Hematopoietic Cell Transplantation Using Treosulfan-Based Conditioning for the Treatment of Bone Marrow Failure Diseases

BMT CTN PROTOCOL 1904

Version 5.0

Study Chairpersons

Lauri Burroughs, M.D. [1,3], Margaret MacMillan, M.D.[2]

Protocol Team:

Anny Chan, PharmD.[3]
Nancy DiFronzo, Ph.D.[4]
Nancy Geller, Ph.D.[4]
Mehdi Hamadani, M.D.[5]
Leslie Kean, M.D., Ph.D.[6]
Brent Logan, Ph.D.[5]
Adam Mendizabal, Ph.D.[7]
Kasiani Myers, M.D.[8]
Eneida Nemecek, M.D., MS, MBA[9]
Brittainy North, MS[7]
Megan Scott, BS[7]
Akiko Shimamura, M.D., Ph.D. [6]
Julie Talano, M.D.[5]
Mark Vander Lugt, M.D.[10]
Adrianna Vlachos, M.D. [11]

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[1]Fred Hutchinson Cancer Research Center
[2]University of Minnesota
[3]Seattle Children's Hospital
[4]National Heart, Lung, and Blood Institute
[5]Medical College of Wisconsin
[6]Boston Children's Hospital
[7]The Emmes Company
[8]Cincinnati Children's Medical Center
[9]Oregon Heath & Science University
[10]University of Michigan
[11]Cohen Children's Medical Center

Lauri Burroughs I am approving this document.

Approval Signature (Protocol Chair/Officer)
Lauri Burroughs 17/Nov/2023 03:32 PM EST

Margaret MacMillan I am approving this document.

Approval Signature (Protocol Chair/Officer)
Margaret MacMillan 16/Nov/2023 05:28 PM EST

Monica Thakar I am approving this document.

Approval Signature (Fred Hutch Medical Monitor)
Monica Thakar 17/Nov/2023 11:45 AM EST

PROTOCOL SYNOPSIS - BMT CTN 1904

Hematopoietic Cell Transplantation Using Treosulfan-Based Conditioning for the Treatment of Bone Marrow Failure Diseases

Study Chairpersons: Lauri Burroughs, M.D. and Margaret MacMillan, M.D.

Study Design: This is a prospective, multicenter phase II study designed to evaluate the outcomes of patients with bone marrow failure diseases (BMFD) undergoing HLA-matched related, HLA-matched unrelated, or single HLA-class 1 allele or HLA-DQB1 antigen or allele mismatched unrelated hematopoietic cell transplantation (HCT) using Treosulfan-based conditioning.

Primary Objectives: The primary objective is to determine the 1-year graft-versus-host disease (GVHD) free, event-free survival (EFS) in patients with BMFD undergoing HCT using Treosulfan-based conditioning. An event will be defined as death due to any cause, graft rejection/failure, or 2nd HCT, whichever occurs first. GVHD will bedefined as grade III-IV acute GVHD or chronic GVHD (using NIH consensus criteria) requiring systemic immune suppression.

Secondary Objectives: Secondary Objectives are to determine:

1. Overall survival at day 100, 6 months, and 1-year post-HCT
2. EFS at 1-year post-HCT
3. Neutrophil recovery at day 42 and platelet recovery at day 100post-HCT
4. Donor chimerism (CD3 and myeloid) at day 28, 100, and 1-year post-HCT
5. Primary graft failure/rejection at day 42 and secondary graft failure/rejection post-HCT
6. Grade II-IV and grade III-IV GVHD at day 100 and chronic GVHD (using NIH consensus criteria) requiring systemic immune suppression at 1-year post-HCT
7. Incidence of grade 3-5 toxicities at day 30 and day 100 post-HCT
8. Incidence of grade 2-3 systemic infections at 6-months post-HCT
9. Incidence of Epstein Barr virus (EBV) reactivation requiring therapy and the incidence of EBV-associated lymphoproliferative disorder by day 180 post-HCT
10. Incidence of cytomegalovirus (CMV) reactivation requiring therapy by day 180 post-HCT

Exploratory Objectives: Exploratory Objectives are to determine:

1. Treosulfan Pharmacokinetic Studies: Treosulfan PK characteristics to determine the optimal dose of Treosulfan and evaluate whether the exposure to Treosulfan determined by area under the curve (AUC) for patients with BMFD impacts engraftment, transplant-related mortality, and overall and event-free survival.
2. Biological Studies: Bank blood and marrow samples (cells, DNA, serum) and establish fibroblast cell cultures as a source ofgermline DNA to investigate a) somatic mutations, b) T cell phenotypes, and c) cytokine levels.
3. Mutational Testing: Collect peripheral blood on patients who lack a known genetic mutation responsible for their bone marrowfailure phenotype in order to perform whole exome or whole genome sequencing with the goal of identifying new mutations for patients with BMFD.
4. Health-related quality of life using PROMIS (Patient Reported Outcomes Measurement Information System) at baseline and at day +180 and day +365 post-HCT. Proxy reports (e.g., parents, guardians) will be collected for patients aged 5 to 8.

Eligibility Criteria:

Patient Inclusions:

1. Patient must be ≥ 1.0 year of age and less than 50.0 years of ageat the time of enrollment (i.e., patient must have celebrated their 1st birthday when enrolled and must NOT have celebrated their 50th birthday when enrolled; 49.99 years).

2. Underlying bone marrow failure disorders treatable by allogenicHCT.

a. Shwachman-Diamond syndrome

i. Criteria for Diagnosis:

1. A pathogenic mutation(s) for Shwachman-Diamondsyndrome
2. For those patients tested but lacking a genetic mutation they must meet both criteria a and b below:

a. Exocrine pancreatic dysfunction as defined by at least one of the following:

i. Pancreatic isoamylase below normal (age ≥3 years old), OR

ii. Fecal elastase <200, AND

b. Bone marrow failure as evidenced by at least one of the following:

i. Intermittent or persistent neutropenia (absolute neutrophil count < 1,500/μL), OR

ii. Hypo-productive anemia with a hemoglobin concentration below the age-related adjusted norms, OR

iii. Unexplained macrocytosis, OR

iv. Platelet count <150,000/μL without alternative etiology, OR

v. Hypocellular bone marrow

ii. Indications for HCT:

1. Severe neutropenia (ANC <500/μL), OR
2. Severe anemia (hemoglobin <8 g/dL) or transfusion-dependent anemia, OR
3. Severe thrombocytopenia (platelet count <20,000/μL) or transfusion-dependent thrombocytopenia, OR
4. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 eligibility review committee (ERC). In addition, patients with severe or recurrent infections will be reviewed by the ERC if they do not meet indications for transplant listed above.

b. Diamond Blackfan anemia

i. Criteria for Diagnosis:

1. A pathogenic mutation for Diamond Blackfan anemia
2. For those patients tested but lacking a genetic mutation the patient must meet criteria ‘a’ and at leastone of the criteria listed in ‘b-f’:

a. History of deficiency of erythroid precursors in an otherwise cellular bone marrow AND,

b. Reticulocytopenia, OR

c. Elevated adenosine deaminase activity, OR

d. Elevated hemoglobin F, OR

e. Macrocytosis, OR

f. Congenital anomalies

ii. Indications for HCT:

1. RBC transfusion dependent anemia despite an adequate trial of steroids; OR
2. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 ERC.

c. Congenital Sideroblastic anemia

i. Criteria for Diagnosis:

1. A pathogenic mutation(s) for sideroblastic anemia
2. For those patients tested but lacking a genetic mutation:
   a. Presence of ringed sideroblasts in the bone marrow excluding acquired causes of ringed sideroblasts such as lead poisoning & zinc toxicity.

ii. Indications for HCT:

1. Severe anemia (hemoglobin <8 g/dL) or transfusion-dependent anemia OR
2. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 ERC.

d. GATA2 mutation with associated marrow failure

i. Criteria for Diagnosis:

1. A pathogenic mutation(s) for *GATA2*

ii. Indications for HCT:

1. Severe neutropenia (ANC <500/μL), OR
2. Severe anemia (hemoglobin <8 g/dL) or transfusion-dependent anemia, OR
3. Severe thrombocytopenia (platelet count <20,000/μL) or transfusion-dependent thrombocytopenia, OR
4. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 ERC. In addition, patients with severe or recurrent infections will be reviewed by the ERC if they do not meet the indications for transplant listed above.

e. SAMD9 or SAMD9L disorders

i. Criteria for Diagnosis:

1. A pathogenic mutation(s) for *SAMD9* or *SAMD9L*

ii. Indications for HCT:

1. Severe neutropenia (ANC <500/μL), OR
2. Severe anemia (hemoglobin <8 g/dL) or transfusion-dependent anemia, OR

3. Severe thrombocytopenia (platelet count <20,000/μL) or transfusion-dependent thrombocytopenia, OR
4. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 ERC.

f. Congenital amegakaryocytic thrombocytopenia

i. Criteria for Diagnosis:

1. A pathogenic mutation(s) for congenital amegakaryocytic thrombocytopenia.
2. For those patients tested but lacking a genetic mutation the patient must meet criteria a and b below:
   a. Thrombocytopenia early in life, AND
   b. History of bone marrow demonstrating megakaryocyte hypoplasia.

ii. Indications for HCT:

1. Severe thrombocytopenia (platelet count <20,000/μL) or transfusion-dependent thrombocytopenia, OR
2. Severe neutropenia defined as an ANC <500/μL, OR
3. Severe anemia (hemoglobin <8 g/dL) or transfusion-dependent anemia, OR
4. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 ERC.

g. Paroxysmal nocturnal hemoglobinuria (PNH)

i. Criteria for Diagnosis:

1. PNH clone size in granulocytes ≥ 10%, AND
2. Complement mediated intravascular hemolysis with an elevated LDH (above institutional upper limits ofnormal)

ii. Indications for HCT:

1. PNH with thrombosis despite adequate medical management, OR
2. PNH with intravascular hemolysis requiring transfusion support despite adequate medical management, OR

3. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 ERC. In addition, patients with PNH and cytopenias may be considered for the protocol eligibility following review by protocol 1904 ERC.

h. **An undefined BMFD:** a patient with a BMFD for whom a genetic mutation responsible for their bone marrow failure phenotype has not been identified (excluding PNH) will be eligible for this clinical trial following approval by BMT CTN 1904 ERC.

i. **A BMFD with a known genetic mutation but not listed above** will be eligible for this clinical trial following approval by BMT CTN 1904 ERC.

