects will be enrolled in the same dose level. If 0 or 1 failure of engraftment is observed among the first 10 subjects of each dose level, 5 additional subjects will be enrolled at the same dose level. If a failure of engraftment is observed among the first 5 subjects, the dose of thiotepa will be increased to twice a day and 10 subjects enrolled at that dose level. If there is 0 or 1 graft failure among the first 10 subjects, additional 5 subjects will be enrolled in twice a day thiotepa arm. If there are 2 or more graft failures among the 10 subjects at twice a day thiotepa dose level in group A, the entire study will be closed. If there are 2 or more graft failures in group B, only that group will be closed to further accrual.

With this design a minimum of 15 subjects will be enrolled at each effective dose level. The study may confirm a different MED of thiotepa for group A and group B.

7.2 Subject Selection

7.2.1 Number of Subjects

Up to 35 subjects will be enrolled in this study. The study aims to enroll a minimum of 15 patients at the dose of thiotepa which is determined to be effective. A minimum of 16 subjects and maximum of 35 subjects will be enrolled in the entire study. The minimum number will be enrolled if the first patient in the group with the low risk of graft failure sustains a graft failure, in which case the dose of thiotepa will be escalated in both group A and group B.

7.2.2 Inclusion Criteria

- Diagnoses:

Subjects with any non-malignant disorder known to benefit from allogeneic transplantation for cure or amelioration of symptoms of the underlying disease.The following groups of diagnoses are eligible:

- Hemoglobinopathies (e.g. thalassemia or sickle cell disease),
- Cytopenias (e.g.Diamond-Blackfan anemia, congenital or acquired neutropenia, congenital or acquired thrombocytopenia, congenital or acquired anemia, and others, regardless clonality),
- Hemophagocytic lymphohystiocytosis,
- Primary immunodeficiencies (e.g. Wiscott Aldrich Syndrome, chronic granulomatous disease, common variable immune deficiency, X-linked lymphoproliferative disease, NK+ severe combined immune deficiencies),
- Metabolic disorders (Hurler's syndrome, mannosidosis, adrenal leuko-dystrophy)
- Other non-malignant disorders for which there is published evidence that HSCT is a curative therapy.

- Donor Requirements
    - Related or unrelated donor who is suitable and willing to donate bone marrow or peripheral blood stem cells. HLA typing should be done by high-resolution typing at A, B, C, DrB1 and DQ loci and the donor should be at a minimum ≥8/10 match (with one antigen/allele mismatch allowed at A, B, or C-loci and other at DQ loci).
    - Cord blood units must be matched at a minimum of 6/8 antigens/alleles at A, B, C and DrB1 loci. High resolution typing at all loci is required. The minimum TNC dose pre-cryopreservation must be ≥3.7 x10^7/kg of recipient's weight, if a single cord blood unit is used, or at least 2x10^7/kg per unit, if two cord blood units are used. The mismatches cannot be at the same loci (e.g. double A mismatch).
- Haploidentical related stem cell donor who is suitable and willing to donate peripheral blood stem cells. T-cell depletion is required if haploidentical donors are used. Adequate organ function defined as:
    - Cardiac: ejection fraction ≥55% or shortening fraction ≥30%
    - creatinine clearance ≥70 ml/min/1.73m^2
    - Pulse oximetry >95% on room air or FEV1/DLCO >60%
    - LFTs < 3 x ULN, Total bilirubin <3 mg/dl (unless due to non-hepatic cause (e.g. Gilbert's syndrome or hemolysis)
    - Lansky/Karnofsky score ≥60%
    - Age: ≥6 months and ≤39 years of age at the time of signing informed consent
- Written informed consent obtained from the subject or parental/guardian permission ± child's assent per institutional guidelines

- Males with female partners of childbearing potential must agree to use physician-approved contraceptive methods (*e.g*., abstinence, condoms, or vasectomy) for at least one month after completion of conditioning.
- Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy for at least 1 month after completion of conditioning. WOCBP include any woman who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or who is not post-menopausal. Post-menopause is defined as:
    - Amenorrhea that has lasted for ≥ 12 consecutive months without another cause, or
    - For women with irregular menstrual periods who are taking hormone replacement therapy (HRT), a documented serum follicle-stimulating hormone (FSH) level of greater than 35 mIU/mL.

7.3 Exclusion Criteria

- Subjects with any of the following will not be eligible for study participation:
    - Diagnoses that do not require myeloablative transplant for cure (e.g. NK- SCID patients), unless they previously did not engraft with non-myeloablative or reduced intensity conditioning transplant.
    - Known or suspected sensitivity to chemotherapy or radiation (e.g Fanconi's anemia, Dyskeratosis congenita, Ligase IV deficiency, etc).
    - Subjects with fast-progressing neurodegenerative disorders (e.g. Krabbe disease or adrenal leukodystrophy with Loes score of ≥10)
    - Cytopenias with increased blasts (>5%)
    - Presence of anti-donor HLA antibodies (positive anti-donor HLA antibody is defined as a 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 with mean fluorescence intensity (MFI)>3000 by solid phase

- Prior allogeneic stem cell transplant, except for patients with immune deficiencies who underwent previous non-myeloablative or reduced intensity transplants.
- Haploidentical donor using in vivo T-cell depletion (e.g. postransplant cyclophosphamide).
- Uncontrolled bacterial, viral, or fungal infection at the time of enrollment. Uncontrolled is defined as currently taking medication and with progression or no clinical improvement on adequate medical treatment.
- Seropositive for HIV
- Active Hepatitis B or C determined by a detectable viral load of HBV or HCV by PCR
- Bridging fibrosis or liver cirrhosis
- Females or males of childbearing potential who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and for at least 1 months after the end of conditioning
- Females who are pregnant or breastfeeding
- History of any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of protocol therapy or that might affect the interpretation of the results of the study or that puts the subject at high risk for treatment complications, in the opinion of the treating physician.
- Subjects demonstrating an inability to understand the study and comply with the study and/or follow-up procedures.

7.4 Inclusion of women, children and minorities

Both men and women and members of all races and ethnic groups are eligible for this trial as long as they meet inclusion criteria.

8.0 STUDY PROCEDURES

8.1 Registration

All subjects must be registered with the UF Health system ion into the trial. The participating site must fax or email the completed study specific eligibility checklist and registration forms, supporting documents and signed informed consent to the Coordinating Center to be entered by a delegated UF Health stem cell transplant study team member. Clarification will be requested for unsigned or incomplete forms. Once documents are received, the designated Research Coordinator will review the documents to confirm eligibility and to complete the registration process. If eligibility cannot be confirmed, the research coordinator will query the site for clarification or additional documents as needed. Subjects failing to meet all study eligibility requirements will not be registered and will be unable to participate in the trial.

8.2. Screening Procedures

Written informed consent must be obtained at least 24 hours prior to starting conditioning regimen.

All pre-transplant evaluations performed as standard of care within 30 days of initiation of conditioning regimen but prior to informed consent, will be accepted for this study and need not be repeated. Neurocognitive testing done up to 90 days prior to transplant will be acceptable as baseline.

Pre-transplant donor and recipient evaluation will follow established standard-of care, FACT recommended guidelines. At a minimum pre-transplant evaluation will include:

- Demographic data, including date of birth, gender, and race
- Vital signs including height, weight, and blood pressure
- Medical history, including major diseases and/or surgeries;
- List of active problems
- Lansky/Karnofsky score
- Pre-transplant disease evaluation will be done per SOC and will be specific for the underlying disease (e.g. liver MRI with quantification of iron for subjects who received > 20 PRBC transfusions previously, brain MRI for subjects with adrenal leukodystrophy, etc.)
- Serum pregnancy test within 7 days of conditioning start for females of child bearing potential
- Echocardiogram
- Pulse oximetry or pulmonary function tests for patients able to cooperate with the test
- Calculated creatinine clearance
- Liver function tests
- Infectious disease testing as per institutional guidelines
- Neurocognitive testing: may be done between 3 months prior to transplant and day -5 – see neurocognitive testing details (section 6.3).
- 3mL of peripheral blood collected in EDTA tubes for pharmacogenomics testing should be obtained prior to initiation of conditioning regimen

8.3 Study Procedures **(**See Appendix A, schedule of assessments for additional details.)

Weekly until day 60, then with every clinic visit until day 100:

- Assessment for AE and SAEs
- Assessment for aGVHD (grade II-IV acute), and moderate and severe cGVHD once patient engrafted
- Assessment for graft failure (blood counts, or as clinically indicated)

Immune Reconstitution Tests: At 100 days and every 3 months subsequently until normal

- Immunoglobulin levels (IgG, IgM, IgA)

- Enumeration of lymphocytes (CD4, CD8, CD19 at a minimum)
- Lymphocyte proliferation to mitogens (start once CD4 > 200/μL)
- Post-immunization titers (obtain at the end of study, if immunization started)

Donor chimerism:

- Obtain at day +30 if ANC has recovered
- Obtain at day +42 or any time that graft failure is suspected
- Obtain at 12 months
- Obtain at 24 months or at the end of study

Neurocognitive testing (Appendix 4)

- Prior to transplant (day -90 to -5)
- At 12 months post-transplant
- At the end of study (if at least 12 months have elapsed since the previous testing)

Any time during follow up:

- Assess for graft failure (blood counts, chimerism if needed)
- Assess for toxicities (AE and SAE) felt to be related to conditioning regimen
- Assess for GVHD (grade II-IV acute and moderate and severe cGVHD)

8.4. Pharmacogenomic Testing

Genomic DNA will be isolated from a blood sample obtained pre-transplant. 15 SNPs listed in Table 8.1 will be genotyped using Quant studio in patients enrolled in the study. Additionally, GSTM1 gene deletion will be tested in these patients. These SNPs have been selected based on the evidence in literature for any functional impact and if they occur with the minimum allele frequency of greater than 5% in a population. SNPs or GSTM1 deletion will be evaluated for association with efficacy and observed toxicities in transplant patients receiving drugs of interest.

Table 8.1 Key candidate genes and SNPs of significance in pharmacokinetics of the drugs used for conditioning

PK genes	Genetic polymorphisms	Drugs impacted
CYP2B6	rs2279343, rs3211371,rs3745274	Thipotepa
CYP3A4	rs35599367	Thipotepa
CYP3A5	rs776746	Thipotepa
GSTA1	rs3957357, rs3957356	Thipotepa, Busulfan
GSTP1	rs1695 and rs1138272	Thipotepa, Busulfan
GSTM1	Deletion	Thipotepa, Busulfan
CYP39A1	rs2277119	Busulfan
DCK	rs2306744, rs11544786, rs9997790	Fludarabine
RRM1/2	rs1042919, rs1561876	Fludarabine

8.5 Data Collection

Other than AEs and SAEs up to day 100 reporting and eligibility documentation, this study will use the following CRFs for data collection.

Table 8.2 – Forms for Data Collection

Time of assessment
Enrollment form
Pre-transplant Evaluation
Post-Transplant Evaluation
Day 100, 180, 1 and 2 years post-transplant

9.0 STUDY TREATMENT

All subjects entering the screening phase will receive a unique subject number. This number will be used to identify the subject throughout the study. Subjects withdrawn from the study will retain their subject number.

9.1 Conditioning Regimen and GVHD Prophylaxis

Dose adjustments of medications used for conditioning will be made for obesity only in children > 2 years of age. Obesity isdefined as BMI ≥95% based on CDC BMI caluculator (see Appendix F for details) This includes thiotepa fludarabine, and the first dose of busulfan. For obese subjects, the first busulfan dose will be given based on adjusted weight until pharmacokinetic (PK) results are available. Once PK values are available, busulfan dose will be modified to target AUC of 20mgxh/L per day for the total AUC of 60 mgxh/L over 3 days. Fludarabin will be targeted to 4.5 mgxh/L/day or 18 mgxh/L over 4 days. Thymoglobulin will not be adjusted for weight; however, capping of thymoglobulin dose in severely obese patients is acceptable at a dose of 150 mg/day.

Starting dose for busulfan and fludarabine will be based on InsightRx platform, whenever feasible, given paucity of dosing for young infants.

Tacrolimus should be adjusted based on levels. Post-transplant Methotrexate and MMF may be adjusted in obese subjects per institutional guidelines.

In children < 12 kg, all drug doses will be calculated per kg rather than per sqm.