Note: The following patients MUST be reviewed by the BMT CTN 1904 ERC in order to determine if they are eligible for this trial:

a. All patients with Shwachman-Diamond syndrome, Diamond Blackfan anemia, congenital sideroblastic anemia, or congenital amegakaryocytic thrombocytopenia who have had genetic testing and a genetic mutation responsible for their disease was not identified.

b. All patients with an undefined BMFD: a patient with a BMFD for whom a genetic mutation responsible for their bone marrow failure phenotype has not been identified, excluding PNH.

c. All patients with a BMFD and a known genetic mutationthat is not listed above

d. All patients with *GATA2* mutation with associated marrow failure

e. All patients with SAMD9 or SAMD9L disorders

f. There may be circumstances where a treating physician will consider a transplant for a patient with a BMFD who does not meet all the criteria listed under “indications for HCT”. In these situations, treating physicians may submit their patient to the BMT CTN 1904 ERC for review in order to determine if the patient is eligible for this clinical trial based on additional clinical or laboratory information.

g. Many patients with BMFD can have bone marrow evaluations that raise concern for possible myelodysplastic syndrome (MDS) including but not limited to dysplastic bone marrow evaluations or cytogenetic abnormalities. However, in patients with BMFD these findings are not necessarily diagnostic or

consistent with MDS. Therefore, given the complexities of diagnosing MDS in patients with BMFD, all patients with bone marrow evaluations concerning for possible MDS should be submitted to the ERC for expert reviewto confirm or exclude MDS. This is particularly important as we do not want to exclude potentially eligible patients due to an incorrect diagnosis of MDS.

3. Patient and/or legal guardian must sign informed consent priorto initiation of conditioning for BMT CTN 1904.

Patient Exclusions:

1. Patients with idiopathic aplastic anemia, Fanconi anemia, dyskeratosis congenita, and congenital neutropenia.
2. Patients with MDS as defined by the World Health Organization (WHO) or leukemia.
3. Prior allogeneic transplant
4. Patient's weight ≤10.0 kg (actual body weight and adjustedbody weight) at time of study enrollment
5. Lansky (patients < 16 years of age) or Karnofsky (patients ≥16years of age) performance <70%
6. Organ Dysfunction defined as follows:
    a. Cardiac:
        i. Left ventricular ejection fraction <50% by echocardiogram or multi-gated acquisition (MUGA) scan.
        ii. For patients unable to obtain a left ventricular ejection fraction, left ventricular shortening fraction <26%.
    b. Pulmonary:
        i. DLCO (corrected/adjusted for hemoglobin), FEV1, and FVC <50% predicted.
        ii. For patients unable to perform pulmonary function tests (PFTs) due to age or developmentaldelay: Oxygen saturation <92% on room air.
        iii. On supplemental oxygen.
    c. Renal:
        i. Estimated creatinine clearance <60 mL/minute/1.73m^2 (estimated per institutional practice).
        ii. Dialysis dependent

d. Hepatic:

i. Conjugated bilirubin > 2x upper limit of normal for age (ULN, unless attributable to Gilbert's syndrome), or

ii. AST or ALT > 4x ULN for age, or

iii. Fulminant liver failure or cirrhosis

7. Iron overload - This exclusion criterion only applies to patientswho are considered at risk for hepatic or cardiac iron overload.Therefore, not all patients enrolled on this protocol will undergo formal hepatic or cardiac iron assessment.

a. For patients ≥ 18 years with a history of significant transfusions defined as ≥8 packed red blood cell transfusions per year for ≥1 year or have received ≥20 packed red blood cell transfusions (lifetime cumulative) will require formal hepatic and cardiac iron measurement.In addition, patients with a prior history of hepatic or cardiac iron overload will also require formal assessmentfor iron overload. Patients are excluded if:

i. Hepatic iron content ≥ 8 mg Fe/g dry weight by liver MRI using a validated methodology (such as T2* MRI or ferriscan) or liver biopsy per institutional practice.

ii. Cardiac iron content < 25 msec by cardiac T2* MRI.

b. For patients < 18 years old with a history of significant transfusions defined as ≥8 packed red blood cell transfusions per year for ≥1 year or have received ≥20 packed red blood cell transfusions (lifetime cumulative) will require formal hepatic iron measurement. In addition, patients with a prior history of hepatic iron overload will also require formal assessment for iron overload. Patientsare excluded if:

i. Hepatic iron content ≥ 8 mg Fe/g dry weight by liver MRI using a validated methodology (such as T2* MRI or ferriscan) or liver biopsy per institutional practice.

8. Uncontrolled bacterial infection within 1 week of study enrollment. Uncontrolled is defined as currently taking medication with no clinical improvement or progression on adequate medical treatment.

9. Uncontrolled viral or fungal infection within 30 days of study enrollment. Uncontrolled is defined as currently taking medication with no clinical improvement or progression on adequate medical treatment.
10. Positive for HIV (human immunodeficiency virus).
11. Presence of clinically significant anti-donor HLA-antibodiesper institutional practice.
12. Prior solid organ transplant.
13. Patients with prior malignancies except resected non-melanoma skin cancer or treated cervical carcinoma in situ.
14. Females who are pregnant or breast-feeding.
15. Females and males of childbearing potential who are unwilling to practice an effective method of contraception or agree to abstinence from the time of signing informed consent through 12 months post-transplant or off tacrolimus whichever is later.
16. Known hypersensitivity to Treosulfan or fludarabine.
17. Known life-threatening reaction (i.e., anaphylaxis) to Thymoglobulin that would prohibit use for the patient as this study requires use of the Thymoglobulin preparation of anti- thymocyte globulin (ATG).

Treatment Description:	Patients will be treated with a preparative regimen of Treosulfan (total dose 30-42 g/m2), fludarabine (total dose 150 mg/m2), and Thymoglobulin (total dose 6 mg/kg). GVHD prophylaxis will be with tacrolimus and methotrexate.
Accrual Objective:	The target sample size is 40 patients.
Accrual Period:	The estimated accrual period is 48 months.
Study Duration:	Patients will be followed for 1-year post-HCT.
Interim Analysis:	No interim analyses for efficacy or futility will be conducted.
Stopping Guidelines:	Monitoring of two key safety endpoints (overall mortality by 100 days post-HCT, graft failure by 100 days post-HCT) will be conducted. Each month, the null hypothesis that the day 100 mortality is less than or equal to 15% is tested using a truncated Sequential Probability Ratio Test (SPRT) for censored exponential data; similarly, the null hypothesis that the graft failure rate is less than or equal to 10% is tested using a binary SPRT.

STUDY SCHEMA

Day	Treatment	Dose
-6	Treosulfan Fludarabine	10-14 grams/m^2/day IV [1,2] 30 mg/m^2/day IV[1]
-5	Treosulfan Fludarabine	10-14 grams/m^2/day IV [1,2] 30 mg/m^2/day IV[1]
-4	Treosulfan Fludarabine Rabbit Anti-thymocyte globulin (ATG; Thymoglobulin®)	10-14 grams/m^2/day IV [1,2] 30 mg/m^2/day IV[1] 1.0 mg/kg/day IV[3]
-3	Fludarabine Rabbit Anti-thymocyte globulin (ATG; Thymoglobulin®)	30 mg/m^2/day IV[1] 2.5 mg/kg/day IV[3]
-2	Fludarabine Rabbit Anti-thymocyte globulin (ATG; Thymoglobulin®)	30 mg/m^2/day IV[1] 2.5 mg/kg/day IV[3]
-1	Rest	
0[4]	HLA-matched related, matched unrelated, or single HLA-class 1 allele or HLA-DQB1 antigen or allele mismatched unrelated marrow or PBSC infusion	

[1] Actual body weight is to be used to calculate BSA for Treosulfan and fludarabine. However, forpatients > 120% of ideal body weight, BSA will be calculated using adjusted body weight **(Appendix D**)

[2]Treosulfan will be dosed according to BSA as follows:

BSA	Treosulfan Dose
< 0.4	10 grams/m^2/day
≥ 0.4 – <0.9	12 grams/m^2/day
≥ 0.9	14 grams/m^2/day

[3]Actual body weight is to be used for calculation of the rabbit ATG (Thymoglobulin) dose

[4]Additional rest days may be added given delays that may occur with stem cell collection, transportation, processing. The actual day the stem cell infusion finishes will remain "Day 0".

Glossary of Abbreviations

ABO	Human Blood Type
AD	Alternative Donor
AE	Adverse Event
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
ATG	Anti-Thymocyte Globulin
AUC	Area Under the Curve
BID	Twice A Day
BM	Bone Marrow
BMF	Bone Marrow Failure
BMFD	Bone Marrow Failure Diseases
BMI	Body Mass Index
BMT CTN	Blood and Marrow Transplant Clinical Trials Network
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
CAMT	Congenital Amegakaryocytic Thrombocytopenia
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CI	Confidence Interval
CIBMTR	Center for International Blood and Marrow Transplant Research
CMV	Cytomegalovirus
CTCAE	Common Terminology Criteria for Adverse Events
CXR	Chest X-Ray
CYP	Cytochrome
DBA	Diamond Blackfan Anemia
DCC	Data Coordinating Center
DEB	Diepoxybutane
DLCO	Diffusing Capacity of Lung for Carbon Monoxide
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr Virus
EFS	Event-Free Survival
EKG	Electrocardiogram
EOI	End of Infusion
ERC	Eligibility Review Committee
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume
FISH	Fluorescence in Situ Hybridization

FVC	Forced Vital Capacity
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
GEFS	GVHD Free, Event Free Survival
GFR	Glomerular Filtration Rate
GI	Gastrointestinal
GVHD	Graft-Versus-Host Disease
HCT	Hematopoietic Cell Transplantation
HHV	Human Herpesvirus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HRQoL	Health Related Quality of Life
HSV	Herpes Simplex Virus
HTLV	Human T-Cell Lymphotropic Virus
IBW	Ideal Body Weight
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
INR	International Normalized Ratio
IRB	Institutional Review Board
IV	Intravenous
IVIG	Intravenous Immune Globulin
LVEF	Left Ventricular Ejection Fraction
LVSF	Left Ventricular Shortening Fraction
MDS	Myelodysplastic Syndrome
MFI	Mean Fluorescence Intensity
MMF	Mycophenolate Mofetil
MOP	Manual of Procedures
MR	Match Related
MRI	Magnetic Resonance Imaging
MSD	HLA-Matched Sibling Donor
MTX	Methotrexate
MUGA	Multigated Acquisition Scan
NCI	National Cancer Institute
NHLBI	National Heart, Lung, And Blood Institute
NIH	National Institutes of Health
NK	Natural Killer
NMDP	National Marrow Donor Program
NS	Normal Saline
OR	Odds Ratio
OS	Overall Survival
PBSC	Peripheral Blood Stem Cell
PCR	Polymerase Chain Reaction
PFT	Pulmonary Function Test

PI	Principal Investigator
PK	Pharmacokinetic
PNH	Paroxysmal Nocturnal Hemoglobinuria
PROMIS	Patient Reported Outcomes Measurement Information System
PT	Prothrombin time
PTT	Partial Thromboplastin Time
PTLD	Post-transplant HCT Lymphoproliferative Disease
RD	Related Donor
RV	Residual Volume
SAE	Serious Adverse Event
SCTOD	Stem Cell Transplant Outcomes Database
SDS	Shwachman-Diamond Syndrome
SOC	System Organ Class
SOS	Sinusoidal Obstructive Syndrome
SPRT	Sequential Probability Ratio Test
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBI	Total Body Irradiation
TLC	Total Lung Capacity
TNC	Total Nucleated Cell Count
TRM	Transplant Related Mortality
UCB	Umbilical Cord Blood
ULN	Upper Limit of Normal
URD	Unrelated Donor
VZV	Varicella Zoster Virus
WHO	World Health Organization

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CHAPTER 1

1 BACKGROUND AND STUDY RATIONALE

1.1 Objectives

The primary objective of this phase II study is to determine the 1-year graft-versus-host-disease (GVHD)-free, event-free survival (EFS) in patients with bone marrow failure diseases (BMFD) undergoing HLA-matched related, matched unrelated, or single HLA-class 1 allele or HLA-DQB1 antigen or allele mismatched unrelated hematopoietic cell transplantation (HCT) using Treosulfan-based conditioning. An event will be defined as death due to any cause, graft rejection/failure, or 2nd HCT whichever occurs first. GVHD will be defined as grade III-IV acute GVHD or chronic GVHD (using NIH consensus criteria) requiring systemic immune suppression.

Secondary objectives are to determine:

1 Overall survival at day 100, 6 months, and 1-year post-HCT.

2 EFS at 1-year post-HCT. An event will be defined as death due to any cause, graft rejection/failure, or 2nd HCT.

3 Neutrophil recovery at day 42 and platelet recovery at day 100 post-HCT.

4 Donor chimerism (CD3 and myeloid) at day 28, 100, and 1-year post-HCT.

5 Primary graft failure/rejection at day 42 and secondary graft failure/rejection post-HCT.

6 Grade II-IV and grade III-IV GVHD at day 100 and chronic GVHD (using NIH consensus criteria) requiring systemic immune suppression at 1-year post-HCT.

7 Incidence of grade 3-5 toxicities at day 30 and day 100 post-HCT.

8 Incidence of grade 2-3 systemic infections at 6-months post-HCT.

9 Incidence of EBV-reactivation requiring therapy and the incidence of EBV-associated lymphoproliferative disorder by day 180 post-HCT.

10 Incidence of CMV reactivation requiring therapy by day 180 post-HCT.

1.2 Background

Allogeneic HCT is effective in the treatment of patients with BMFD and, for many, is the only known curative therapy. Historically, patients with BMFD often had poor outcomes following HCT using conventional myeloablative conditioning regimens, such as busulfan combined with cyclophosphamide, due to underlying comorbidities which increased risk for transplant related toxicities and mortality.[1-4] In addition, many patients with BMFD have normocellular or even hypercellular marrows that, along with a competent immune system, increase their risk for graft rejection. Thus, it can be difficult to effectively lower the intensity of the conditioning regimen without increasing the risk for graft failure. Therefore, less toxic conditioning regimens that are effective at establishing multi-lineage engraftment are needed for this group of BMFD patients.

Treosulfan is a pro-drug of an alkylating agent structurally related to busulfan but with a different mode of alkylation. Treosulfan has both cytotoxic and immunosuppressive properties and has been increasingly used in HCT conditioning regimens in both pediatric and adult patients with hematologic malignancies and non-malignant diseases.[5-10] Treosulfan has several characteristics that make it particularly appealing for use in HCT, including the fact that it is water soluble and bypasses hepatic enzyme activation, thereby leading to a decreased risk for sinusoidal obstructivesyndrome (SOS) as well as its highly predictable pharmacokinetics in adults. Treosulfan combinedwith fludarabine has been used by European groups for almost a decade as a high intensity, yet reduced toxicity, regimen with low transplant related mortality (TRM). In the US, there have beenseveral prospective studies using Treosulfan combined with fludarabine for the treatment of hematologic malignancies, which have also demonstrated low TRM.[5-7,11] However, there have been few prospective multi-center trials using Treosulfan-based conditioning for non-malignant diseases including patients with BMFD. Although most of the data comes from retrospective analyses, these studies have shown a very low TRM in patients with various non-malignant diseases.[8-10]

Burroughs and colleagues at the Fred Hutchinson Cancer Research Center have been leading a phase II prospective study using Treosulfan-based conditioning in patients with non-malignant diseases including 23 patients with BMFD (NCT00919503). Outcomes of the first 14 patients are published.[12] Conditioning consisted of Treosulfan (14 grams/m^2, days -6 to -4; total dose 42 grams/m^2), fludarabine (30 mg/m^2/day, days -6 to -2; total dose 150 mg/m^2), +/- rabbit ATG (thymoglobulin; 1 mg/kg/day, day -4, 2.5 mg/kg days -3 and -2; total dose 6 mg/kg; n=20) followed by HLA-matched sibling (n=5) or HLA-matched unrelated donor (URD) (n=15) bone marrow (BM, n=16), peripheral blood stem cell (PBSC, n=5), or sibling BM plus sibling cord blood (n=2) grafts. Three patients received a single class 1 allele (n=2) or DQB1 antigen (n=1) mismatched graft. GVHD prophylaxis consisted of tacrolimus and methotrexate (MTX). Outcomes were encouraging. With a median follow-up of 2 years, the 2-year overall survival (OS) and event-free survival (EFS) were both 96%. The 1-year GVHD-free, EFS was 87%. The median time to neutrophil recovery was 22 (range, 13-26) days. The median time to platelet recovery defined as a platelet count >50,000 without platelet transfusions x 7 days was 24 (range, 13-82) days. The cumulative incidences of grades II-IV and III-IV acute GVHD at day 100 and NIH chronic GVHD at 1 year were 39%, 4% and 9%, respectively. None of the patients experienced graft rejection, with 22 patients having full (≥95%) and one patient having stable mixed donor CD33 myeloid engraftment.

These primarily single center results indicate that the combination of Treosulfan, fludarabine, and rabbit anti-thymocyte globulin (ATG) is effective at establishing donor engraftment with low toxicity and excellent survival in patients with BMFD. Preliminary data from this trial provides strong support for a multi-center prospective clinical trial. Therefore, the primary objective of this clinical trial is to evaluate the 1-year GVHD-free, EFS in patients with BMFD undergoing HCT using Treosulfan-based conditioning in a multi-center prospective clinical trial.

1.3 Clinical Data to Date

1.3.1 Hematopoietic Cell Transplantation for Marrow Failure Diseases

Allogenic HCT is currently the only definitive curative therapy for many BMFD, however approaches vary significantly based on underlying etiology. Traditional myeloablative approaches such as busulfan and cyclophosphamide, or total body irradiation (TBI) containing regimens have historically been used but have been associated with increased morbidity and mortality.

HCT for Diamond Blackfan anemia (DBA):

One of the earliest publications on HCT outcomes for patients with DBA was by Vlachos and colleagues who reported on the HCT outcomes in 20 patients with DBA.[13] Conditioning was primarily busulfan-based (n=10) or TBI-based (n=8) followed by HLA-matched sibling donor (MSD) (n=8) or URD BM or umbilical cord blood (UCB) grafts (n=11; of note HLA-match at the time was 6/6). Superior 5-year OS was seen in recipients of HLA-matched sibling grafts (OS 87.5%) versus recipients of alternative donor (AD) grafts (OS 14.1%) which led to the recommendation for HCT if a matched sibling was available and to use caution with other donors. In addition, since most patients who received TBI died, there was a recommendation to avoid TBI.

Vlachos and colleagues published a follow-up report in 2010 from the DBA Registry that demonstrated continued encouraging survival following HLA-matched sibling grafts (77.3%) and slightly higher but still inferior survival following AD grafts (OS 31.5%).[14] However, they highlighted that survival following AD HCT had improved overtime with an OS of 85.7% in patients who underwent HCT after the year 2000. In addition, they reported improved OS for patients <9 years of age at time of HCT (OS 90%) versus > 9 years of age (OS 70%). Similar results were reported in an International Bone Marrow Transplant Registry (IBMTR) series of 61 patients with DBA who underwent HCT following primarily busulfan/cyclophosphamide (n=44) or TBI (n=13) based conditioning. The 3-year survival OS was 64% with superior OS in MSD (OS 76%) versus AD (OS 39%).[4] Similar to prior studies, TBI was associated with inferior outcomes.

More recently the Italian group reported on 30 patients with DBA who underwent HCT following predominantly busulfan-based conditioning with a 5-year OS of 74.4%[3] and transplant related mortality (TRM) of 25.6%. Patients younger than 10 years and those transplanted after 2000 showed a significantly higher OS and lower risk of TRM. Importantly, there was no difference in OS between donor types.

HCT for Shwachman-Diamond syndrome (SDS):

Historically patients with SDS who underwent HCT following myeloablative regimens had OS rates of approximately 60% irrespective of donor source, with mortality largely secondary to myelodysplastic syndrome (MDS), leukemia or organ toxicity including cardiotoxicity, as well as neurologic and pulmonary toxicities.[2,15-18] In a review of the European experience with HCT in SDS, Cesaro et al. reported treatment-related mortality of 35.5%, with a significantly higher rate in patients receiving TBI-containing regimens compared with those receiving a non–TBI-containing regimen (67% vs 20%).[1] While overall HCT outcomes using myeloablative strategies have improved over time, these regimens can still be associated with significant morbidity and mortality, especially in subsets of higher risk patients with concomitant risk factors commonly seen in BMFD populations such as infections or iron overload. Additionally, late effects after HCT also remain a significant concern especially after myeloablative regimens.[19,20]

HCT for amegakaryocytic thrombocytopenia, sideroblastic anemia and GATA2 mutation with associated marrow failure:

The literature on HCT for congenital amegakaryocytic thrombocytopenia is largely old and consists primarily of case reports or small case series using a variety of donor sources and preparative regimens. Historically, more aggressive myeloablative regimens have been used including TBI combined with cyclophosphamide[21-23] or busulfan combined with cyclophosphamide.[24,25] While effective at curing the disease, patients are at risk of death from transplant related complications including graft rejection as reviewed by Frangoul and colleagues and more recently by Tarek et al.[25,26] King et al. reported on the outcomes of 15 patients with amegakaryocytic thrombocytopenia who underwent HCT with HLA-MSD (n=6), matched URD (n=3), or mismatched RD (n=6).[27] Patients died from bronchiolitis obliterans (n=1), graft failure (n=2), MDS/AML (n=1) and not reported (n=1).