Table 9.1 Conditioning Schedule

Day -8	Admit, start hydration at least 12 hours prior to Thiotepa, Start anti-seizure prophylaxis (Levetiracetam or Ativan)
Day -7	Thiotepa 5 mg/kg x1 (first dose level) Thiotepa 5 mg/kg q 12 hours x 2 (2^{nd} dose level) Skin care per institutional guidelines
Day -6 to -3	Fludarabine 40 mg/m^2/dose daily iv over 30 min or 1.3 mg/kg/dose IV if <12 kg or InsightRx dose targeting 4.5 mgxh/L/day 18 mgxh/L total dose
Day -6 Day -4, -3	Busulfan iv over 3 hours, given following Fludarabine. Use weight-based dosing or InsightRx platform targeting 20 mgxh/L/day 60 mgxh/L total dose **PK on day -6 after the first dose**. NOTE **Busulfan is skipped on day -5**
Day -3 to -1	rATG (Thymoglobulin®) 2mg/kg x 3 for subjects receiving bone marrow, or **peripheral blood** stem cells as stem cell source (total 6 mg/kg). **Subjects receiving cord blood or T-cell depleted product will receive 2 mg/kg x 2 on days -2 and -1 only (total 4 mg/kg)** **May cap daily dose to 150 mg for severely obese patients**
Day -2	Begin Tacrolimus (0.03 mg/kg/day, initial maximum dose 1 mg/day) IV continuous infusion, adjust based on levels
Day 0	Stem Cell Infusion
Day +1	Methotrexate 5 mg/m^2/dose IV (0.15 mg/kg)
Day +3	Methotrexate 5 mg/m^2/dose IV
Day +6	Methotrexate 5 mg/m^2/dose IV
Day +11	Methotrexate 5 mg/m^2/dose IV

Cord blood transplant recipients will not receive post-transplant Methotrexate; their GVHD prophylaxis will consist of Tacrolimus as described in the table and Mycophenolate Mofetil (MMF) 15 mg/kg PO TID up to 1 gm TID (or iv equivalent), starting on day -2 and continuing until day +35.

Subjects receiving T-cell depleted transplant (using CliniMACS® or similar T-cell depletion) will not receive any pharmacologic GVHD prophylaxis. The infused T-cell dose (CD3+) in these

transplants should not exceed $3x10^4$ CD3+ cells/kg of recipient's weight if CD34+ selection is done Patients receiving αβ T-cell depletion will receive CD3+ and CD19+ doses and/or Rituxan as prescribed in the αβ T-cell depletion protocol.

9.2 Treatment Schedule/Administration

9.2.1 Thiotepa

The initial Thiotepa dose is 5 mg/kg. It will be administered IV over 2 hours on Day -7. In case of dose escalation, Thiotepa will be administered IV twice 12 hours apart on Day -7. Hydration and skin care prior to and during Thiotepa will follow institutional guidelines.

9.2.2 Fludarabine

Fludarabine dose 40 mg/m^2, or 1.3 mg/kg in children <12 kg, or dose targeting the AUC of 4.5 mgxh/L/day for the total AUC of 18 mgxh/L over 4 days. InsightRx platform will be a preferred way of determining the dose for infants. AFludarabine will be administered over 60 minutes intravenously on days -6 to -3 immediately prior to busulfan. Potential study participants who have an estimated or measured creatinine clearance <70 mL/min/1.73 m^2 are excluded from enrollment, thus there will be no adjustment in the fludarabine dose for renal insufficiency; however InsightRx platform will adjust the dose based on patient's creatinine level.

Pentamidine should be avoided with fludarabine.

9.2.3 Busulfan (including PK)

First dose will be based on weight following European Medicines Agency IV busulfan dose guidelines (Palmer et al, 2016), or based on InsightRx platform individualized recommendation. Once PK results are available dose will be based on PK results to target AUC of 20 mgxh/L±10% / day for the total AUC of 60 mgxh/L over 3 doses.). **Note that there are only 3 doses of busulfan in this study.**.

Table 9.2 – European Medicines Agency IV Busulfan Dose Guidelines

Weight:	Dose:
<9kg	4 mg/kg/dose
≥9 to <16 kg	4.8 mg/kg/dose
≥16 to<23 kg	4.4 mg/kg/dose
≥23 to <34 kg	3.8 mg/kg/dose
≥34 kg	3.2 mg/kg dose

Administration: Busulfan is to be administered intravenously via a central venous catheter as a 3-hour infusion once daily on days -6, -4 and -3 for a total of 3 doses. Use of alternative (ie, divided) dosing schedules is NOT permitted on this study. Please note: the timing of busulfan PK sample collection is based upon this 3-hour infusion. Busulfan is administered at a final concentration of approximately 0.5 mg/mL in D5W or 0.9% NaCl over 3 hours via central line. Flush line with D5W or 0.9% NaCl before and after busulfan administration. Do not use polycarbonate syringes or filters.

Busulfan PK studies are required, with first dose pharmacokinetics performed to achieve an average daily area under the curve (AUC) for busulfan of 20 mgxh/L/day for the total AUC of 60 mgxh/L over 3 doses.

For the first dose, busulfan should be started early enough to allow that pharmacokinetic studies can be sent out the same day so that the doses on days -4 and -3 may be adjusted. Busulfan should be adjusted if first dose PK results indicate that the level is >10% outside of targeted AUC.. Decision about adjustment will be made in discussion with the pharmacologist and institutional PI. In general, the busulfan dose should not be increased by more than 50% of the starting dose. When adjusting busulfan, the goal is to achieve the outlined AUC for future doses (e.g. do not try to make up for already given busulfan dose).

Anti-seizure prophylaxis should start on admission and continue for a minimum of 48 hours after completion of Busulfan. Subjects at a high risk for seizures may continue with anti-seizure prophylaxis during therapy with calcineurin inhibitors. Institutions may use lorazepam or levetiracetam for seizure prophylaxis, phenytoin is not allowed. For subjects who are already on anti-seizure medications, consult neurologist and pharmacologist regarding continuation of patient's anti-seizure medications. This is allowed, assuming there are no interactions with the conditioning regimen medications.

The following medications should be avoided with busulfan:

- phenytoin or fosphenytoin (increased clearance of busulfan)
- acetaminophen (decreased clearance of busulfan)
- itraconazole – reduced clearance of busulfan (fluconazole and voriconazole had no effect on the clearance of busulfan)
- metronidazole – reduced clearance of busulfan.

Busulfan PK Samples

Samples should not be drawn from the lumen used to infuse busulfan. In case infusion runs more or less than 3 hours, draw one sample immediately when infusion ends. Then draw the next sample 15 minutes later and continue to draw 4, 5, 6, and 8 hours samples by counting from beginning of the infusion. Busulfan PKs is sent to central laboratory as standard of care test (Philadelphia or Seattle). It can also be done at any other laboratory that performs busulfan PK, assuming the above guidelines are followed.

Table 9.3 – Busulfan PK Sample Timing

PK Sample timing from START of 1st Busulfan dose
3 hours (end of infusion)
3 hours 15 minutes
4 hours
5 hours
6 hours
8 hours

9.2.4 Thymoglobulin®

Thymoglobulin® (rATG) will be given in this study at 2 different dose levels (4 mg/kg and 6 mg/kg, see conditioning regimen). Thymoglobulin will be infused through a 0.22-micron filter over 4-6 hours with pre-medications to include: steroids, oral or intravenous (IV) acetaminophen and diphenhydramine. It is recommended that the first day infusion be 6 hours. An anaphylaxis kit will be kept at bedside during Thymoglobulin administration. Steroids should be administered per institutional standard to prevent infusion toxicity (suggested steroid dose is 1 mg/kg of Solumedrol prior to infusion with repeating the dose of 1 mg/kg 4-6 hours later only if required by patient's symptoms).

9.2.5 GVHD Prophylaxis

1. Mycophenolate Moefetil for CORD BLOOD recipients
   MMF will be given at a dose of 15 mg/kg PO TID with the maximum daily dose not to exceed 3 grams (1 gram PO TID). MMF prophylaxis will be used only for CORD BLOOD recipients. It will begin on day -2 and continue until day +35, unless active GVHD is present. At day 35 MMF can be discontinued a once.

2. Tacrolimus
   Tacrolimus IV or PO should be started on Day -2 and administered per institutional standards. The recommended IV starting dose is 0.03 mg/kg/day with PO equivalent acceptable. May change to oral dosing once therapeutic levels are achieved or per institutional standards. Young children may require more frequent (e.g. three times a day) oral dosing to maintain appropriate levels. If Tacrolimus is not tolerated,

cyclosporine may be substituted IV or PO. Immunosuppression will be withdrawn as per institutional guidelines.

3. Methotrexate
   Methotrexate IV push at 5 mg/m^2 (0.15 mg/kg for children < 10 kg) will be given on Day 1 (a minimum of 24 hours after stem cell infusion) and repeated on Day +3, +6 and +11. Institutional guidelines will be used for adjustment of methotrexate for toxicities.

9.3 Stem Cell Infusion

Stem cell dose and infusion will follow institutional guidelines. The following are general guidelines for stem cell doses. If cord blood is used, a minimum of $3.7x10^7$/kg of cryopreserved nucleated cells should be available for transplant or at least 2x10^7/kg per unit, if two cord blood units are used. The mismatches cannot be at the same loci (e.g. double A mismatch). Use cord blood bank or institutional guidelines for instructions about the washing, dilution and infusion of cord blood products. There are no limits to cord blood dose. If in vitro T-cell depletion is used, infuse >5 and <$20x10^6$/kg of CD34+ cells and a fixed dose of $3x10^4$/kg of CD3+ cells. For peripheral blood stem cells, infuse a minimum of 2 and the maximum of $8x10^6$/kg of CD34+ cells. For bone marrow infusion, the target is $5x10^8$ /kg, (acceptable range 2-8) of total viable nucleated cells (TVNC). Follow institutional guidelines for ABO incompatible stem cell products.

9.4 G-CSF

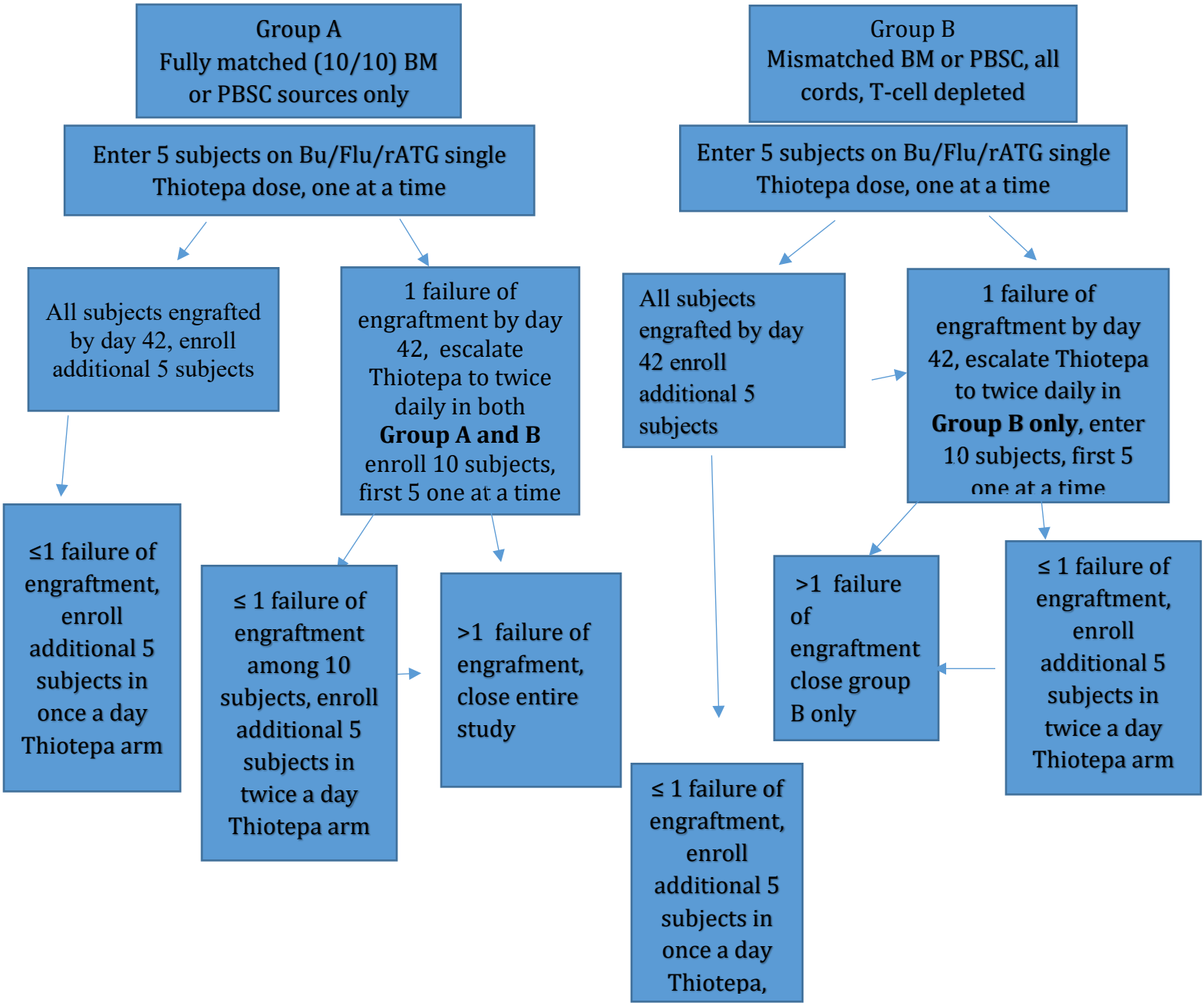
G-CSF may be started at day +15 if there is no evidence of ANC recovery, or it can be used per institutional guidelines.