Similar to amegakaryocytic thrombocytopenia, the literature published on HCT outcomes for sideroblastic anemia is also largely remote and is primarily case reports or small case series.[28-30] Conditioning consisted primarily of busulfan combined with cyclophosphamide; however, more recently Kim et al. published on the successful outcome of HCT using busulfan combined with fludarabine in a patient with congenital sideroblastic anemia.[31] The literature published to date on HCT outcomes for patients with GATA2 mutation is largely in the setting of MDS or infections. However, patients with GATA2 mutation can have evidence of marrow failure as well.[12,32,33] Future studies are needed evaluating outcomes for this group of patients.

HCT for paroxysmal nocturnal hemoglobinuria (PNH):

Myeloablative conditioning has also been used for HCT in paroxysmal nocturnal hemoglobinuria (PNH). The European Group for Blood and Marrow Transplantation reported outcomes of 211 patients with PNH who underwent HCT between 1978 and 2007 with predominantly myeloablative conditioning regimens with an overall 5-year HCT survival of 68% with higher mortality in those with history of thromboembolic events.[34] Graft failure occurred in 7% and treatment-related mortality was largely related to infection and GVHD. More recently Cooper et al reported on 55 patients, roughly half of whom received a myeloablative regimen with an OS of 70% at 5 years and infection and hemorrhage as the leading causes of mortality.[35]

Motivation to decrease toxicity and treatment-related mortality in patients with BMFD has led to the use of reduced-intensity conditioning. Reduced intensity HCT regimens have demonstrated excellent success in patients with idiopathic aplastic anemia both in the MSD and URD settings.[36] There remains limited experience with the use of reduced-intensity HCT in patients with BMFD outside this setting and is mainly limited to case reports and small series.[37-41] In one series Hegenbart et al. report long-term disease-free survival in 4 of 7 patients with PNH using fludarabine and 2 Gy of TBI.[42] Pantin et al. at the NIH also reported a cohort of 17 consecutive PNH patients transplant using a reduced-intensity cyclophosphamide/fludarabine +/-anti-thymocyte globulin regimen with an OS of 88% at a median follow-up of 6 years; however, 47% and 71% had acute and chronic GVHD, respectively.[43] In SDS, Bhatla et al. reported a retrospective series of 7 patients transplanted predominantly for marrow failure using an alemtuzumab, fludarabine, and melphalan approach without graft failure and OS of 100%.[44] One patient with DBA and history of iron overload with liver cirrhosis[45] and another with amegakaryocytic thrombocytopenia[41] have also been reported after matched URD HCT using this approach with survival at 21 and 9 months follow-up, respectively. In summary, although data are limited using reduced-intensity conditioning for patients with marrow failure, preliminary

outcomes are encouraging and support further study with the goal of decreasing both early and late toxicities.

1.3.2 HCT for Bone Marrow Failure Diseases – Unpublished Data from the CIBMTR

From 2015-2017, 92 patients (less than 50 years of age) with BMFD [Diamond Blackfan anemia (n=27), Shwachman-Diamond syndrome (n=4), congenital amegakaryocytic thrombocytopenia (n=13), sideroblastic anemia (n=12), paroxysmal nocturnal hemoglobinuria (n=28), and other BMFD (n=8)] underwent HCT and had their information recorded in the Center for International Blood and Marrow Transplant Research (CIBMTR) database**.** Busulfan-based conditioning was most commonly used (n=46; 50%) followed by TBI-based regimens (n=21; 23%). In addition, the majority of patients received serotherapy with either alemtuzumab or ATG (n=52; 57%). Donor sources included MSD (n=32), other related donor (n=2), and HLA-matched (8/8 matched; n=47) or partially matched (7/8 matched; n=11) URD grafts. BM was the primary hematopoietic cell source (n=70; 76%). The day 100 and 1-year OS and 1-year EFS were 92.4%, 84.7%, and 81.4%, respectively. Day 100 graft failure was 3.8% for patients who received ATG versus 7.5% for those that did not. The 1-year GVHD-free (defined as extensive chronic GVHD) EFS was 65% in patients who received serotherapy versus 62.4% in those that did not. Although encouraging, this data supports that studies of novel approaches are needed in order to decrease transplant related toxicity and mortality while maintaining engraftment in patients with BMFD.

1.3.3 Initial Experience with Treosulfan-Based Conditioning for Allogeneic HCT in Patients with Non-Malignant Diseases: European Experience

There have been several large retrospective studies published on the HCT outcomes following Treosulfan-based conditioning for patients with non-malignant diseases. Greystoke and colleagues published a retrospective analysis of 32 pediatric patients undergoing HCT for non-malignant diseases including primary immunodeficiency (n=18), metabolic disorders (n=9), osteopetrosis (n=4) and thalassemia (n=1).[8] Patients received a total Treosulfan dose of 36 g/m^2 (n=6) or 42 g/m^2 (n=26) given in three divided doses on consecutive days. A variety of other conditioning agents were used depending on the stem cell source and donor type including fludarabine (150 mg/m^2), cyclophosphamide (200 mg/kg), plus or minus alemtuzumab or ATG. HLA-matched or mismatched RD or URD BM (n=17), PBSC (n=9), and UCB (n=5) grafts were used as the stem cell source. Post-HCT immunosuppression included cyclosporine alone or combined with mycophenolate mofetil (MMF), methylprednisolone, or methotrexate (MTX). Twenty-eight patients (87.5%) established donor cell engraftment; however, 4 patients required a 2nd HCT. With a median follow up of 471 days, 27 (84%) of the patients survive. One patient died early (day +4) due to SOS and four other deaths were late due to disease progression, GVHD or infection. Although multiple different donors and conditioning regimens were used, these results were encouraging in pediatric patients with non-malignant disorders.

More recently, Slatter and colleagues published the outcomes of 160 pediatric patients with primary immunodeficiency diseases who underwent HCT following conditioning with Treosulfan, fludarabine +/- alemtuzumab (n=124).[46] Median age was 1.36 (range, 0.09-18.25) years. Donor sources included matched URD (n=73), mismatched URD (n=54), MSD (n=12), other matched family (n=17) or haploidentical (n=4) grafts. Stem cell sources included PBSC (n=70), BM (n=49), or UCB (n=41). With a median follow-up of 4.3 years, the 2-year OS and EFS were 88.3% and 88.1%, respectively. None of the patients developed SOS. Deaths were primarily due to infection or GVHD. Most patients had full donor or high-level (50-94%) donor T cell and myeloid engraftment regardless of hematopoietic cell source; however, superior myeloid engraftment was

seen with PBSC grafts. This study demonstrates excellent OS and EFS with a low rate of early mortality in patients with primary immunodeficiency disorders following Treosulfan-based conditioning.

Bernardo et al published the outcomes of 60 patients with thalassemia (median age, 7 years; range 1-37) who underwent HCT following Treosulfan, fludarabine, thiotepa +/- ATG.[47] Twenty patients received HLA-matched sibling grafts and 40 patients received an unrelated donor graft. With a median follow up of 36 months, the 5-year OS and thalassemia free survival was 93% and 84%, respectively. The cumulative incidence of graft failure was 9% and TRM was 7%. None of the patients developed SOS. In addition, there was a low incidence of acute grade II-IV, III-IV, and chronic GHVD (14%, 7%, and 2%, respectively). This study demonstrated excellent OS with low TRM in pediatric and adult patients with thalassemia.

Limited data has been published on HCT outcomes following Treosulfan-based conditioning in patients with BMFD. Crazzolara et al. published on 3 patients with DBA who underwent HCT, 2 of whom received Treosulfan-based conditioning.[48] Both patients were young (age 7 and 9 months) at time of HCT and received a MSD or matched URD BM grafts. Indications for HCT included anemia combined with severe neutropenia. Both patients are alive with 100% donor engraftment 3 and 4 years post-HCT. Sauer et al. published on 3 patients with SDS who underwent HCT following conditioning with Treosulfan, fludarabine, melphalan combined with either alemtuzumab or thymoglobulin and either MSD (n=1), HLA matched URD (n=1) or UCB (n=1) grafts.[49] All 3 patients achieved 100% donor engraftment. Two of the three patients are alive with 16 and 24 months of follow-up. One patient died following UCB HCT of idiopathic pneumonitis syndrome 98 days after HCT.

Slatter and colleagues published one of the largest retrospective series on HCT outcomes following Treosulfan-based conditioning in 316 pediatric patients with non-malignant diseases.[10] Diagnoses included BMFD (n=24), primary immunodeficiency disorders (n=144), hemoglobinopathies (n=70), metabolic disorders (n=39), histiocytic disorders (n=32), autoimmune disease (n=2), and not specified (n=5). The conditioning consisted of Treosulfan combined with fludarabine (n=106), cyclophosphamide (n=98), fludarabine + thiotepa (n=104), or fludarabine + melphalan (n=8). GVHD prophylaxis consisted primarily of cyclosporine combined with MMF (n=110) or MTX (n=101). Patients received grafts from BM (n=167), BM plus UCB (n=8), BM plus PBSC (n=3), UCB (n=50), PBSC (n=87) or PBSC plus UCB (n=1). The incidence of graft failure was low at 5.1% (n=16). The OS and EFS were 83% and 76%, respectively. The 3-year OS and EFS for patients with BMFD were 83% and 83%, respectively. Multivariate analysis showed no association of TRM with age at HCT, dose of Treosulfan given, other agents used in combination with Treosulfan, type of donor, stem cell source, or second or subsequent transplant. Only 5% of patients developed grade 1 or 2 SOS and none developed grade 3 or 4 SOS. Similar to prior studies, Treosulfan-based conditioning was associated with excellent OS, EFS and low TRM in a wide variety of non-malignant diseases including patients with BMFD.

1.3.4 Phase II Study of Treosulfan and Fludarabine for Non-Malignant Diseases in the United States- Protocol 2256[50]

The Fred Hutchinson Cancer Center has been conducting a prospective clinical trial using Treosulfan-based conditioning in patients with non-malignant diseases since 2009. Results of the first 31 patients have been published. Patients received HLA-MSD (n=4) or URD (n=27) grafts following conditioning with Treosulfan (total dose: 42 g/m2), fludarabine (total dose: 150 mg/m2), ± thymoglobulin (rabbit ATG, 6 mg/kg; n=22). GVHD prophylaxis consisted of tacrolimus and

MTX. All patients engrafted. Day 100 TRM was 0%. With a median follow-up of2 years, the 2-year OS was 90%. The cumulative incidence of grades II-IV and III-IV acute GVHDat day 100 and chronic extensive GVHD at 1-year were 62%, 10% and 39%, respectively. Patientswho received thymoglobulin had a significantly lower incidence of grade III-IV acute GVHD (0%versus 33%; P = 0.005). These results demonstrated that the combination of Treosulfan, fludarabine, and thymoglobulin was associated with low toxicity, low grade III-IV acute GVHD, and improved OS in patients with various non-malignant diseases and supported the need for continued study in order to gain more robust data on a larger number of patients with rare non- malignant diseases.

To date, 80 patients with various non-malignant diseases have undergone HCT following conditioning with Treosulfan, fludarabine, ± rabbit ATG (Thymoglobulin; n=71) and HLA-MSD (n=13) or URD (n=67) grafts. Six patients received a single HLA-class 1 (n=4) or DQB1 antigen/allele (n=2) mismatched URD graft. BM was the primary stem cell source (n=66). GVHD prophylaxis consisted of MTX and tacrolimus. At a median follow-up of 4 years, 73 of 80 patients survive for a 4-year OS of 91% and day 200 TRM of 3%. Of the 80 patients with non-malignant diseases, 23 had BMFD including DBA (n=9), SDS (n=4), PNH (n=5), GATA 2 (n=2), and undefined BMFD (n=3). Median age of this cohort was 15 (range, 1.2-22) years. With a median follow-up of 2 years, the 2-year OS and EFS were 96% (**Figure 1**). The cumulative incidences of grades II-IV and III-IV acute GVHD at day 100 and NIH chronic GVHD at 1 year were 39%, 4% and 9%, respectively. The 1-year GVHD-free, EFS (an event was defined as death from any cause, disease recurrence, rejection/graft failure, or 2nd HCT) was 87% (**Figure 1**). None of the patients experienced graft rejection, with 22 patients having full (≥95%) and one patient having stable mixed donor CD33 myeloid engraftment. In terms of T-cell engraftment, 22 patients had full (>95%) or high-level mixed (50%-94%) donor T-cell engraftment and one patient had stable low-level (6%-49%) donor T-cell engraftment. These results indicate that the combination of Treosulfan, fludarabine, and rabbit ATG is effective at establishing donor engraftment with low toxicity, low grade III-IV acute GVHD, and improved survival in patients with BMFD. Preliminary data from this trial provides strong support for a multi-center prospective clinical trial.

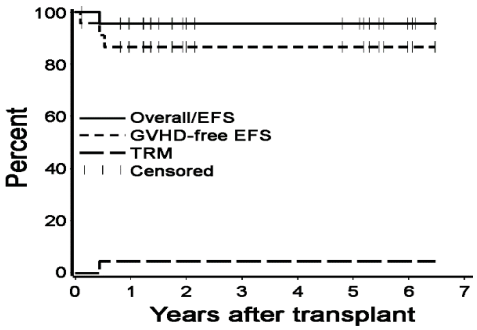


Figure 1. OS, EFS and GVHD-free EFS in 23 patients with BMFD who underwent HCT using Treosulfan-based conditioning.

1.3.5 Treosulfan

Treosulfan (Ovastat®, L-treitol-1,4-bis-methanesulfonate, dihydroxybusulfan, TREO; Medac GmbH, Hamburg, Germany) is a water-soluble intravenous busulfan analog approved for therapy of advanced ovarian carcinoma in Europe. Under physiologic conditions, Treosulfan is spontaneously activated into epoxide species that cause cross-linking of DNA molecules and cytotoxicity in rapidly proliferating cells, both malignant and non-malignant, such as normal hematopoietic cells.[51] Contrary to busulfan, Treosulfan does not require enzymatic activation, thus bypassing hepatic metabolism. The hydroxyl groups in positions 2 and 3 of the molecule account for differences in pharmacological activity between Treosulfan and busulfan (**Figure 2**). Busulfan directly alkylates nucleophilic centers, while Treosulfan induces alkylation of nucleophilic centers by intramolecular epoxide formation. Clinical pharmacokinetic studies havedemonstrated that Treosulfan has a similar half-life to busulfan (1.8 to 2 hours) and a higher, dose-independent, cumulative renal excretion (50% vs. 20%).[52,53] Pharmacokinetic studies of both single and multiple intravenous infusions of Treosulfan have demonstrated low inter-patient and inter-day variability. There is a high linear correlation between the area under the curve and Treosulfan dose (r2=0.9227), which compares favorably with that of oral and intravenous busulfan.[54,55] On preclinical studies in mice, rats, dogs and primates, Treosulfan resulted in at least 10 times lower acute and chronic toxicity than busulfan.[56]

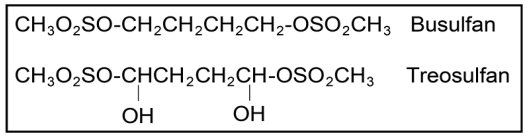


Figure 2: Molecular structures of busulfanand Treosulfan

The pronounced effect of Treosulfan on primitive and committed hematopoietic stem cells and its immunosuppressive effects have been demonstrated in allogeneic murine transplant models.[51] At doses 80-88% lower than those used for humans in clinical trials, Treosulfan was at least as effective as busulfan in depleting hematopoietic cell subsets (**Figure 3**). Treosulfan was capable of inducing full donor engraftment and immune tolerance across major histocompatibility complex (MHC) barriers. Marrow suppression is the limiting toxicity for conventional chemotherapy with Treosulfan at doses over 10 g/m^2. With autologous stem cell rescue, the Treosulfan dose could be escalated up to 47 g/m^2 before dose-limiting toxicity including mucositis, diarrhea, dermatitis or metabolic acidosis was observed.[57] Some initial phase I/II studies of Treosulfan-containing regimens for allogeneic HCT conducted in Europe and the United States are summarized above. Overall, hundreds of patients with malignant and non-malignant diseases have been conditioned with Treosulfan-containing regimens in preparation for allogeneic HCT.

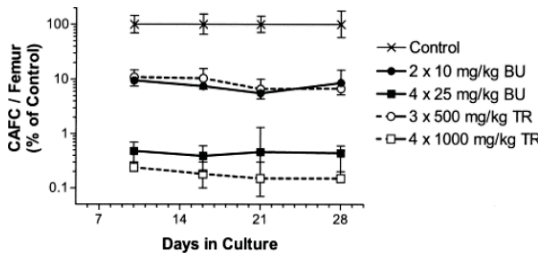


Figure 3: Survival of different cobblestone area-forming cells (CAFC) subsets remaining in the femoral bone marrow after treatment with different doses of busulfan and Treosulfan. Error bars=95% CI.

1.3.5.1 Treosulfan Dose Based on Pharmacokinetic Data

Treosulfan in combination with fludarabine is currently approved by the European Medical Agency as a conditioning treatment prior to allogeneic HCT in adult patients with malignant and non-malignant diseases, and in pediatric patients older than one month with malignant diseases. The pharmacokinetics of Treosulfan have been well described in adults, with a maximum tolerated total dose of 42 g/m^2 administered in three doses of 14 g/m^2 IV daily. Pediatric pharmacokinetic data are scarce and more variable, with previous studies in a limited number of patients showing more inter-patient variability in children compared to adults. A recently completed study of Treosulfan-based conditioning for children with hematologic malignancies and an ongoing study for children with non-malignant diseases conducted in Europe collected pharmacokinetics data in this patient population. Data generated from these studies confirm the need for body surface area (BSA)-based dosing for children under the following schema: a) 10 gram/m^2/day for BSA < 0.4 m^2, b) 12 gram/m^2/day for BSA $\geq$ 0.4 to <0.9 m^2 and c) 14 gram/m^2/day for BSA $\geq$ 0.9 m^2. This is the recommended dosing schema by Medac GmbH for pediatric patients as outlined in Medac GmbH internal report dated 02-April-2020: Population Pharmacokinetic Modeling of Treosulfan in Pediatric Patients – Final Analysis Report as well as in the updated Investigator's Brochure (IB) version 13.

1.4 Exploratory Endpoints

1.4.1 Treosulfan Pharmacokinetic Studies

As stated above, studies have demonstrated highly predictable pharmacokinetics (PK) in adult patients; however, limited data are available for pediatric patients. In addition, very little data are available on the relationship between Treosulfan exposure and HCT outcomes including early and late toxicities and engraftment. Van der Stoep recently published on Treosulfan pharmacokinetics and the drug's relationship with regimen-related toxicity and early clinical outcome in 77 pediatric patients with hematological malignancies (n=12) or non-malignant diseases (n=65) who underwent HCT.[58] Conditioning consisted of Treosulfan, fludarabine, +/- thiotepa (n=52) followed by MSD n=27, matched URD ($\geq$9/10; n=36), or haploidentical (n=14) grafts. Hematopoietic cell sources varied and included BM (n=50), PBSC (n=20), UCB (n=6), and BM plus UCB (n=1). Van der Stoep and colleagues reported low intra-patient variability (13.9%) but high inter-patient variability [56%; <1-year old, (n=12) and 33%; ≥1 to 21 years of age (n=65)] among 77 pediatric patients. This is in contrast to that seen in adult patients where there is low intra- and low inter-patient variability. In addition, high Treosulfan exposure (>1650 mg*h/L) was associated with an increased risk of developing grade 2 or higher mucositis [OR 4.40; 95% CI 1.19–16.28, p=0.026], increased risk of skin toxicity, including erythematous rash and skin exfoliation, [OR 4.51; 95% CI 1.07-18.93; p=0.040], and increased risk of developing multiple toxicities [OR 4.52; 95%CI 1.32-15.53, p=0.016] compared to the lower exposure (<1350 mg*h/L) group. No correlation was observed with other early clinical outcome parameters including engraftment, acute GVHD and donor chimerism. The authors highlighted that their study included a heterogeneous group of diseases and additional data in more homogeneous and single disease groups with longer follow-up are needed in order to better understand the relationship between AUC and long-term outcomes. We plan on collecting Treosulfan PK samples from the peripheral blood on patients enrolled on this clinical trial. The goal of this research will be to try and gain a better understandingof the relationship between the exposure to Treosulfan AUC to engraftment, transplant related mortality, overall and event-free survival for patients with BMFD. This information will help guidefuture studies using Treosulfan-based conditioning in patients with non-malignant diseases. This is an optional research study and Treosulfan PK data will not be used to adjust the patient's Treosulfan dose.