9.5 Allowed Concomitant Therapy

All standard of care supportive care treatments (e.g. anti-emetics, seizure prophylaxis, pain medications, antimicrobials and transfusions) are allowed. All standard of care supportive care treatments (e.g. anti-emetics, seizure prophylaxis, pain medications, antimicrobials and transfusions) are allowed.
Patients on this study are allowed to be on concomitant trials for GVHD therapy, donor source selection, T-cell depletion, and others, assuming that the outlined conditioning regimen is permitted on those trials.

9.6 Thiotepa Escalation based on graft failure

STUDY SCHEMA I: PHASE I/II THIOTEPA DOSE ESCALATION



The above study schema will be used for thiotepa dose escalation based on failure of engraftment by day 42.

Definition of engraftment failure used for dose escalation in this study includes:

- Failure to achieve sustained ANC recovery of >0.5x10^9/L with evidence of donor chimerism by day 42, in light of absence of myelosupressive medications (e.g. ganciclovir), or
- Engraftment with subsequent loss of donor chimerism to <5% by day 42, or
- Need for the 2^{n} conditioned transplant by day 42 to correct manifestations of disease or cytopenia.

9.7 Toxicity based stopping rules

Table 9.1 – Toxicity based stopping rules

N of enrolled Subjects	≤10	11-13	14-16	17-19	20
N of subjects with Grade 4 or 5 toxicities that will trigger stopping rules	≥4	≥5	≥6	≥7	≥8
N of subjects with grade 5 toxicities that will trigger stopping rules	≥2	≥3	≥3	≥4	≥4

T**oxicities up to 100 days post-transplant** will be used as triggers for stopping the study for excessive toxicities. If toxicity stopping rules have been met, the DSMB will review data and make recommendations regarding study modifications and conditions for study continuation.

Graft failure is not counted in these grade 4 and 5 toxicities as study modifications for graft failure already exist.

The rate of acceptable combined Grade 4 and 5 toxicities is ≤40%, or ≤20% Grade 5 toxicities.

DLTs will be followed **cumulatively for each dose level** (5 mg/kg of thiotepa or 10 mg/kg of thiotepa).

DLTs (CTCAE version 5.0 criteria) are defined as:

Grade 5 toxicities: any death that is in the opinion of study investigator possibly, probably or definitely related to toxicities of the conditioning regimen.

Grade 4 toxicities: any grade 4 neurologic, cardiac, GI, lung, infections or any other system toxicity, that is in the **opinion of investigator possibly, probably or definitely related to conditioning regimen.**

Severe VOD will also be considered Grade 4 toxicity (defined as VOD with renal or pulmonary dysfunction).

Acute GVHD of grade IV will also be considered as grade 4 toxicity.

Post-transplant expected hematopoietic and immunologic toxicities will not be considered DLTs and do not require reporting.

If the enrollment is brisk following the first 5 subjects enrollment in each dose level and each group the enrollment will be held so that at any given time 5 or fewer subjects are <100 days post-transplant

9.7 Supportive Care Guidelines

Subjects should receive full supportive care, including transfusions of blood products, antibiotics, anti-emetics, antidiarrheals, analgesics, etc., when appropriate, according to institutional standard of care guidelines. Available disease-specific supportive care guidelines should be followed (e.g. for sickle cell disease including reduction of Hemoglobin S to <35% prior to start of conditioning, strict control of blood pressure, continuation of anti-seizure prophylaxis during calcineurin inhibitor use and maintenance of normal magnesium levels throughout transplant).

9.8 Infectious Disease Monitoring

Infectious disease monitoring should follow institutional guidelines. In patients receiving mismatched transplants, follow-up for reactivation of viruses (CMV, EBV, HHV6, adenovirus, etc.) should continue at a minimum until the absolute CD4 lymphocyte count reaches 200/microL.

10. TREATMENT DISCONTINUATION

10.1 Removal of Subjects From Study

Any patients who initiate conditioning regimen will be considered a study subject.

Subjects who are not initiated on study drug, but have signed an informed consent will be considered screening failures. A record of these subjects will be maintained by the study site.

10.2 Criteria For Study Treatment Discontinuation

The Investigator will make every reasonable effort to keep each subject in the study unless it is in the subject's best interests to discontinue participation. If a subject is removed from the study or declines further participation, a description of the reason(s) for withdrawal from the study must be recorded on the case report form (CRF). The Investigator should also ensure that all subjects are followed up for survival status until 24 months post-transplant.

10.3 Replacement of Subjects

There will be no replacement of subjects on this study.

11. BIOLOGICAL SPECIMENS AND CORRELATIVES

11.1 Source of Specimens

Peripheral blood specimens for pharmacogenomics will be obtained for the purposes of this study and sent to Dr. Jatinder's laboratory. All other blood tests will be done as SOC.

11.2 Preparation, Shipment and Storage of Specimens

See Appendix C for collection, processing and shipping instructions for all tissue and blood specimens.

12. STUDY DRUG INFORMATION

This study uses commercial agents. The current known toxicities for each commercial agent is provided in the drug package insert.

See Appendix D for current study drug information. Always refer to the drug insert for the most up to date information.

13. ADVERSE EVENT REPORTING REQUIREMENTS

13.1 Purpose

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents.

This study uses commercial agents and AE reporting will follow Cancer Therapy Evaluation Program (CTEP) guidelines for reporting of AE that occur during therapy with commercial agents.

13.2 Determination of Reporting Requirements

Reporting requirements should include the following considerations: 1) the characteristics of the adverse event including the *grade* (severity); 2) the *relationship to the study therapy* (attribution); and 3) the *prior experience* (expectedness) of the adverse event.

Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. In some cases, an agent obtained commercially may be used for indications not included in the package label. In addition, NCI may on some occasions distribute commercial supplies for a trial. Even in these cases, the agent is still considered to be a commercial agent and the procedures described below should be followed.

Determine the prior experience: Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered **unexpected,** for reporting purposes only, when either the type of event or the severity of the event is not listed in:

- the current known toxicities for each commercial agent as provided described in Appendix D, or
- the drug package inserts.

The following AE will be reported within 5 calendar days of learning about them for participants in the interventional trial:

- Grade **3 and 4 toxicities that are unexpected and related** (possibly, probably or definitely related) to study treatment and occur within 100 days of transplant, will be reported.
- Use the assigned protocol number and the protocol-specific subject ID provided during trial registration on all reports.

CTCAE term (AE description) and grade: The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting and are located on the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

All serious adverse events (SAE) that occur within 100 days of transplant will be reported within 5 days of learning about them. A serious adverse events is one that:

- Results in death
- Is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below for exceptions)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event, defined as a medical event that may not be immediately life-threatening or result in death or hospitalization but, based on appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed above. Examples of such events include but are not limited to intensive treatment in an emergency department or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization. “Medically important” should be marked only if no other serious criteria are met.

In addition to the above, the study also requires reporting of following Adverse Events of Special Interest within 5 days of learning of the event throughout study participation:

- Graft failure
- New malignancy that occurs any time following treatment
- Grade 4 toxicity of any organ that occurs at any time during study follow-up and is felt to be related to conditioning regimen (e.g. late pulmonary toxicity)
- GVHD (acute grade 2-4) and cGVHD of moderate and severe grade should be reported throughout participation in this study

- Grade 5 toxicity (death) should be reported throughout participation in the study
- Severe VOD with multiorgan involvement

Secondary Malignancy

A **secondary malignancy** is a cancer caused by treatment for a previous malignancy (eg, treatment with investigational agent/intervention, radiation or chemotherapy). A metastasis of the initial neoplasm is not considered a secondary malignancy.

All secondary malignancies that occur following treatment need to be reported to this study.

Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy
- Myelodysplastic syndrome
- Treatment related secondary malignancy

14. STATISTICAL METHODS

14.1 Sample Size Determination

This is a feasibility Phase I/II study of a new HSCT conditioning regimen for pediatric non-malignant disorders. Engraftment is the primary endpoint. The study is designed to examine two dose levels of thiotepa and determine the minimum effective dose in two groups: (A) low risk of graft failure group, and (B) high risk of graft failure group. Initially, 5 subjects will be enrolled in each group and receive once daily thiotepa. If all 5 engraft by day 42, 5 more subjects will be enrolled at this dose. Then, if there are 0 or 1 failures out of 10, 5 more subjects will be enrolled at this dose in each cohort. For 1 or more failures among the first 5 subjects in a cohort, the dose will be increased to twice daily. If the graft failure is encountered among the first 5 subjects from group A, the dose will be increased to twice daily for both groups. If graft failure is encountered among the first 5 subjects in group B, the dose of thiotepa will be increased for this group only. Ten additional subjects will be enrolled in twice a day thiotepa dose level. If there are 0 or 1 graft failure among 10 subjects, additional 5 subjects will be enrolled in twice a day thiotepa dose level. For 2 or more failures among the first 10 subjects at twice a day dose level, the group will be closed if this occurs in group B (high risk of graft failure), or the entire study will be closed if this occurs in group A. This scheme results in a minimum of 15 subjects at each MED. We would like to determine the dose such that engraftment is ≥90%. With 5 subjects enrolled, if the true engraftment rate is 90%, there is a 59% chance all will engraft and the dose will not be increased. If the true engraftment rate is less, 80% (or 70%), there is only a 33% (or 17%) chance that the dose will not be increased. Similarly, after 10 subjects are enrolled, if the true engraftment rate is 90%, there is a 73% chance that the dose will not be increased. And, if the true engraftment rate is less, 80% (or 70%), there is only a 37% (or 15%) chance that the dose will not be increased.

This feasibility study is primarily descriptive and the width of a 95% confidence interval can be used to determine how precisely the parameters can be estimated for a specified outcome and a given sample size. By group, the sample size will be 15 or 10. The table below illustrates the precision for estimating engraftment rate by day 42 for the combined sample and by group assuming the engraftment rate is approximately 90%.

	N	# with engraftment	% with engraftment	95% confidence interval	width of confidence interval
combined	30	27	90%	(0.73-0.98)	0.25
	25	21	84%	(0.64-0.95)	0.31
	20	18	90%	(0.68-0.99)	0.31
by group	15	13	87%	(0.60-0.98)	0.38
	10	9	90%	(0.55-01.00)	0.45

14.2 Analysis of Primary Endpoint

Determine the minimum effective dose (MED) of thiotepa in combination with reduced-dose busulfan, fludarabine and rATG required to achieve engraftment in >90% subjects undergoing hematopoietic stem cell transplantation for non-malignant disorders. The MED will be determined for subjects stratified in group A (lower risk of graft failure) and group B (high risk of graft failure). The two groups will be merged if the group A requires escalation to the higher dose.

Effective dose of Thiotepa in combination with busulfan/fludarabine and rATG is defined as a dose that results in engraftment of >90% study subjects by day 42 with acceptable toxicity, defined as ≤10% grade 5 toxicities and ≤40% combined grade 4 and 5 toxicities across all subjects treated with the same dose level.

Definition of engraftment failure used for dose escalation in this study includes:

- Failure to achieve sustained ANC recovery of >0.5x10^9/L with evidence of donor chimerism by day 42, in light of absence of myelosupressive medications (e.g. ganciclovir), or
- Engraftment with subsequent loss of donor chimerism to <5% by day 42, or
- Need for the 2nd conditioned transplant by day 42 to correct manifestations of disease or cytopenia.

14.3 Analysis of Secondary Endpoints

- **Evaluate the risk of early (by day 42) and late (by day 365) graft rejection/failure**

  Proportion and associated confidence interval (CI) of all subjects who initiated conditioning regimen and have sustained engraftment failure (as outlined above) at 42 days and at 1 year post transplant.

- **Evaluate 12 and 24 month disease free survival (DFS)/event-free-survival and overall survival (OS) of children undergoing HSCT using busulfan/fludarabine/rATG/thiotepa conditioning regimen**

  Proportion and associated confidence interval (CI) of all subjects who initiated conditioning regimen and are alive (OS) and without evidence of underlying disease (DFS) at 12 months post-transplant will be calculated. KM estimates of OS and DFS will also be used. OS is defined as time from transplant (day 0) until date of death or date of last contact for those who have not died. DFS/event-free survival is defined as time from transplant (day 0) until disease recurrence/progression, or date of death from any cause, or date of last contact for those who are disease-free. Disease progression will follow-standard of care definitions for each disorder. In general, disease-free-survival implies improvement of reversible manifestations of a disorder and arrest in further progression of symptoms in light of presence of donor engraftment and immune reconstitution.

- **Evaluate transplant-related mortality at 100 days and 12 months post-transplant**

  Proportion and associated CI of subjects who initiated conditioning regimen and who die due to a cause unrelated to the underlying disease. Time to transplant-related mortality is defined as time from transplant (day 0) until date of transplant related mortality. Those who die from other causes will be censored at date of death; those who were alive at last contact will be censored at their date of last contact.

- **Rate of acute (grade II-IV) graft-versus-host disease by 12 months post-transplant**

  Proportion and associated CI of subjects who reached engraftment (sustained ANC>500 x 3 days by day 42) and developed acute GVHD grade II-IV (by modified Glucksberg criteria) (Appendix B).