1.4.2 Biological Studies

Recent attention has focused on genomic analysis of germline and somatic mutations for risk stratification to inform treatments tailored to the individual patient. A subset of patients with BMFD are at high risk for regimen-related toxicities. In addition, a subset of patients with BMFD develop malignancies, predominantly solid tumors, after HCT. Identification of patients at risk for these complications would allow appropriate adjustment of HCT regimens and institution of surveillance strategies for early tumor detection. Such genomic studies also stand to advance our understanding of the molecular pathogenesis of bone marrow failure in order to develop novel treatments. Recent studies have also elucidated potential roles for T -cell phenotypes, cytokine levels and somatic mutations in disease pathogenesis and prognostic stratification. In collaboration with Dr. Akiko Shimamura at Dana Farber and Boston Children's Cancer and Blood Disorders Center, this study will collect blood and marrow samples (cells, DNA, serum) and establish fibroblast cell cultures as a source of germline DNA to investigate: 1) somatic mutations, 2) T cell phenotypes, 3) cytokine levels. These biological studies will be correlated with clinical outcomes for study subjects. Dr. Shimamura has a biorepository of over 600 samples from patients with BMFD which includes patients who have not required bone marrow transplant. These study subjects will provide important controls for these studies.

1.4.3 Mutational Testing

Over the past 5-10 years, there have been significant strides made in identifying new genetic mutations in patients with inherited BMFD. However, there remain a fair number of patients who are diagnosed with an underlying BMFD but lack a known genetic mutation responsible for their bone marrow failure phenotype. One common reason is that commercial genetic panels are very different and do not analyze for all the known genetic mutations for these diseases. As has been mentioned above, a definite diagnosis is important for choosing the appropriate HCT regimen and for future management and surveillance of these patients. Dr. Adrianna Vlachos of Cohen Children's Medical Center, in collaboration with Dr. David Bodine at the National Human Genome Research Institute, will collect peripheral blood on patients who lack a known genetic mutation responsible for their bone marrow failure phenotype with the goal of identifying new mutations for patients with BMFD.

1.4.4 Quality of Life and Late Effects

Quality of life (QOL) refers to every dimension of life except for its length and includes physical abilities, symptoms, social well-being, psycho-emotional status, and spiritual/existential qualities. It reflects how well people feel, what they can accomplish, how satisfied they are with their lives, and whether their lives have meaning and purpose. HCT survivors generally report high global QOL following HCT, but many specific symptoms[59] and limitations on their daily activities[60].

The purpose of the QOL component of this trial is to explore the long-term QOL implications of HCT using Treosulfan-based conditioning on patients with BMFD. While GVHD-free, EFS is the primary endpoint of this study, QoL will be an important exploratory endpoint as well. It is also possible that immunologic recovery, peri-transplant experiences and complications, speed of physical recovery, and expectations may influence ultimate QOL.

It is very important that data collection is centralized, patients' response burden is minimized and QOL assessments are fully integrated into the trial to maximize the chance of complete data collection. With this goal in mind, the number of survey items will be minimized and focused on answering the research questions.

CHAPTER 2

2 STUDY DESIGN

2.1 Study Overview

This is a prospective, multicenter phase II study designed to evaluate the outcomes of patients with BMFD undergoing HLA-matched related, matched unrelated, or single HLA-class 1 allele or HLA-DQB1 antigen or allele mismatched unrelated HCT using Treosulfan-based conditioning.

2.2 Hypotheses and Objectives

2.2.1 Hypothesis

Treosulfan, fludarabine, and rabbit ATG conditioning followed by allogeneic HCT will result in excellent 1-year GVHD-free, EFS in patients with BMFD.

2.2.2 Objectives

Primary Objective:

The primary objective of this phase II study is to assess the 1-year GVHD-free, EFS in patients with BMFD undergoing HCT using Treosulfan-based conditioning. An event will be defined as death due to any cause, graft rejection/failure, or 2nd HCT whichever occurs first. GVHD will be defined as grade III-IV acute GVHD or chronic GVHD (using NIH consensus criteria) requiring systemic immune suppression.

Secondary Objectives:

Secondary objectives will be to determine:

1. Overall survival at day 100, 6 months, and 1-year post-HCT.
2. EFS at 1-year post-HCT
    a. An event will be defined as death due to any cause, graft rejection/failure, or 2nd HCT.
3. Neutrophil recovery at day 42 and platelet recovery at day 100 post-HCT.
4. Donor chimerism (CD3 and myeloid) at day 28, 100, and 1-year post-HCT.
5. Primary graft failure/rejection at day 42 and secondary graft failure/rejection post-HCT.
6. Incidence of grade II-IV and grade III-IV GVHD at day 100 and chronic GVHD requiring systemic immune suppression at 1-year post-HCT.
7. Acute GVHD will be defined as grade II-IV and grade III-IV. [61]
8. Chronic GVHD will be defined as chronic GVHD (using NIH consensus criteria) requiring systemic immune suppression.[62]
9. Incidence of grade 3-5 toxicities at day 30 and day 100 post-HCT.
10. Incidence of grade 2-3 systemic infections by day 180 post-HCT.
11. Incidence of EBV-reactivation requiring therapy and the incidence of EBV-associated lymphoproliferative disorder by day 180 post-HCT.
12. Incidence of CMV reactivation requiring therapy by day 180 post-HCT.

Exploratory Objectives:

1. Treosulfan Pharmacokinetic Studies: Treosulfan PK characteristics to determine the optimal dose of Treosulfan and evaluate whether the exposure to Treosulfan determined byarea under the curve (AUC) for patients with BMFD impacts engraftment, transplant-related mortality, and overall and event-free survival.
2. Biological Studies: Collect blood and marrow samples (cells, DNA, serum) and establish fibroblast cell cultures as a source of germline DNA to investigate: 1) somatic mutations, 2) T cell phenotypes, and 3) cytokine levels.
3. Mutational Testing: Collect peripheral blood on patients who lack a known genetic mutation responsible for their bone marrow failure phenotype in order to perform whole exome or whole genome sequencing with the goal of identifying new mutations for patients with BMFD.
4. Health-related quality of life using PROMIS at baseline, day +180 and day +365 post-HCT.

2.3 Patient Eligibility Criteria for Enrollment

2.3.1 Patient Inclusion Criteria

1. Patient must be ≥ 1.0 year of age and less than 50.0 years of age at the time of enrollment (i.e., patient must have celebrated their 1st birthday when enrolled and must NOT have celebrated their 50th birthday when enrolled; 49.99 years).
2. Underlying BMFD treatable by allogenic HCT.

a. Shwachman-Diamond syndrome

i. Criteria for Diagnosis:

1. A pathogenic mutation(s) for Shwachman-Diamond syndrome
2. For those patients tested but lacking a genetic mutation they must meet both a and b below:
   a. Exocrine pancreatic dysfunction as defined by at least one of the following:
      i. Pancreatic isoamylase below normal (age ≥3 years old), OR
      ii. Fecal elastase <200, AND
   b. Bone marrow failure as evidence by at least one of the following:
      i. Intermittent or persistent neutropenia (absolute neutrophil count < 1,500/μL), OR
      ii. Hypo-productive anemia with a hemoglobin concentration below the age-related adjusted norms, OR
      iii. Unexplained macrocytosis, OR
      iv. Platelet count <150,000/μL without alternative etiology, OR
      v. Hypocellular bone marrow

ii. Indications for HCT:

1. Severe neutropenia (ANC <500/μL), OR

2. Severe anemia (hemoglobin <8 g/dL) or transfusion-dependent anemia, OR
3. Severe thrombocytopenia (platelet count <20,000/μL) or transfusion-dependent thrombocytopenia, OR
4. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 eligibility review committee (ERC). In addition, patients with severe or recurrent infections will be reviewed by the ERC if they do not meet the indications for transplant listed above.

b. Diamond Blackfan anemia

i. Criteria for Diagnosis:

1. A pathogenic mutation for Diamond Blackfan anemia
2. For those patients tested but lacking a genetic mutation the patient must meet criteria a and at least one of the criteria listed in b-f:
    a. History of deficiency of erythroid precursors in an otherwise cellular bone marrow AND,
    b. Reticulocytopenia, OR
    c. Elevated adenosine deaminase activity, OR
    d. Elevated hemoglobin F, OR
    e. Macrocytosis, OR
    f. Congenital anomalies

ii. Indications for HCT:

1. RBC transfusion dependent anemia despite an adequate trial of steroids; OR
2. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 ERC.

c. Congenital Sideroblastic anemia

i. Criteria for Diagnosis:

1. A pathogenic mutation(s) for sideroblastic anemia
2. For those patients tested but lacking a genetic mutation:
    a. Presence of ringed sideroblasts in the bone marrow excluding acquired causes of ringed sideroblasts such as lead poisoning & zinc toxicity.

ii. Indications for HCT:

1. Severe anemia (hemoglobin <8 g/dL) or transfusion-dependent anemia OR
2. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 ERC.

d. *GATA2* mutation with associated marrow failure

i. Criteria for Diagnosis:

1. A pathogenic mutation(s) for *GATA2*

ii. Indications for HCT:

1. Severe neutropenia (ANC <500/μL), OR
2. Severe anemia (hemoglobin <8 g/dL) or transfusion-dependent anemia, OR
3. Severe thrombocytopenia (platelet count <20,000/μL) or transfusion-dependent thrombocytopenia, OR
4. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 ERC. In addition, patients with severe or recurrent infections will be reviewed by the ERC if they do not meet indications for transplant listed above.

e. SAMD9 or SAMD9L disorders

i. Criteria for Diagnosis:

1. A pathogenic mutation(s) for *SAMD9* or *SAMD9L*

ii. Indications for HCT:

1. Severe neutropenia (ANC <500/μL), OR
2. Severe anemia (hemoglobin <8 g/dL) or transfusion-dependent anemia, OR
3. Severe thrombocytopenia (platelet count <20,000/μL) or transfusion-dependent thrombocytopenia, OR
4. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 ERC.

f. Congenital amegakaryocytic thrombocytopenia

i. Criteria for Diagnosis:

1. A pathogenic mutation(s) for congenital amegakaryocytic thrombocytopenia.
2. For those patients tested but lacking a genetic mutation the patient must meet criteria a and b below:
   a. Thrombocytopenia early in life, AND
   b. History of bone marrow demonstrating megakaryocyte hypoplasia.

ii. Indications for HCT:

1. Severe thrombocytopenia (platelet count <20,000/μL) or transfusion-dependent thrombocytopenia, OR
2. Severe neutropenia defined as an ANC <500/μL, OR
3. Severe anemia (hemoglobin <8 g/dL) or transfusion-dependent anemia, OR
4. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 ERC.

g. Paroxysmal nocturnal hemoglobinuria

i. Criteria for Diagnosis:

1. PNH clone size in granulocytes ≥ 10%, AND
2. Complement mediated intravascular hemolysis with an elevated LDH (above institutional upper limits of normal)

ii. Indications for HCT:

1. PNH with thrombosis despite adequate medical management, OR
2. PNH with intravascular hemolysis requiring transfusion support despite adequate medical management, OR
3. Additional clinical or laboratory data may be considered for protocol eligibility following review by protocol 1904 ERC. In addition, patientswith PNH and cytopenias may be considered for the protocol eligibilityfollowing review by protocol 1904 ERC.

h. An undefined BMFD: a patient with a BMFD for whom a genetic mutation responsible for their bone marrow failure phenotype has not been identified (excluding PNH) will be eligible for this clinical trial following approval by BMT CTN 1904 ERC.

i. A BMFD with a known genetic mutation but not listed above will be eligible for this clinical trial following approval by BMT CTN 1904 ERC.

3. Patient and/or legal guardian must sign informed consent prior to initiation of conditioning for BMT CTN 1904.

Note: The following patients MUST be reviewed by the BMT CTN 1904 ERC in order to determine if they are eligible for this trial:

1. All patients with Shwachman-Diamond syndrome, Diamond Blackfan anemia, congenital sideroblastic anemia, or congenital amegakaryocytic thrombocytopenia who have had genetic testing and a genetic mutation responsible for their disease was not identified.
2. All patients with an undefined BMFD: a patient with a BMFD for whom a genetic mutation responsible for their bone marrow failure phenotype has not been identified, excluding PNH.
3. All patients with a BMFD and a known genetic mutation that is not listed above
4. All patients with *GATA2* mutation and associated marrow failure
5. All patients with SAMD9 or SAMD9L disorders
6. There may be circumstances where a treating physician will consider a transplant for a patient with a BMFD who does not meet all the criteria listed under “indications for HCT”. In these situations, treating physicians may submit their patient to the BMT CTN1904 ERC for review in order to determine if the patient is eligible for this clinical trial based on additional clinical or laboratory information.
7. Many patients with BMFD can have bone marrow evaluations that raise concern for possible myelodysplastic syndrome (MDS) including but not limited to dysplastic bone marrow evaluations or cytogenetic abnormalities. However, in patients with BMFD these findings are not necessarily diagnostic or consistent with MDS. Therefore, given the complexities of diagnosing MDS in patients with BMFD, all patients with bone marrow evaluations concerning for possible MDS should be submitted to the ERC for review to confirm or exclude MDS. This is particularly important as we do not want to exclude potentially eligible patients due to an incorrect diagnosis of MDS.

2.3.2 Patient Exclusion Criteria

1. Patients with idiopathic aplastic anemia, Fanconi anemia, dyskeratosis congenita, and congenital neutropenia

2. Patients with MDS as defined by the WHO or leukemia
3. Prior allogeneic HCT
4. Patient's weight ≤10.0 kg (actual body weight and adjusted body weight) at time of studyenrollment Lansky (patients <16 years of age) or Karnofsky (patients ≥16 years of age) performance
<70%
5. Organ Dysfunction defined as follows:
    a. Cardiac:
        i. Left ventricular ejection fraction <50% by echocardiogram or multi-gated acquisition (MUGA) scan.
        ii. For patients unable to obtain a left ventricular ejection fraction, left ventricular shortening fraction of <26%.
    b. Pulmonary:
        i. DLCO (corrected/adjusted for hemoglobin) <50%, FEV1 < 50%predicted, and FVC <50% predicted.
        ii. For patients unable to perform PFTs due to age or developmental delay: Oxygen (O2) saturation <92% on room air.
        iii. On supplemental oxygen
    c. Renal:
        i. Estimated creatinine clearance <60 mL/minute/1.73m^2 (estimated per institutional practice).
        ii. Dialysis dependent
    d. Hepatic:
        i. Conjugated bilirubin > 2x upper limit of normal for age (ULN, unless attributable to Gilbert's syndrome), or
        ii. AST or ALT > 4x ULN for age, or
        iii. Fulminant liver failure or cirrhosis
6. Iron overload - This exclusion criterion only applies to patients who are considered at risk for hepatic or cardiac iron overload. Therefore, not all patients enrolled on this protocol will undergo formal hepatic or cardiac iron assessment.
    a. For patients ≥ 18 years with a history of significant transfusions defined as ≥8 packed red blood cell transfusions per year for ≥1 year or have received ≥20 packed red blood cell transfusions (lifetime cumulative) will require formal hepatic and cardiac iron measurement. In addition, patients with a prior history ofhepatic or cardiac iron overload will also require formal assessment for iron overload. Patients are excluded if:
        i. Hepatic iron content ≥ 8 mg Fe/g dry weight by liver MRI using a validated methodology (such as T2* MRI or ferriscan) or liver biopsy perinstitutional practice.
        ii. Cardiac iron content < 25 msec by cardiac T2* MRI.
    b. For patients < 18 years old with a history of significant transfusions defined as ≥8 packed red blood cell transfusions per year for ≥1 year or have received ≥20 packed red blood cell transfusions (lifetime cumulative) will require formal hepatic iron measurement. In addition, patients with a prior history of liver iron overload will also require formal assessment for iron overload. Patients are excluded if:

i. Hepatic iron content ≥ 8 mg Fe/g dry weight by liver MRI using a validated methodology (such as T2* MRI or ferriscan) or liver biopsy perinstitutional practice.

7. Uncontrolled bacterial infection within 1 week of study enrollment. Uncontrolled is defined as currently taking medication with no clinical improvement or progression on adequate medical treatment.
8. Uncontrolled viral or fungal infection within 30 days of study enrollment. Uncontrolled is defined as currently taking medication with no clinical improvement or progression on adequate medical treatment.
9. Positive for HIV (human immunodeficiency virus).
10. Presence of clinically significant anti-donor HLA-antibodies per institutional practice.
11. Prior solid organ transplant.
12. Patients with prior malignancies except resected non-melanoma skin cancer or treated cervical carcinoma in situ.
13. Females who are pregnant or breast-feeding.
14. Females and males of childbearing potential who are unwilling to practice an effective method of contraception or agree to abstinence from the time of signing informed consent through 12 months post-transplant or off tacrolimus whichever is later.
15. Known hypersensitivity to Treosulfan or fludarabine.
16. Known life-threatening reaction (i.e., anaphylaxis) to Thymoglobulin that would prohibit use for the patient as this study requires use of the Thymoglobulin preparation of ATG.

2.4 Donor Selection Criteria

2.4.1 Donor Inclusion

1. HLA-matched related donor
    a. HLA-matched sibling: Must be a minimum HLA-6/6 matched to the recipient at HLA-A, -B (serologic typing) and DRB1 (high-resolution typing).
    b. HLA-matched related (phenotypic match): Fully matched for HLA-A, -B, -C, -DRB1, and DQB1 by high-resolution typing.
    c. **If a genetic mutation is known for the patient**, the HLA-matched related donor [either HLA-matched sibling or HLA-matched related (phenotypic match)] must be screened for the same genetic mutation if clinically appropriate and should be confirmed to not have the same genetic disease (this does not include patients with PNH). Consult the protocol team with questions.
    d. **If a patient has an undefined BMFD** (a patient with a BMFD for whom a genetic mutation responsible for their bone marrow failure phenotype has not been identified), the HLA-matched related donor [either HLA-matched sibling or HLA-matched related (phenotypic match)] must have an evaluation as directed by the treating physician to confirm that the donor does not have the same underlying disease. This will include a complete blood count (CBC) with differential and potentially a bone marrow evaluation or other studies as directed by the treating physician.

2. Unrelated donor:
    a. Fully matched for HLA-A, -B, -C, -DRB1, and DQB1 by high-resolution typing, OR
    b. Mismatched for a single HLA-class 1 allele (HLA-A, -B, or -C) by high-resolution typing; OR
    c. Mismatched for a single HLA DQB1 allele or antigen by high-resolution typing.

    Note: DP matching per intuitional practice.

Donor selection recommendations: in the case where there are multiple donor options, donors should be selected based on the following priority numbered below:

1. Unaffected fully HLA-matched sibling
2. Unaffected fully phenotypically HLA-matched related donor
3. Fully HLA-matched unrelated donor
4. Unrelated donor with single allele or antigen level mismatch at DQB1
5. Unrelated donor with single allele level mismatch at class 1 (HLA-A, -B, or -C)

2.5 Treatment Plan

2.5.1 Conditioning Schema

Table 1: Conditioning Schema for Patients with an HLA-matched related or unrelated donor. Unrelated donors can have a single allele mismatch at HLA-class 1 or an antigen or allele mismatch at DQB1.		
Day	**Treatment**	**Dose**
-6	Treosulfan Fludarabine	10-14 grams/m^2/day IV[1,2] 30 mg/m^2/day IV[1]
-5	Treosulfan Fludarabine	10-14 grams/m^2/day IV[1,2] 30 mg/m^2/day IV[1]
-4	Treosulfan Fludarabine Rabbit Anti-thymocyte globulin (ATG; Thymoglobulin®)	10-14 grams/m^2/day IV[1,2] 30 mg/m^2/day IV[1] 1.0 mg/kg/day IV[3]
-3	Fludarabine Rabbit Anti-thymocyte globulin (ATG; Thymoglobulin®)	30 mg/m^2/day IV[1] 2.5 mg/kg/day IV[3]
Table 1: Conditioning Schema for Patients with an HLA-matched related or unrelated donor. Unrelated donors can have a single allele mismatch at HLA-class 1 or an antigen or allele mismatch at DQB1.		
-2	Fludarabine Rabbit Anti-thymocyte globulin (ATG; Thymoglobulin®)	30 mg/m^2/day IV[1] 2.5 mg/kg/day IV[3]
-1	Rest	
0[4]	HLA-matched related, matched unrelated, or single HLA-class 1 allele or HLA-DQB1 antigen or allele mismatched unrelated marrow or PBSC infusion	

[1] Actual weight is to be used to calculate BSA for Treosulfan and fludarabine. However, for patients > 120% of ideal body weight, BSA will be calculated using adjusted body weight **(Appendix D**)

[2]Treosulfan will be dosed according to BSA as follows:

BSA	Treosulfan Dose
< 0.4	10 grams/m^2/day
≥ 0.4 – < 0.9	12 grams/m^2/day
≥ 0.9	14 grams/m^2/day

[3]Actual body weight is to be used for calculation of the rabbit ATG (Thymoglobulin) dose

[4]Additional rest days may be added given delays that may occur with stem cell collection, transportation, processing. The actual day the stem cell infusion finishes will remain "Day 0".