- **Rate of chronic graft-versus host disease by 24 months post-transplant**

  Proportion and associated CI of subjects who reached engraftment and developed moderate or severe GVHD by 2014 NIH Consensus Criteria (Appendix B)

- **Transplant-related complications at 12 and 24 months post-transplant**

  Complications gathered via CIBMTR post-transplant form will be tabulated and described by treatment received.

14.4 Analysis of Exploratory Endpoints

Statistical analysis of pharmacogenomics, immune reconstitution, and neurocognitive exploratory endpoints may include descriptive statistics, analyses of variance, regression analyses and other appropriate statistical tests.

14.5 Analysis of Safety Data

All reported AEs and SAEs will be tabulated and described.

14.6 Interim Analysis

No formal interim analysis is planned. Refer to toxicity based stopping rules section (9.7) for description of ongoing analyses for toxicity and graft failure and plan for temporary suspension of enrollment while assessing for toxicities.

15. DATA AND SAFETY MONITORING

15.1 Data Safety Monitoring Board (DSMB)

This protocol will be reviewed and monitored by an independent DSMB chartered for this study in accordance with FDA guidelines, policies, and procedures. The committee will review and monitor study progress, toxicity, safety and other data from this trial. The review will be done at a minimum twice a year, and more frequently if requested by investigators or DSMB members. Questions about subject safety or protocol performance will be addressed with the investigator, statistician and study team members. Should any major concerns arise; the DSMB will offer recommendations regarding whether or not to suspend the trial. DSMB Charter contains full description of DSMB and sponsor's responsibilities related to safety.

Briefly, the independent chartered DSMB activities will include:

- Review of clinical trial conducted for progress and safety
- Review of all adverse events as defined in the protocol Notification of the investigators of recommended action

15.2 Principal Investigator Responsibilities

As part of the responsibilities assumed by conducting this study, the Principal Investigator (PI) agrees to maintain and have available for monitoring adequate case records (accurate source documents and CRFs) for the subjects treated under this protocol.

The PI will be primarily responsible for monitoring of adverse events, protocol violations, and other immediate protocol issues. The study coordinator will collect information on subjects enrolled through the use of electronic or paper adverse event (AE) forms, CRFs, and Informed Consent forms.

16. EMERGENCY PROCEDURES

16.1 Emergency Contact

In emergency situations, the treating physician should contact the Principal Investigator by telephone at the number listed on the title page of the protocol.

16.2 Emergency Treatment

During and following a subject's participation in the study, the treating physician and/or institution should ensure that adequate medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the study.

17. ADMINISTRATIVE, ETHICAL, AND REGULATORY CONSIDERATIONS

17.1 Good Clinical Practice

The procedures set out in this study protocol pertaining to the conduct, evaluation, and documentation of this study are designed to ensure that the Principal Investigator and Co-Investigators abide by Good Clinical Practice (GCP), as described in International Conference on Harmonization (ICH) Guideline E6 and in accordance with the general ethical principles outlined in the Declaration of Helsinki.

The study will be conducted in compliance with the protocol. The protocol, any amendments, and the subject informed consent will receive Institutional Review Board (IRB) approval before initiation of the study.

The Principal Investigator will conduct all aspects of this study in accordance with applicable national, state, and local laws of the pertinent regulatory authorities.

All potential serious breaches must be reported immediately to the independent DSMB and IRB of record, if applicable. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.

17.2 Institutional Review Board

Before study initiation, the investigator must have written and dated approval from the IRB for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects. The investigator should also provide the IRB with a copy of the Investigator Brochure or product labeling, information to be provided to subjects, and any updates. The investigator should provide the IRB with reports, updates, and other information (e.g., amendments, and administrative letters) according to regulatory requirements or institution procedures.

17.3 Compliance with Laws and Regulations

It is intended that the proposed study be conducted according to the International Conference on Harmonization E6 Guideline for Good Clinical Practice (GCP) and the Declaration of Helsinki. Please refer to the International Conference on Harmonization and GCP:

> http://www.fda.gov/oc/gcp/guidance.html; Declaration of Helsinki: http://www.fda.gov/oc/health/helsinki89.html; Code of Federal Regulations, Title 21: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch. cfm]

The study will be registered with ClinicalTrials.gov by the Protocol Development Officer or assigned designee. All studies must be registered no later than 21 days after enrollment of the first participant. The Protocol Development Officer will maintain the responsibility of updating trials registered with ClinicalTrials.gov; per the FDA's updating requirements, information must be updated at least every twelve months and the registry must be updated within thirty days of any changes in recruitment status or completion of the study.

17.4 Delegation of Investigator Responsibilities

The Principal Investigator will ensure that all persons assisting with the study are adequately informed about the protocol, any amendments to the protocol, the study treatments, and their study-related duties and functions. The Principal Investigator will maintain a list of Co-Investigators and other appropriately qualified persons to whom he/she has delegated significant study-related duties.

Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks. This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure; debarment).

17.5 Subject Information and Informed Consent

Before being enrolled in this clinical trial, the subject must consent to participate after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to him or her. An informed consent document that includes both information about the study and the consent form will be prepared and given to the subject. This document will contain all ICH, GCP, and locally required regulatory elements. The document must be in a language understandable to the subject and must specify the person who obtained informed consent.

After reading the informed consent document, the subject must give consent in writing. The written informed consent will be obtained prior to conducting any study-related procedures or tests. The subject's consent must be confirmed at the time of consent by the personally dated signature of the person conducting the informed consent discussions. A copy of the signed consent document must be given to the subject.

The PI will retain the original signed consent document. The PI will not undertake any measures specifically required only for the clinical study until valid consent has been obtained.

17.6 Confidentiality

All records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

Should direct access to medical records require a waiver or authorization separate from the subject's statement of informed consent, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

Subjects will be told that the IRB, the independent DSMB, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection law.

17.7 Protocol Amendments

Once the study has started, amendments should be made only in exceptional cases. Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to subjects. A protocol amendment intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, provided the IRB is notified within five business days. All amendments will be submitted to the IRB and written verification that the amendment was submitted and subsequently approved is to be obtained. Study projected dose escalations will be communicated to sites as a memo.

17.8 Case Report Forms

The Principal Investigator, or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study-specific Case Report Forms (CRFs) will document safety and treatment outcomes for safety monitoring and data analysis.

An electronic case report form (eCRF) is required and must be completed for each included subject. The completed dataset is the sole property of the University of Florida and should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from the University of Florida.

17.9 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed subject consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

All study documentation will be maintained for at least 6 years from the date of final study publication. No study records may be destroyed without prior authorization from UF.

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19. APPENDICES

APPENDIX A: SCHEDULE OF ASSESSMENTS

Visit: / Procedure:	SCREENING (PRE-TRANSPLANT EVALUATION[1])	TREATMENT (DAY -7 to DAY 11)	(WEEKLY UNTIL DAY 60 then with every visit until 100 daysPOST TRANSPLANT)	ON STUDY (DAY 100 AND EVERY 3 MONTHS UNTIL NORMAL)	12 and 24 months post-transplant (or end of study)[12]
Informed Consent[2]	X				
Demographics[3]	X				
Vital Signs[4]	X				
Complete Medical History	X				
Neurocognitive assessment (up to age 16 years at the time of testing)	X				X
Lansky/Karnofsky Score	X				X
Pre-Transplant Disease Evaluation[5]	X				
Pregnancy Test (Serum)[6]	X				
Echocardiogram	X				
Calculated Creatinine Clearance	X				
Liver Function Tests	X				
PFTs or O2 saturation	X				
Peripheral blood specimen for Pharmacogenomic studies	X				
Protocol Treatment:					
Busulfan		X[7]			
Fludarabine		X[7]			
Thiotepa		X[7]			
Anti-thymocyte globulin		X[7]			

Tacrolimus		**X**[7]			
Methotrexate – IV only		**X**[7]			
Mycophenolate mofetil		**X**[7]			
PK Sampling (Busulfan only)		**X**[8]			
Immune Reconstitutions Studies				**X**	**X**
Chimerism testing[9]			**X**[9]		**X**
SAE Reporting[10]		**X**[10]			
GVHD Assessment[10]			**X**[10]		
CIBMTR forms reporting[11]	**X**		**X**[11]		**X**

[1] All pre-transplant evaluations performed as SOC within 30 days of initiation of conditioning regimen but prior to informed consent, will be accepted for this study and need not be repeated. Neurocognitive testing up to 90 days prior to start of conditioning will be accepted.
[2] Written informed consent must be obtained at least 24 hours prior to subject starting conditioning regimen.
[3] Demographics include date of birth, gender, rac and ethnicity.
[4] Vital signs include height, weight, blood pressure, and pulse oximetry.
[5] Pre-transplant disease evaluation will be determined by treating physician, it is disease specific and will follow SOC guidelines
[6] Pregnancy test to be completed within 7 days of starting conditioning for WOCBP.
[7] Refer to section 7.1 for treatment schedule and administration.
[8] Busulfan PK collection obtained at the following time points from the start of first busulfan dose: 3 hours (end of infusion), 3 hours 15 minutes, 4 hours, 5 hours, 6 hours, and 8 hours.
[9]Obtain at day 30 if ANC has recovered, or at day 42 if there is failure of engraftment, obtain any time there is suspected failure of engraftment and then at 12 months and end of study
[10]Assess for SAEs and GVHD weekly until day 60, then at every clinic visit
[11]See section 6.3 for CIBMTR forms requirements, post transplant forms are due on day 100, 180 and 12 and 24 months
[12]Survival status will also be collected at year 3 and 4, if the study stays open.

APPENDIX B: MODIFIED GLUCKSBERG STAGING CRITERIA FOR ACUTE GRAFT VERSUS HOST DISEASE

Stage	Skin	Liver (bilirubin)	Gut (stool output/day)
0	No GVHD rash	< 2 mg/dL	Adult: < 500 mL/day Child: < 10 mL/kg/day
1	Maculopapular rash < 25% BSA	2-3 mg/dL	Adult: 500-999 mL/day Child: 10-19.9 mL/kg/day ***Or persistent nausea, vomiting, or anorexia, with a positive upper GI biopsy.***
2	Maculopapular rash 25% – 50% BSA	3.1-6 mg/dL	Adult: 1000-1500 mL/day Child: 20-30 mL/kg/day
3	Maculopapular rash > 50% BSA	6.1-15 mg/dL	Adult: >1500 mL/day Child: > 30 mL/kg/day
4	Generalized erythroderma plus bullous formation and desquamation > 5% BSA	>15 mg/dL	Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume).

For GI staging: The "adult" stool output values should be used for patients > 50 kg in weight. Use 3 day averages for GI staging based on stool output. If stool and urine are mixed, stool output is presumed to be 50% of total stool/urine mix.
For Stage 4 GI: the term "severe abdominal pain" will be defined as:
(a) Pain control requiring institution of opioid use, or an increase in on-going opioid use, PLUS
(b) Pain that significantly impacts performance status, as determined by the treating MD.
If colon or rectal biopsy is +, but stool output is < 500 mL/day (< 10 mL/kg/day), then consider as GI Stage 0.

There is no modification of liver staging for other causes of hyperbilirubinemia
Overall Clinical Grade (based on the highest stage obtained):
Grade 0: No Stage 1-4 of any organ
Grade I: Stage 1-2 skin and no liver or gut involvement
Grade II: Stage 3 skin, or Stage 1 liver involvement, or Stage 1 GI
Grade III: Stage 0-3 skin, with Stage 2-3 liver, or Stage 2-3 GI
Grade IV: Stage 4 skin, liver or GI involvement

APPENDIX B2: CGVHD GRADING AS MIDLE, MODERATE AND SEVERE BY NIH 2014 CONSENSUS CONFERENCE CRITERIA

Refer to published details on diagnostic signs and symptoms and scores by involved organs (Jagasia et al, 2015)

Mild	• 1 or 2 organs or sites (except lung) with score 1
	  • Mild oral symptoms, no decrease in oral intake
	  • Mild dry eyes, lubricant eyedrops ≤ 3x/day

Moderate	• 3 or more organs with score 1
	• At least 1 organ or site with score 2
	  • 19-50% body surface area involved or superficial sclerosis
	  • Moderate dry eyes, eyedrops > 3x/day or punctal plugs
	• Lung score 1 (FEV1 60-79% or dyspnea with stairs)

Severe	• At least 1 organ or site with score 3
	  • > 50% body surface area involved
	  • Deep sclerosis, impaired mobility or ulceration
	  • Severe oral symptoms with major limitation in oral intake
	  • Severe dry eyes affecting ADL
	• Lung score 2 (FEV1 40-59% or dyspnea walking on flat ground)

APPENDIX C: STUDY REQUIRED SPECIMENS, VOLUMES, HANDLING AND SHIPPING INSTRUCTIONS

Specimen description	Diagnosis
Pharmacogenomic studies – 3 mL in EDTA tube	X

After being obtained, blood specimens in EDTA tubes should be kept in the refrigerator until shipping. The specimens should be shipped on ice package overnight. Specific collection, packaging and shipping instructions are as follows:
Procedure:

1. Draw sample into labeled purple top (EDTA) tube
    a. Peripheral blood: 3mL
2. Samples should be slowly inverted 8 to 10 times to ensure the mixing of the sample and the anti-coagulant liquid inside the tube
3. Label tube with:
    a. Subject identifier (see below)
    b. Specimen type (peripheral blood)
    c. Date of collection
4. Secure and tape the top of the tube by wrapping the cap with parafilm to prevent spilling during shipment.
5. Individually wrap each tube with paper towels and rubber band to prevent freezing of sample and absorb any leaks.
6. Seal each wrapped tube in biohazard bag/secondary receptacle to prevent sample from leaking.
7. Place samples in the rigid outer container along with frozen gel packs.
8. Secure the outer container with packing tape and attach the provided shipping label.

Notes:

- Samples should be sent as soon as they are obtained. If sample cannot be sent immediately, it can be stored at 4C for up to 24 hours.
- Do not provide any protected health information (PHI) on the sample or in the shipment if possible.
- Include a marking on the package that properly identifies the shipment as "Exempt Human Specimen" as appropriate to comply with current IATA and ICAO regulations.

Samples are to be labeled using the following format:

Study Center Identifier –Subject ID- Specimen Number – Sample Source

- Subject IDs to start at 01 and increase sequentially with each newly consented subject.

- Specimen numbers to start at 01 for the diagnostic/baseline specimen and increase sequentially with each newly collected sample.
- Sample source:
    - Peripheral Blood (PB)
- **For this study specimens will be labeled: e.g. UFP-01(first subject)-01-PB (all subjects will be having only one specimen and peripheral blood will be the only source).**

- Contact Dr. Jatinder Lamba Ph.D, prior to shipping. Ship to the following address:

University of Florida, Department of
Pharmacotherapy and Translational Research
Office PG-25 HSC Building
Lab PG-06 HSC Building
1345 Center Drive
PO box# 100486
Gainesville, FL-32610
Office Phone: (352) 273-6425
Lab Phone: (352) 294 5745
Fax: (352) 273-6121
Email: jlamba@cop.ufl.edu
Email: jatinderklamba@ufl.edu

APPENDIX D: PROTOCOL DRUGS

1. **Busulfan Source and Pharmacology (**Busulfan-Injection (07/01/2015) (Busulfex® NSC #750)

Busulfan is a non-cell-cycle-specific bi-functional alkylating agent. In aqueous media, busulfan hydrolyzes to release methanesulfonate groups. This produces reactive carbonium ions that interact with cellular thiol groups and nucleic acids to form DNA cross-links. Busulfan injection is 100% bioavailable by definition of intravenous administration. The elimination of busulfan appears to be independent of renal function, presumably reflecting the extensive metabolism of the drug in the liver, since less than 2% of the administered dose is excreted in the urine unchanged within 24 hours. The drug is metabolized by enzymatic activity to at least 12 metabolites, among which tetrahydrothiophene, tetrahydrothiophene 12-oxide, sulfolane, and 3-hydroxysulfolane were identified. These metabolites do not have cytotoxic activity. Irreversible binding to plasma proteins (primarily albumin) is approximately 32.4%. Busulfan has a plasma terminal elimination half-life (t1/2) of about 2.6 hours and demonstrates linear kinetics. It is rapidly distributed into tissue and crosses the blood-brain and the placental barriers. CSF concentrations are approximately equal to those in plasma. Itraconazole reduced busulfan clearance by up to 25% in patients receiving itraconazole compared to patients who did not receive itraconazole. Higher busulfan exposure due to concomitant itraconazole could lead to toxic plasma levels in some patients. Fluconazole had no effect on the clearance of busulfan.

Busulfan Formulation and Stability

Each ampoule or vial of busulfan injection contains 60 mg (6 mg/mL) of busulfan, N,N-dimethylacetamide (DMA) 33% vol/vol and Polyethylene Glycol 400, 67% vol/vol. Store refrigerated at 2°- 8°C, (36°- 46°F).

Busulfan Guidelines for Administration

Dilute busulfan injection to a final concentration of approximately 0.5 mg/mL with NS or D5W. The drug should not be infused with any other drug or IV solution other than NS or D5W. Always add the busulfan to the diluent, not the diluent to the busulfan injection. Mix thoroughly by inverting several times.

Do not use polycarbonate syringes or filter needles with busulfan injection. Busulfan injection diluted in NS or D5W is stable at room temperature (25°C) for up to 8 hours but the infusion must be completed within that time. Busulfan injection diluted in NS is stable at refrigerated conditions 2°- 8°C (36°-46°F) for up to 12 hours but the infusion must be completed within that time. Busulfan injection contains N,N-dimethylacetamide, which is incompatible with many closed-system transfer devices (CSTDs); the plastic components of CSTDs may dissolve and result in subsequent leakage and potential infusion of dissolved plastic into the subject.

Busulfan injection should be administered by IV infusion through a central venous catheter. Subjects receiving busulfan in a conditioning regimen for bone marrow transplant must receive seizure prophylaxis. If phenytoin is used, it should be given 12 hours prior to the start of busulfan, then daily during busulfan administration and for 48 hours after completion of busulfan. In dose-finding studies of busulfan where patients received concomitant busulfan and phenytoin, phenytoin reduced busulfan plasma AUC by approximately 15%. Use of other anticonvulsants may result in higher busulfan plasma AUCs, and an increased risk of sinusoidal obstruction syndrome, (SOS, formerly VOD) or seizures. After an initial dose of busulfan injection, blood levels are monitored with bone marrow transplant patients in order to achieve a target area-under-the-curve (AUC) plasma concentration.

Busulfan Supplier

Busulfan is commercially available from various manufacturers. Reference the package insert for further information

Busulfan Toxicities

Please refer to Table 19.1 for busulfan toxicities.

Table 19.1- Busulfan Toxicities:

	Common (Occurs in 21-100 children out of every 100)	**Occasional** (Occurs in 5-10 children out of every 100)	**Rare** (Occurs in < 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 & 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 anti-seizure 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 & azoospermia, amenorrhea, ovarian failure		Secondary malignancy, breast enlargement, cataracts, idiopathic pulmonary syndrome (cough, dyspnea, pleural effusion, infiltrates, & hypoxemia), bronchopulmonary dysplasia with interstitial pulmonary fibrosis & pneumonitis, myocardial fibrosis, osteonecrosis
Unknown Frequency & Timing:	Fetal toxicities and teratogenic effects of busulfan and its solvent have been noted in animals. Toxicities include multiple anomalies and low birth weight. It is unknown whether the drug or its solvent is excreted in breast milk.		

(L) Toxicity may also occur later

2. **Fludarabine Source and Pharmacology** (Fludarabine (05/09/2011) (Fludara®, fludarabine phosphate, (2-fluror-ara-AMP) NSC# 3121887)

Fludarabine phosphate is a synthetic purine nucleoside. It differs from the physiologic nucleosides, adenosine, in that the sugar moiety is arabinose instead of ribose, and by the addition of a fluorine atom to the purine base adenine. Fludarabine is also a fluorinated nucleotide analog the antiviral agent vidarabine, (ara-A). The addition of fluorine results in increased aqueous solubility and resistance to enzymatic degradation by adenosine deaminase. Fludarabine (2-fluoro-ara-A) is commercially available as the monophosphate salt (2-fluoro-ara-

AMP). The monophosphorylation increases the drug's aqueous solubility while maintaining pharmacologic activity. The chemical name for fludarabine phosphate is 9HPurin-6-amine, 2-fluoro-9-(5-0-phosphono β-D-arabino-furanosyl) (2-fluoro-ara-AMP) and the molecular weight is 365.2. Fludarabine is a purine antagonist antimetabolite. In vivo, fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then it is phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibitingDNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted. Phase I studies in humans have demonstrated that within several minutes after IV infusion, fludarabine phosphate is converted to the active metabolite 2-fluror-ara-A and becomes undetectable. Therefore, PK studies have focused on 2-fluror-ara-A. Fludarabine phosphate 25 mg/m2 infused intravenously over 30 minutes to adult cancer patients, showed a moderate accumulation of 2-fluoro-ara-A. During a 5-day treatment schedule, 2-fluoro-ara-A plasma trough levels increased by a factor of about 2. Fludarabine is widely distributed. The volume of distribution at steady state (Vss) reported after daily administration of 25 mg/m2 for 5 days to adults averaged at 96-98 L/m2. Tissue distribution studies in animals indicate that the highest concentrations of the drug are in liver, kidney, and spleen. Although the extent to which fludarabine and/or its metabolites distribute into the CNS in humans has not been determined to date, severe neurologic toxicity (e.g., blindness, coma) has been reported in patients receiving the drug, particularly in high dosages. There is evidence from animal studies that fludarabine distributes into the CNS and that a toxic metabolite (2-fluoroadenine, possibly formed by bacteria in the GI tract), can be absorbed systematically via enterohepatic circulation and distributed into CSF. According to in vitro data, about 19-29% of fludarabine is bound to plasma proteins. Following IV administration, fludarabine phosphate is dephosphorylated rapidly to fludarabine. Plasma concentrations of fludarabine decline in a linear, dose-independent manner. The elimination profile of fludarabine also has been reported to be either biphasic or triphasic; however, reported terminal elimination half-lives have been similar. In adult cancer patients receiving fludarabine 25 mg/m2 as a 30-minute IV infusion daily for 5 days, a terminal half-life of about 20 hours was reported. In a limited number of pediatric patients, the plasma concentration profile of fludarabine exhibited both monoexponential and biexponential decay, with a mean t1/2 of 10.5 hours in patients with monoexponential elimination and a t1/2 of 1.2-1.4 and 12.4-19 hours, respectively, in patients with biexponential elimination. Renal clearance accounts for about 40% of the total body clearance of fludarabine. Renal elimination appears to become more important at high dosages of the drug. The dose of fludarabine needs to be adjusted in patients with moderate renal impairment. The use of fludarabine in combination with pentostatin is not recommended due to the risk of severe pulmonary toxicity.

Fludarabine Formulation and Stability

Fludarabine phosphate injection is available as sterile lyophilized powder and in solution. Each single-dose vial of powder contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. After reconstitution, the pH range for the final product is 7.2-8.2. The single-dose solution vial contains 25 mg/mL, 2 mL of fludarabine phosphate. It may contain mannitol and is preservative-free. Fludarabine phosphate vials should be stored refrigerated at 2-8ºC (36-46ºF).

Fludarabine Guidelines for Administration

Prior to administration fludarabine phosphate powder should be reconstituted with Sterile Water for Injection and further diluted in D5W or NS to a concentration of 10–25 mg/mL. Fludarabine 25 mg/mL solution should be further diluted in the same manner. Fludarabine phosphate powder should be reconstituted with 2 mL of Sterile Water for Injection. The solid cake should fully dissolve in 15 seconds or less. The resulting concentration is 25 mg/mL. When reconstituted to a final concentration of 25 mg/mL, the drug is stable for at least 16 days at room temperature and normal light conditions. The manufacturer recommends that the solution be used within 8 hours after reconstitution. Prior to administration, fludarabine 25 mg/mL solution or the reconstituted 25 mg/mL solution should be further diluted in 100 mL or 125 mL of D5W or NS. When diluted to a final concentration of 1 mg/mL, fludarabine is stable for at least 16 days at room temperature and normal light conditions. The manufacturer recommends that the diluted solution be used within 8 hours after preparation. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

Fludarabine Supplier

Fludarabine is commercially available from various manufacturers. Reference the package insert for further information.

Fludarabine Toxicities

Please refer to Table 19.2 for fludarabine toxicities.

Table 19.2 - Fludarabine Toxicities:

	Common (Occurs in 21-100 subjects out of every 100)	**Occasional** (Occurs in 5-20 subjects out of every 100)	**Rare** (Occurs in < 5 subjects out of every 100)
Immediate: (Within 1-2 days of receiving drug)	Fever, fatigue, weakness, pain, nausea, vomiting, anorexia, cough, dyspnea	Edema including peripheral edema, chills, rash, diarrhea, rhinitis, diaphoresis, malaise, abdominal pain, headache, back pain, myalgia, stomatitis, flu-like syndrome	Anaphylaxis, tumor lysis syndrome, dehydration*
Prompt: (Within 2-3 weeks, prior to next course)	Myelosuppression (anemia, neutropenia, thrombocytopenia) infection (urinary tract infection, herpes simplex infection, pneumonia upper respiratory)	Weight loss, gastrointestinal bleeding, hemoptysis, paresthesia, allergic pneumonitis, bronchitis, pharyngitis, visual disturbance, hearing loss, hyperglycemia	Sinusitis, dysuria, opportunistic infections and reactivation of latent viral infections like Epstein-Barr virus (EBV), herpes zoster and John Cunningham (JC) virus (progressive multifocal leukoencephalopathy [PML])[L], EBV associated lymphoproliferative disorder, pancytopenia (can be prolonged), pulmonary hypersensitivity [a] (dyspnea, cough, hypoxia, interstitial pulmonary infiltrate), pulmonary toxicity (acute respiratory distress syndrome) [ARDS], pulmonary fibrosis, pulmonary hemorrhage, respiratory distress, respiratory failure), pericardial effusion, skin toxicity (erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, pemphigus), liver failure, renal failure, hemorrhage, transfusion-associated GVHD has occurred following transfusion of non-irradiated blood products, phlebitis*, sleep disorder*, cerebellar syndrome*, depression*, mentation impaired*, alopecia*, pruritus*, seborrhea*, esophagitis*, constipation*, mucositis*, dysphagia* hesitancy*, cholelithiasis*, abnormal liver function tests*, osteoporosis*, arthralgia*, abnormal renal function test*, proteinuria*, epistaxis*, hemorrhagic cystitis* eosinophilia*

Delayed: (Any time later during therapy, excluding the above conditions)			Neurotoxicity (increased with high doses): seizures, agitation, confusion, weakness, visual disturbances, optic neuritis, optic neuropathy, photophobia, blindness, paralysis, coma, death, peripheral neuropathy [a]); autoimmune phenomena: thrombocytopenia/idiopathic thrombocytopenic purpura (ITP), Evans syndrome, hemolytic anemia, acquired hemophilia
Late: (Any time after completion of treatment)			Myelodysplastic syndrome/acute myeloid leukemia (mainly associated with prior or concomitant or subsequent treatment with other anticancer treatments), skin cancer (new onset or exacerbation)
Unknown Frequency & Timing:	**Pregnancy Category D** Based on its mechanism of action, fludarabine phosphate can cause fetal harm when administered to a pregnant woman. Fludarabine phosphate was embryo-lethal and teratogenic in both rates and rabbits.		

(L) Toxicity may also occur later.

* Reported in ≤ 3% of subjects. Since these are not considered life threatening, they are not included in the consent form.

[a] These effects were not reported in children.

3. **<u>Thiotepa Source and Pharmacology</u>** <u>(Thiotepa 11/15/2016) (Tepadina, Tespa, Thiophosphamide, Triethylenethiophosphoramide Tspa, WR-45312) NSC #6396</u>

Thiotepa is a cytotoxic agent of the polyfunctional type, related chemically and pharmacologically to nitrogen mustard. The radiomimetic action of thiotepa is believed to occur through the release of ethylenimine radicals, which, like irradiation, disrupt the bonds of DNA. One of the principal bond disruptions is initiated by alkylation of guanine at the N-7 position, which severs the linkage between the purine base and the sugar and liberates alkylated guanines. Thiotepa is desulfurated by cytochrome P-450 enzymes such as 2B1 and 2C11 which catalyze the conversion of thiotepa to tepa. Tepa is less toxic than thiotepa and has been demonstrated to produce alkali-labile sites in DNA, rather than cross-links. These findings indicate that tepa reacts differently from thiotepa and produces monofunctional alkylation of DNA. A second metabolite of thiotepa, a mercapturic acid conjugate, is formed via glutathione conjugation. Monochloro tepa is the third metabolite found in the urine. Following short intravenous infusion (less than 5 minutes), peak concentrations of thiotepa were measured within 5 minutes. At steady state, the volume of distribution was independent of dose and ranged from 0.3 to 1.6 liters per kilogram (L/kg). Approximately 4.2% of the original dose is eliminated in the urine within 24 hours as tepa. The elimination half-life of thiotepa ranges from 2.3 to 2.4 hours. The half-life of tepa ranged from 3 to 21.1 hours in one study.

Thiotepa Formulation and Stability

Thiotepa for Injection USP, for single use only, is available in vials containing 15 mg of non-pyrogenic, sterile lyophilized powder. Store in a refrigerator at 2°-8°C (36°-46°F). PROTECT FROM LIGHT AT ALL TIMES. Note: FDA is allowing temporary importation of a European thiotepa product (Tepadina®). Verify product, storage, and preparation instructions prior to dispensation and administration. Refer to specific product labeling for details. Tepadina®: Store intact vials under refrigeration at 2°C to 8°C (36°F to 46°F). Protect from light; do not freeze. Reconstituted solution (10 mg/mL) is stable for 8 hours when stored at 2°C to 8°C (36°F to 46°F). Solution further diluted for infusion is stable for 24 hours when stored at 2°C to 8°C (36°F to 46°F), or for 4 hours when stored at 25°C (77°F).

Thiotepa Guidelines for Administration

Reconstitute Thiotepa for Injection with 1.5 mL of Sterile Water for Injection resulting in a drug concentration of approximately 10 mg/mL. (As per manufacturer's information: Actual content per vial 15.6 mg; withdrawable amount 14.7 mg/1.4 mL; approximate reconstituted concentration: 10.4 mg/mL). When reconstituted with Sterile Water for Injection, solutions of thiotepa should be stored at refrigerated temperatures 2°-8°C (36°-46°F) protected from light and used within 8 hours. The reconstituted solution is hypotonic and should be further diluted with Sodium Chloride Injection (0.9% NaCl) prior to use. Thiotepa at a concentration of 1-5 mg/mL in 0.9% NaCl is stable for 24 hours at room temperature. At concentration of 0.5mg/mL it is stable for only one hour and stability decreases significantly at concentrations of less than 0.5mg/mL. Therefore, solutions diluted to 0.5mg/mL should be used immediately. In order to eliminate haze, filter solutions through a 0.22 micron filter [Polysulfone membrane (Gelman's Sterile Aerodisc®, Single Use) or triton-free mixed ester of cellulose/PVC (Millipore's MILLEX®- GSFilter Unit)] prior to administration. Filtering does not alter solution potency. Reconstituted solutions should be clear. Solutions that remain opaque or precipitate after filtration should not be used. Tepadina®: Reconstitute each 15 mg vial with 1.5 mL SWFI, or each 100 mg vial with 10 mL SWFI, to a concentration of 10 mg/mL. Gently mix by repeated inversions. Solution may be clear or opalescent; do not use if particulate matter is present. Further dilute reconstituted solution for IV infusion in 500 mL NS (1,000 mL NS if dose > 500 mg). If dose is < 250 mg, dilute in an appropriate volume of NS to achieve a final concentration of 0.5 to 1 mg/mL. When thiotepa is given in bone marrow transplant doses, bath the patient and change linen frequently (≥ 2 baths/day) to avoid the contact dermatitis and discoloration of the skin that is seen with high dose.

Thiotepa Supplier

The FDA is allowing temporary importation of Tepadina® (thiotepa), from Adienne Pharma & Biotech. Product may be ordered directly through Adienne Pharm & Biotech. Reference the package insert for further information. Please review the letter regarding importation of thiotepa on the FDA website:
http://www.fda.gov/downloads/Drugs/DrugSafety/DrugShortages/UCM251666.pdf

Thiotepa Toxicities

Please refer to Table 19.3 for thiotepa toxicities.

Table 19.3 - Thiotepa Toxicities:

	Common (Occurs in 21-100 subjects out of every 100)	**Occasional** (Occurs in 5-20 subjects out of every 100)	**Rare** (Occurs in < 5 subjects out of every 100)
Immediate: (Within 1-2 days of receiving drug)	Nausea, vomiting, anorexia, fatigue, weakness	Pain at the injection site, dizziness, headache, blurred vision, abdominal pain, contact dermatitis, rash	Anaphylaxis, laryngeal edema, wheezing, hives
Prompt: (Within 2-3 weeks, prior to next course)	Myelosuppression; at higher doses in conditioning regimens for BMT: mucositis, esophagitis	At higher doses in conditioning regimens for BMT: encephalopathy (inappropriate behavior, confusion, somnolence), increased liver transaminases, increased bilirubin, hyperpigmentation of the skin (bronzing effect)	Febrile reaction, conjunctivitis, dysuria, urinary retention
Delayed: (Any time later during therapy, excluding the above conditions)	Gonadal dysfunction/infertility, azoospermia, amenorrhea		Alopecia, secondary malignancy
Unknown Frequency & Timing:	Fetal and teratogenic toxicities: Carcinogenic and teratogenic effects of thiotepa have been noted in animal models at doses ≤ those used in humans. It is not known if thiotepa is excreted into human breast milk.		

(L) Toxicity may also occur later.

4. **Anti-thymocyte Source and Pharmacology (**Anti-thymocyte Globulin (Rabbit) (05/06/11) (Rabbit ATG, RATG – Rabbit, Antithymocyte Globulin, Thymoglobulin®) NSC #720095)

Anti-thymocyte globulin (rabbit) is a purified, pasteurized, gamma immune globulin, obtained by immunization of rabbits with human thymocytes. This immunosuppressive product contains cytotoxic antibodies directed against antigens expressed on human T-lymphocytes. The mechanism of action by which polyclonal anti-lymphocyte preparations suppress immune responses is not fully understood. Possible mechanisms by which anti-thymocyte globulin (rabbit) may induce immunosuppression in vivo include: T-cell clearance from the circulation and modulation of T-cell activation, homing, and cytotoxic activities. Anti-thymocyte globulin (rabbit) includes antibodies against T-cell markers such as CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, HLA Class I heavy chains, and (beta) 2 microglobulin. In vitro, anti-thymocyte globulin (rabbit) (concentrations > 0.1 mg/mL) mediates T-cell suppressive effects via inhibition of proliferative responses to several mitogens. In patients, T-cell depletion is usually observed within a day from initiating anti-thymocyte globulin (rabbit) therapy. After an intravenous dose of 1.25 to 1.5 mg/kg/day (over 4 hours for 7-11 days) 4-8 hours post-infusion, anti-thymocyte globulin (rabbit) levels were on average 21.5 mcg/mL (10-40 mcg/mL) with a half-life of 2-3 days after the first dose, and 87 mcg/mL (23-170 mcg/mL) after the last dose. During an anti-thymocyte globulin (rabbit) Phase III randomized trial, anti-rabbit antibodies developed in 68% of the Thymoglobulin-treated patients (108 of 163 patients evaluated). The volume of distribution is 0.12 L/kg. Approximately 1% of the dose excreted in the urine.

Anti-thymocyte Formulation and Stability

Each vial contains anti-thymocyte globulin (rabbit) 25 mg and inactive ingredients: glycine (50 mg), mannitol (50 mg), sodium chloride (10 mg). The reconstituted preparation contains approximately 5 mg/mL of anti-thymocyte globulin (rabbit), of which > 90% is rabbit gamma immune globulin (IgG). The reconstituted solution has a pH of 7 ± 0.4. Each anti-thymocyte globulin (rabbit) lot is released following potency testing (lymphocytotoxicity and E-rosette inhibition assays), and cross-reactive antibody testing (hemagglutination, platelet agglutination, anti-human serum protein antibody, antiglomerular basement membrane antibody, and fibroblast toxicity assays) on every fifth lot.

Anti-thymocyte Guidelines for Administration

Reference the Treatment and Dose Modifications sections of the protocol. Reconstitute anti-thymocyte globulin (rabbit) with the supplied diluent, SWFI, immediately before use. Anti-thymocyte globulin (rabbit) should be used within 4 hours after reconstitution if kept at room

temperature. Allow Thymoglobulin and SWFI vials to reach room temperature before reconstituting the lyophilized product. 1. Reconstitute each vial of anti-thymocyte globulin (rabbit) lyophilized powder with 5 mL of SWFI. 2. Inject SWFI slowly into the vial containing the lyophilized powder. 3. Rotate vial gently until powder is completely dissolved. Each reconstituted vial contains 25 mg or 5 mg/mL of anti-thymocyte globulin (rabbit). 4. Inspect solution for particulate matter after reconstitution. Should some particulate matter remain, continue to gently rotate the vial until no particulate matter is visible. If particulate matter persists, discard this vial. Dilution: 1. Transfer the contents of the calculated number of anti-thymocyte globulin (rabbit) vials into the bag of infusion solution (saline or dextrose). Recommended volume: per one vial of anti-thymocyte globulin (rabbit), use 50 mL of infusion solution (total volume usually between 50 to 500 mL). 2. Mix the solution by inverting the bag gently only once or twice. Administer by intravenous infusion through a high-flow vein. Anti-thymocyte globulin (rabbit) should be infused over a minimum of 6 hours for the first infusion and over at least 4 hours on subsequent days of therapy. Administer through an in-line 0.22 μm filter. Premedication with corticosteroids, acetaminophen, and/or an antihistamine 1 hour prior to the infusion is recommended and may reduce the incidence and intensity of side effects during the infusion. Always keep appropriate resuscitation equipment at the patient's bedside while anti-thymocyte globulin (rabbit) is being administered. Observe the patient continuously for possible allergic reactions throughout the infusions.

Anti-thymocyte Supplier

Anti-thymocyte is commercially available from various manufacturers. See package insert for further information.

Anti-thymocyte Toxicities

Please refer to Table 19.4 for anti-thymocyte toxicities.

Table 19.4 – Anti-thymocyte 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)	Fever, chills, rash, dyspnea w/wo bronchoconstriction, hypertension, tachycardia, peripheral edema, nausea, vomiting, diarrhea, abdominal pain, myalgia, headache	Pruritis, dizziness	Anaphylaxis, swelling, redness or phlebitis at the infusion site (peripheral veins)
Prompt: (Within 2-3 weeks, prior to next course)	Thrombocytopenia, leukopenia, pain, hyperkalemia, asthenia, infection	Malaise	Abnormal liver function test
Delayed: (Any time later during therapy, excluding the above conditions)	Antibody development to rabbit ATG (may persist for 1 year)	Serum sickness (L) which can have some or all of the following symptoms: glomerulonephritis, fever, myalgia, arthralgia, and periorbital edema (incidence reduced with use of corticosteroid medication)	Secondary malignancy
Unknown Frequency and Timing:	Animal reproduction studies have not been conducted with anti-thymocyte globulin (rabbit). It is not known whether anti-thymocyte globulin (rabbit) can cause fetal harm when administered to a pregnant woman or affect reproductive capacity. It is unknown whether the drug is excreted in breast milk.		

(L) Toxicity may also occur later.

5. **Tacrolimus Source and Pharmacology** (Tacrolimus (05/10/2011) (FK-506, Prograf®) NSC #717865

Tacrolimus is a macrolide immunosuppressant produced by Streptomyces tsukubaensis. Tacrolimus is a potent immunosuppressive agent which prolongs the survival of the host and transplanted grafts in animal transplant models of liver, kidney, heart, bone marrow, small bowel and pancreas, lung and trachea, skin, cornea, and limb. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-

FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (such as interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (immunosuppression). Additionally, tacrolimus may inhibit cellular activities such as nitric oxide synthetase activation and apoptosis, and may potentiate the action of corticosteroids in these processes. Tacrolimus activity is primarily due to the parent drug. The plasma protein binding of tacrolimus is approximately 99% and is independent of concentration over a range of 5-50 ng/mL. Tacrolimus is bound mainly to albumin and alpha-1-acid glycoprotein, and has a high level of association with erythrocytes. The t½ in adult patients ranges from 11-19 hours. The pharmacokinetics of tacrolimus have been studied in pediatric liver transplant patients (0.7 to 13.2 years of age). Following the IV administration of a 0.037 mg/kg/day dose to 12 pediatric patients, mean terminal half-life, volume of distribution and clearance were 11.5 ± 3.8 hours, 2.6 ± 2.1 L/kg and 0.138 ± 0.071 L/hr/kg, respectively. Following oral administration to 9 pediatric patients, the absolute bioavailability was 31 ± 21%. Whole blood trough concentrations from 31 patients less than 12 years old showed that pediatric patients needed higher doses than adults to achieve similar tacrolimus trough concentrations. Tacrolimus is extensively metabolized by the mixed-function oxidase system, primarily the cytochrome P-450 system (CYP3A) in the liver and to a lesser extent in the intestinal mucosa. The major metabolite identified in incubations with human liver microsomes is 13-demethyl tacrolimus. The main route of elimination is via the biliary tract and excretion in faeces. The mean clearance in renal dysfunction and mild hepatic dysfunction is the same as normal volunteers. Severe hepatic dysfunction (Pugh score > 10) led to a substantially decreased clearance. A retrospective comparison of Black and Caucasian kidney transplant patients indicated that Black patients required higher tacrolimus doses to attain similar trough concentrations; there were no gender-based differences. The absorption of tacrolimus from the gastrointestinal tract is incomplete and variable exhibiting large intra- and inter-patient variability. Administration with food significantly decreases the rate and extent of absorption Drugs that stimulate or inhibit hepatic p-450 enzymes will alter clearance of tacrolimus and close attention to potential drug interactions is crucial.

Tacrolimus Formulation and Stability

Injection: Tacrolimus is available as a sterile solution (tacrolimus injection) containing the equivalent of 5 mg anhydrous tacrolimus per 1 mL. Each mL also contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80% v/v. Store between 5°C and 25°C (41°F and 77°F).

Oral: Tacrolimus is available for oral administration as capsules containing the equivalent of 0.5 mg, 1 mg or 5 mg of anhydrous tacrolimus. Inactive ingredients include lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate. The 0.5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide, the 1 mg capsule shell contains gelatin and titanium dioxide, and the 5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide. Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F).

Tacrolimus Toxicities

Please refer to Table 19.5 for tacrolimus toxicities.

Table 19.5 - Tacrolimus 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)	Headache (L), hypertension (L), nausea, vomiting, anorexia, immunosuppression (L), diarrhea, constipation, fever	Chest pain	Anaphylaxis with the injection, allergic reaction, hypotension, asthma, dyspnea, increased cough, flu like syndrome, pleural effusion, seizure (L), tachycardia, angina
Prompt: (Within 2-3 weeks, prior to next course)	Tremor (L), renal dysfunction (acute with decrease in GFR, impaired urinary concentrating ability, and sodium retention), elevated creatinine/BUN, anemia, insomnia, asthenia, pain (abdominal, back pain), hyperglycemia, hypomagnesemia (L), hyper/hypokalemia (L), hypophosphatemia, paresthesia	Alopecia, dizziness, elevated LFTs, UTI, peripheral edema, rash, pruritis, hyperlipidemia, hypercholesteremia	Dyspepsia, dysphagia, gastritis, esophagitis, flatulence, CNS abnormalities (confusion (L), somnolence (L), depression (L), anxiety, anxiousness, abnormal dreams, emotional labiality, hallucinations, psychosis, hypertonia, incoordination, neuropathy, nervousness encephalopathy), coagulation disorder, leukopenia (L), thrombocytopenia, polycythemia, anemia, leukocytosis, infections (bacterial, fungal, viral – sepsis, cellulites, fungal dermatitis, herpes simplex, sinusitis, pharyngitis, abscess, pneumonia, bronchitis, peritonitis), hyperbilirubinemia (L), thrombosis, phlebitis, arthralgia, myalgia, electrolyte abnormalities

Delayed: (Any time later during therapy, excluding the above conditions)			Acne, exfoliative dermatitis, skin discoloration, photosensitivity reaction, skin ulcer, delayed wound healing, hirsuitism (hypertrichosis) (L), gingival hyperplasia, abnormal vision, amblyopia, ear pain, otitis, tinnitus, GI hemorrhage, GI perforation, cholelithiasis, cholestatic jaundice, chronic renal dysfunction, renal failure, post-transplant diabetes mellitus (L), myocardial hypertrophy, elevated liver function tests, liver damage, ascites
Late: (Any time after the completion of treatment)			Lymphoproliferative disorders, skin malignancies
Unknown Frequency and Timing:	Fetal toxic effects of tacrolimus have been noted in animals. Tacrolimus is transported across the placenta and its use during pregnancy has been associated with neonatal hyperkalemia and renal dysfunction. Tacroimus is excreted in human milk, nursing should be avoided.		

(L) Toxicity may also occur later.

6. **Methotrexate Source and Pharmacology** (Methotrexate – IV only (02/29/2012) (MTX, amethopterin) NCS #000740

A folate analogue which reversibly inhibits dihydrofolate reductase, the enzyme that reduces folic acid to tetrahydrofolic acid. Inhibition of tetrahydrofolate formation limits the availability of one carbon fragments necessary for the synthesis of purines and the conversion of deoxyuridylate to thymidylate in the synthesis of DNA and cell reproduction. The polyglutamated metabolites of MTX also contribute to the cytotoxic effect of MTX on DNA repair and/or strand breaks. MTX cytotoxicity is highly dependent on the absolute drug concentration and the duration of drug exposure. MTX is actively transported across cell membranes. At serum methotrexate concentrations exceeding 0.1 μmol/mL, passive diffusion becomes a major means of intracellular transport of MTX. The drug is widely distributed

throughout the body with the highest concentration in the kidney, liver, spleen, gallbladder and skin. Plasma concentrations following high dose IV MTX decline in a biphasic manner with an initial half-life of 1.5-3.5 hours, and a terminal half life of 8-15 hours. About 50% is bound to protein. MTX is excreted primarily by the kidneys via glomerular filtration and active secretion into the proximal tubules. Renal clearance usually equals or exceeds creatinine clearance. Small amounts are excreted in the feces. There is significant entero-hepatic circulation of MTX. The distribution of MTX into third-space fluid collections, such as pleural effusions and ascitic fluid, can substantially alter MTX pharmacokinetics. The slow release of accumulated MTX from these third spaces over time prolongs the terminal half-life of the drug, leading to potentially increased clinical toxicity.

Methotrexate Formulation and Stability

Methotrexate for Injection is available as a lyophilized powder for injection in 1000 mg vials. The powder for injection contains approximately 7 mEq sodium in the 1000 mg vial. Methotrexate for Injection is also available as a 25 mg/mL solution in 2, 4, 8, 10, and 40 mL preservative free vials and 2 and 10 mL vials with preservative. The 2, 4, 8, 10, and 40 mL solutions contain approximately 0.43 0.86, 1.72, 2.15, and 8.6 mEq sodium per vial, respectively. The preserved vials contain 0.9% benzyl alcohol as a preservative. Sterile methotrexate powder or solution is stable at 20º-25ºC (68º-77ºF); excursions permitted to 15º-30ºC (59º- 86 Fº). Protect from light.

Methotrexate Guidelines for Administration

Reference the Treatment and Dose Modifications sections of protocol. Leucovorin rescue may be necessary with certain doses of methotrexate. For IV use: Powder for injection: Dilute 1000 mg vial with 19.4 mL of preservative free SWFI, D5W or NS to a 50 mg/mL concentration. The powder for injection may be further diluted in NS or dextrose containing solutions to a concentration of ≤ 25 mg/mL for IV use.

Do not use the preserved solution for high dose methotrexate administration due to risk of benzyl alcohol toxicity. Methotrexate dilutions are chemically stable for at least 7 days at room temperature but contain no preservative and should be used within 24 hours. Diluted solutions especially those containing bicarbonate exposed to direct sunlight for periods exceeding 4 hours should be protected from light. High dose Methotrexate requires alkalinization of the urine, adequate hydration and leucovorin rescue. Avoid probenecid, penicillins, cephalosporins, aspirin, proton pump inhibitors, and NSAIDS as renal excretion of MTX is inhibited by these agents.

Methotrexate Supplier

Methotrexate is commercially available from various manufacturers. Reference the package insert for further information.

Methotrexate Toxicities

Please refer to Table 19.6 for methotrexate toxicities.

Table 19.6 - Methotrexate 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)	Transaminase elevations	Nausea, vomiting, anorexia	Anaphylaxis, chills, fever, dizziness, malaise, drowsiness, blurred vision, acral erythema, urticaria, pruritis, toxic epidermal necrolysis, Stevens-Johnson Syndrome, tumor lysis syndrome, seizures[1], photosensitivity
Prompt: (Within 2-3 weeks, prior to next course)		Myelosuppression, stomatitis, gingivitis, photosensitivity, fatigue	Alopecia, folliculitis, acne, renal toxicity (ATN, increased creatinine/BUN, hematuria), enteritis, GI ulceration and bleeding, acute neurotoxicity[1] (headache, drowsiness, aphasia, paresis, blurred vision, transient blindness, dysarthria, hemiparesis, decreased reflexes), diarrhea, conjuntivitis
Delayed: (Any time later during therapy, excluding the above conditions)		Learning disability[1] (L)	Pneumonitis, pulmonary fibrosis (L), hepatic fibrosis (L), osteonecrosis (L), leukoencephalophthy[1] (L), pericarditis, pericardial effusions, hyperpigmentation of the nails
Late: (Any time after the completion of treatment)			Progressive CNS deterioration[1]
Unknown Frequency and Timing:	Methotrexate crosses the placenta. Fetal toxicities and teratogenic effects of methotrexate have been noted in humans. The toxicities include: congenital defects, chromosomal abnormalities, severe newborn myelosuppression, low birth weight, abortion, and fetal death. Methotrexate is excreted into breast milk in low concentrations.		

[1] *May be enhanced by HDMTX and/or cranial irradiation*

(L) Toxicity may also occur later.

7. **Mycophenolate Mofetil Source and Pharmacology (**Mycophenolate Mofetil – (12/05/16)) CellCept®, MMF, RS-61443) NSC# 724229

Mycophenolate (MMF) is the morpholinoethyl ester of mycophenolic acid (MPA), an antibiotic with immunosuppressant properties isolated from Penicillium spp. The chemical name for oral

mycophenolate mofetil is 2-morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate. It has an empirical formula of C23H31NO7 and a molecular weight of 433.50. Mycophenolate mofetil is a white to off-white crystalline powder which is slightly soluble in water (43 μg/mL at pH 7.4); the solubility increases in acidic medium (4.27 mg/mL at pH 31 3.6). The intravenous product is the hydrochloride salt of mycophenolate mofetil. The chemical name for the hydrochloride salt of mycophenolate mofetil is 2-morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride. It has an empirical formula of C23H31NO7 HCl and a molecular weight of 469.96.

MMF has been used in a variety of solid organ and hematopoietic stem cell transplant settings for the prevention of acute rejection. MMF is a prodrug which, after oral administration, is rapidly and primarily hydrolyzed by the liver to the biologically active metabolite mycophenolic acid. MPA is metabolized principally by glucuronyl transferase to form the pharmacologically inactive phenolic glucuronide of MPA (MPAG). In vivo, MPAG is converted to MPA via enterohepatic recirculation. Mycophenolic acid inhibits nucleic acid synthesis and produces a potent, noncompetitive, and reversible inhibition of inosine monophosphate dehydrogenase (IMPDH), blocking the de novo synthesis of guanosine nucleotides without being incorporated into DNA. Both T and B lymphocytes rely on this de novo pathway for purine synthesis. As a result, the proliferative responses of T and B lymphocytes to both mitogenic and allospecific stimulation are inhibited. Other rapidly dividing cell lines are capable of recycling purine nucleotides via the "salvage" pathway, which is not blocked by mycophenolic acid.

In vitro and in vivo studies have demonstrated the ability of mycophenolic acid to block proliferative responses of T and B lymphocytes, inhibit antibody formation and the generation of cytotoxic T-cells, and suppress antibody formation by B lymphocytes. Mycophenolic acid prevents the glycosylation of lymphocyte and monocyte glycoproteins that are involved in intercellular adhesion of these cells to endothelial cells, and may inhibit recruitment of leukocytes into sites of inflammation and graft rejection. Antirejection effects have been attributed to decreased recruitment of activated lymphocytes to the graft site.

The mean absolute bioavailability of oral mycophenolate mofetil relative to intravenous mycophenolate mofetil (based on MPA AUC) was 94% in a small sample of healthy, adult volunteers. In this group the mean (±SD) apparent volume of distribution of MPA was approximately 3.6 (±1.5) and 4.0 (±1.2) L/kg following intravenous and oral administration, respectively. At clinically relevant concentrations, MPA is 97% bound to plasma albumin. MPAG is 82% bound to plasma albumin at MPAG concentration ranges that are normally seen in stable renal transplant patients; however, at higher MPAG concentrations (e.g., patients with renal impairment), the binding of MPA may be reduced as a result of competition between MPAG and MPA for protein binding. A negligible amount of the agent (< 1% of dose) is excreted as MPA in the urine. Most of the administered dose (~87%) is excreted in the urine as MPAG. Bile acid sequestrants (e.g., cholestyramine) reduce the AUC of MPA by interfering with the enterohepatic circulation of the drug.

Mycophenolate mofetil can cause fetal harm when administered to a pregnant woman. Use of MMF during pregnancy is associated with an increased risk of first trimester pregnancy loss and an increased risk of congenital malformations, especially external ear and other facial abnormalities including cleft lip and palate, and anomalies of the distal limbs, heart, esophagus,

and kidney. According to the package labeling, the National Transplantation Pregnancy Registry (NTPR) presents data on 33 MMF-exposed pregnancies in 24 transplant patients. Of these, there were 15 spontaneous abortions and 18 live-born infants. Four of the 18 infants had structural malformations (22%). In postmarketing data (collected 1995-2007) of 77 women exposed to systemic MMF during pregnancy, 25 had spontaneous abortions and 14 had a malformed infant or fetus. Six of 14 malformed offspring had ear abnormalities. Because these postmarketing data are reported voluntarily, it is not always possible to reliably estimate the frequency of particular adverse outcomes. These malformations seen in offspring were similar to findings in animal reproductive toxicology studies. In animal reproductive toxicology studies, there were increased rates of fetal resorptions and malformations in the absence of maternal toxicity. Female rats and rabbits received MMF doses equivalent to 0.02 to 0.9 times the recommended human dose for renal and cardiac transplant patients, based on body surface area conversions. In rat offspring, malformations included anophthalmia, agnathia, and hydrocephaly. In rabbit offspring, malformations included ectopia cordis, ectopic kidneys, diaphragmatic hernia, and umbilical hernia. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

WOCBP should have a negative serum or urine pregnancy test with a sensitivity of at least 25 mIU/mL within 1 week prior to beginning therapy (manufacturer's recommendation). MMF should not be initiated until a negative pregnancy test report is obtained. WOCBP taking MMF must receive contraceptive counseling and use effective contraception. It is recommended by the manufacturer that the patient begin using two chosen methods of contraception 4 weeks prior to starting MMF, unless abstinence is the chosen method. She should continue contraceptive use during therapy and for 6 weeks after stopping MMF. Subjects should be aware that MMF reduces blood levels of the hormones in the oral contraceptive pill and could theoretically reduce its effectiveness.

An approved medication guide (www.fda.gov/downloads/Drugs/DrugSafety/UCM170919.pdf) must be dispensed with each refill of mycophenolate mofetil.

Mycophenolate Mofetil Formulation and Stability

Mycophenolate mofetil is available in the following preparations:

- Capsule: 250 mg
- Tablet: 500 mg
- Powder for suspension, oral: 200 mg/mL (following reconstitution)
- Powder for reconstitution, injection: 500 mg per vial

A delayed release tablet (mycophenolic acid, Myfortic) is also commercially available. This preparation is not interchangeable with mycophenolate mofetil (Cellcept®, MMF) due to differences in absorption. This tablet is not discussed in this monograph and should not be used by subjects treated on this protocol.

Inactive ingredients in the 250 mg capsules include the following: croscarmellose sodium, magnesium stearate, povidone (K-90) and pregelatinized starch. The capsule shells contain black iron oxide, FD&C blue #2, gelatin, red iron oxide, silicon dioxide, sodium lauryl sulfate, titanium dioxide, and yellow iron oxide.

Inactive ingredients in the 500 mg tablets include black iron oxide, croscarmellose sodium, FD&C blue #2 aluminum lake, hydroxypropyl cellulose, hydroxypropyl methylcellulose,

magnesium stearate, microcrystalline cellulose, polyethylene glycol 400, povidone (K-90), red iron oxide, talc, and titanium dioxide; may also contain ammonium hydroxide, ethyl alcohol, methyl alcohol, n-butyl alcohol, propylene glycol, and shellac.

Inactive ingredients in the powder for oral suspension include aspartame, citric acid anhydrous, colloidal silicon dioxide, methylparaben, mixed fruit flavor, sodium citrate dihydrate, sorbitol, soybean lecithin, and xanthan gum.

The injectable product is available as a sterile, white to off-white, lyophilized powder in vials containing mycophenolate mofetil hydrochloride for administration by intravenous infusion only. Each vial contains the equivalent of 500 mg mycophenolate mofetil as the hydrochloride salt. Inactive ingredients include polysorbate 80 (25 mg), citric acid (5 mg), and sodium hydroxide to adjust the pH. Reconstitution and dilution with D5W yields a final solution of mycophenolate mofetil that is slightly yellow in color.

Mycophenolate Mofetil Toxicities

Please refer to Table 19.7 for mycophenolate mofetil toxicities.

Table 19.7 – Mycophenolate Mofetil Toxicities:

Incidence	Toxicities
Common (> 20% of patients)	Hypertension, edema (face, limbs, trunk), rash maculo-papular, cholesterol high, hyperglycemia, hyperkalemia, hypocalcemia, hypokalemia, hypomagnesemia, abdominal pain, constipation, diarrhea, nausea, vomiting, anorexia, dyspepsia, anemia, white blood cell decreased, platelet count decreased, back pain, anxiety, generalized muscle weakness, dizziness, headache, insomnia, tremor, creatinine increased, dyspnea, cough, fever, pleural effusion, alanine aminotransferase increased, alkaline phosphatase increased, aspartate aminotransferase increased, blood bilirubin increased, GGT increased, pain, paresthesia, infection[1]
Occasional (4-20% of patients)	Sepsis, paresthesia, urinary tract pain, urinary frequency, phlebitis (IV only), thrombosis (IV only)
Rare (≤ 3% of patients)	Neoplasms benign, malignant, and unspecified (including cysts and polyps) – Other, [Malignant epithelial neoplasm of skin, non-melanoma; lymphoproliferative disease or lymphoma], gastric ulcer, gastrointestinal hemorrhage, gastric perforation, mucositis oral, thromboembolic event, infective endocarditis, renal calculi, pulmonary fibrosis, pneumonitis, neutrophil count decreased, leukoencephalopathy, colitis, pancreatitis, pure red cell aplasia
Pregnancy & Lactation	**Pregnancy Category D** **Mycophenolate is associated with an increased risk of congenital malformations and spontaneous abortions when used during pregnancy.** Adverse events have been reported in animal studies at doses less than the equivalent recommended human dose. Data from the National Transplantation Pregnancy Registry (NTPR) have observed an increase in structural malformations (including ear malformations) in infants born to mothers taking mycophenolate during pregnancy. Spontaneous abortions have also been noted. Females of childbearing potential should have a negative pregnancy test within 1 week prior to beginning therapy. Two reliable forms of contraception should be used beginning 4 weeks prior to, during, and for 6 weeks after therapy. The effectiveness of hormonal contraceptive agents may be affected by mycophenolate. It is unknown if mycophenolate is excreted in human milk. Due to potentially serious adverse reactions, the decision to discontinue the drug or discontinue breast-feeding should be considered. Breast-feeding is not recommended during therapy or for 6 weeks after treatment is complete.

[1] *Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.*

APPENDIX E. STANDARD OF CARE NEUROPYSCHOLOGY TESTING BY AGE

Neurocognitive testing will be obtained prior to transplantation and then at 1 year post-transplant and at study exit (assuming that at least 12 months has elapsed since the previous testing).

Due to complexity of scheduling neurocognitive testing, the study allows that testing be completed up to 90 days prior to transplant for baseline. Neurocognitive testing is not a part of eligibility criteria and patients who cannot obtain the test may be enrolled, but lack of testing of eligible patients will be considered protocol violation.

The following standardized measures will be used to assess broad neurocognitive/developmental functioning:

For ages 6–35 months: Bayley Scales of Infant & Toddler Development-3rd Edition (Bayley-3), which assesses development across cognitive, language (expressive/receptive) and motor (fine & gross) domains; Bayley Social Emotional and Adaptive Questionnaire (parent rating scale).

For ages 3-6 years:

- Intelligence- Wechsler Preschool & Primary Scale of Intelligence, 4th Edition (WPPSI-IV)
- Memory- Ages 3-4: NEPSY-II, Narrative Memory; Ages 5-6: Children's Memory Scale (CMS), Dot Locations & Stories
- Visuoconstruction/graphomotor- Beery Buktenica Test of Visuo-motor Integration (VMI-6)
- Attention- Ages 4-6: Conners' Kiddie Continuous Performance Test-2nd Edition
- Receptive language/school readiness- Bracken Basic Concept Scale-3rd Edition-Receptive
- Emotional, behavioral & adaptive functioning- Behavior Assessment Scale for Children-3rd Edition, parent rating scales

For ages 7-15 years:

- Intelligence- Wechsler Intelligence Scale for Children, 5th Edition (WISC-V)
- Memory- Children's Memory Scale (CMS), Dot Locations & Stories
- Visuoconstruction/graphomotor- Beery Buktenica Test of Visuo-motor Integration (VMI-6)
- Attention- Conners' Continuous Performance Test-3rd Edition
- Executive Functioning- Ages 8+: Delis Kaplan Executive Function System (DKEFS), Trail Making Test & Verbal Fluency
- Emotional, behavioral & adaptive functioning- Behavior Assessment Scale for Children-3rd Edition, parent rating scales & self-report form (ages 8+)

Appendix E

Pt Name:

MR#

Dosing Weight Adjustments in Pediatric BMT Patients

Actual Weight (BW) = _____kg taken on _____ (Must be within 2 weeks of admission for patients > 12kg, within one week for ≤ 12 kg)

Height (Ht): _____ cm = _____inches taken on _____

BSA _____m^2

Age _____

Ht in cm divided by 2.54 = Ht in inches

5ft = 60inches

BMI % _____
(CDC BMI calculator for children and teens
https://www.cdc.gov/healthyweight/bmi/calculator.html:)

Ideal Body Weight (IBW) Calculations

☐	<2yo or <12 kg	Use actual body weight, calculate all chemotherapy using weight rather than BSA (divide BSA with 30 to get dose per kg)
☐	2-17.99 years old AND < 5ft	IBW= $\frac{\text{(height in cm}^2 \text{ x 1.65)}}{1000}$
☐	2-17.99 years old AND > 5ft	☐ IBW (male) = 39 + (2.27 x height in inches over 5 feet) ☐ IBW (female) = 42.2 + (2.27 x height in inches over 5 feet)
☐	≥ 18 years old	☐ IBW (male) = 50 + (2.3 x height in inches over 5 feet) ☐ IBW (male) = 50 – (2.3 x height in inches UNDER 5 feet) ☐ IBW (female) = 45.5 + (2.3 x height in inches over 5 feet) ☐ IBW (female)= 45.5 – (2.3 x height in inches UNDER 5 feet)

If the calculated BMI using CDC calculator is ≥95%, use adjusted body weight for calculation of chemotherapy but not serotherapy. Serotherapy may be adjusted in severely obese patients at MD discretion.

☐ Do not need to weight adjust, all children <12 kg

☐ Weight adjust according to calculation below:

Adjusted Body Weight (Adj Wt) =

IBW (_____) + [(BW _____ – IBW _____) x 0.4] = _____kg

Adjusted Body Surface Area = _____m^2