2.5.1.1 Conditioning for HLA-Matched Related, Matched Unrelated, or Single HLA-Class 1 Allele or HLA-DQB1 Antigen or Allele Mismatched Unrelated Donors

2.5.1.1.1 Treosulfan

Treosulfan will be administered intravenously over 120 minutes for three consecutive days (day -6 to -4). Actual body weight is to be used to calculate BSA for Treosulfan (**Appendix D**). However, for patients >120% of ideal weight, use adjusted weight to calculate BSA. See **Table 2** for dosing guidelines. Pharmacokinetic evaluation will be performed on days -6 and -5 (**see Appendix E**). On days -6 to -4, Treosulfan should be administered prior to fludarabine.

Table 2: Treosulfan dose based on BSA

BSA	Dose
< 0.4	10 grams/m^2/day
≥ 0.4 – < 0.9	12 grams/m^2/day
≥ 0.9	14 grams/m^2/day

Note: The BSA should NOT be rounded to the "tenths" position. For example, if the patient's BSA is 0.88 the BSA should not be rounded up to 0.9, it should remain 0.88. Therefore, if a patient had a BSA of 0.88, the Treosulfan dose based on BSA would be 12grams/m^2/day. However, if a patient's BSA is 0.848 this may be rounded to 0.85 per institutional practice.

Drug-Drug Interactions (DDI): DDI analysis predicted a weak (AUC ratio ≥ 1.25 and < 2) to moderate (AUC ratio ≥ 2 and< 5) interaction for CYP3A4, a weak interaction for CYP2C19, and a negligible (AUC ratio < 1.25) interaction for P-gp. Given the short duration of treatment with Treosulfan and its short t1/2 (2 hours), no dose adjustment of Treosulfan is recommended when inhibitors of CYP3A4, CYP2C19, or P-gp are co-administered. While the reduced dose of Treosulfan (10 g/m²/d × 3) also reduces the potential for DDI, the PBPK-based analysis still predicts a weak inhibitor potential for Treosulfan with CYP3A4 and CYP2C19. Considering overall timing of treatments and the respective PK properties of the concomitantly used drugs (e.g., t1/2), the interaction potential can be reduced to "no interaction" (AUC ratio < 1.25), if all concomitantly used CYP3A4 and CYP2C19 substrate drugs are dosed 2 hours before or 8 hours after the 2-hour I.V. infusion of Treosulfan.

Note: The protocol recommends using methylprednisolone as a pre-medication prior to rabbit ATG and potentially again 4 and 8 hours after rabbit ATG. Although Methylprednisolone is a

major CYP3A4 substrate, this medication may be administered after Treosulfan even within the 8-hour time referenced above.

Voriconazole, posaconazole, itraconazole, and isavuconazole should be discontinued 72 hours prior to the start of conditioning and should not be administered during conditioning due to risk for drug-drug interaction/toxicities. Voriconazole, posaconazole, itraconazole, and isavuconazole may be administered if needed following conditioning.

2.5.1.1.2 Fludarabine

Fludarabine will be administered intravenously at a dose of 30 mg/m^2/day over 60 minutes for five consecutive days (day –6 to –2; total dose received 150 mg/m^2). Actual body weight is to be used to calculate BSA for fludarabine (**Appendix D**). However, for patients > 120% of ideal weight, BSA will be calculated using adjusted weight. On days –6 to –4, fludarabine should be administered after Treosulfan is given. Fludarabine (Fludara®, Berlex Laboratories, Inc., Richmond, CA) is commercially available in the U.S. Details of the product's description, preparation; storage and stability are found in the drug's package insert.

2.5.1.1.3 Thymoglobulin® (Rabbit Anti-Thymocyte Globulin; RATG)

Thymoglobulin® will be administered intravenously over three consecutive days (days -4, -3, -2) at a dose of 1.0 mg/kg on day -4, and then 2.5 mg/kg on days -3 and -2 for a total dose of 6.0 mg/kg. Thymoglobulin® will be given intravenously over a minimum of 6 hours for the first two doses and may be infused over 4 hours for the subsequent dose if there are no clinical concerns per institutional practice. Thymoglobulin (rabbit ATG) doses should be based on actual body weight. On days, -4 to -2, Thymoglobulin® should be administered after fludarabine.

Thymoglobulin premedications and subsequent dosing should follow local institutional practice with suggested recommendation for pre-medication with methylprednisolone, diphenhydramine, and acetaminophen (**Table 3a**). Skin tests are not required prior to Thymoglobulin® administration. We also recommend that medications for significant allergic reactions be available at the bedside in case of allergic reaction per standard of care, institutional practice (**Table 3b**).

Table 3a: Recommended Thymoglobulin Pre-Medications and Subsequent Dosing				
	Pre-Medications 30-60 minutes priorto the start of the thymoglobulin infusion on days -4, -3, and -2.		**Subsequent dosing 4 hours after the start of the thymoglobulin infusion. The same doses may be repeated at 8hours after the start of the thymoglobulin infusion.**	
	Pediatric:	**Adults:**	**Pediatric:**	**Adults:**
Methylprednisolone	1 mg/kg/dose IV	1 mg/kg/dose IV	0.5 mg/kg/dose IV	0.5 mg/kg/doseIV
Table 3a: Recommended Thymoglobulin Pre-Medications and Subsequent Dosing				
Diphenhydramine	1 mg/kg/dose IV (max 50 mg)	50 mg IV	1 mg/kg/dose IV (max 50 mg)	50 mg IV
Acetaminophen	12.5 mg/kg/dose PO (max 650 mg)	650 mg PO	12.5 mg/kg/dose PO(max 650 mg)	650 mg PO

Table 3b: Medication Recommendations for Thymoglobulin® Significant Allergic Reactions		
	Pediatric:	**Adults ≥ 40 kg:**
Diphenhydramine	1 mg/kg IV (max 50 mg)	50 mg IV
Hydrocortisone	4 mg/kg IV (max 250 mg)	250 mg IV

Note: Thymoglobulin® is the required preparation of ATG for this study. Patients will not be eligible if the treating center plans to use other preparations of ATG.

2.5.2 Infusion of Marrow or PBSC Product Handling & Infusion

2.5.2.1 Bone Marrow

Bone marrow will be the preferred stem cell source for related and unrelated donors; however, PBSC will be allowed as clinically indicated. Donors will undergo a bone marrow harvest according to institutional practice. Institutional procedures should be followed for requesting and receiving marrow units for infusion. Monitoring immediately prior to, during infusion of bone marrow cells, and thereafter will be according to institutional practice. Pre-medication prior to infusion of bone marrow will be according to institutional practice. The goal total nucleated cell count (TNC) for bone marrow grafts is $\geq 3.5 \times 10^8$ TNC/kg (actual recipient weight).

2.5.2.2 PBSC

PBSC collection and infusion will be according to institutional practice. The goal CD34 cell count for PBSC grafts is $\geq 5 \times 10^6$ CD34/kg (actual recipient weight). The recommended maximum CD34 count is 10×10^6 CD34/kg (actual recipient weight).

2.5.3 GVHD Prophylaxis

GVHD prophylaxis will consist of tacrolimus and methotrexate.

2.5.3.1 Tacrolimus

2.5.3.1.1 Tacrolimus Starting Dose/Administration

1. Tacrolimus will be administered intravenously (IV; oral dosing is not allowed for the initial tacrolimus dose) beginning on day -2 at the following recommended starting dose:
    a. For patients <18 years old:
        i. Continuous tacrolimus infusion initial dosing: 0.03 mg/kg/day by continuous IV infusion. Centers may transition from continuous tacrolimus infusion to intermittent tacrolimus dosing (i.e., every 12 or every 8 hours per day) per institutional practice.
        ii. Intermittent tacrolimus initial dosing: 0.015 mg/kg IV every 12 hours (for an initial total daily dose of 0.03 mg/kg/day) or 0.01 mg/kg IV every 8 hours (for an initial total daily dose of 0.03 mg/kg/day for patients <6 years old).
    b. For patients ≥18 years old:
        i. Continuous tacrolimus infusion dosing: 0.02-0.03 mg/kg/day by continuous IV infusion. Centers may transition from continuous tacrolimus infusion to intermittent tacrolimus dosing (i.e., every 12 hours or every 8 hours) per

institutional practice.

ii. Intermittent tacrolimus dosing: Tacrolimus should be administered at an initial dose of 0.015 mg/kg IV every 12 hours (for an initial total daily dose of 0.03 mg/kg/day).

2. Initial tacrolimus doses are calculated using actual body weight except for those patients who are greater than 100% ideal body weight in which case calculation of dose using adjusted body weight is recommended (**see Appendix D**).
3. The starting tacrolimus dose may be modified to account for possible drug interactions (e.g., concurrent CYP3A4 inhibitor use) according to institutional practice.
4. The same lumen of the central catheter should be used for all tacrolimus infusions.
5. Monitor closely for an acute allergic reaction for the first 30 minutes after starting tacrolimus IV infusion and at frequent intervals thereafter.

2.5.3.1.2 Serum Tacrolimus Levels

1. The following frequency is recommended for following Tacrolimus levels post-transplant:
   - Day +1 through day +28: Minimum twice weekly
   - Day +29 through day +100: Minimum weekly
   - Day +101 through day +180 or initiation of the tacrolimus taper whichever is later: minimum every other week then monthly per institutional practice
   - If there are concerns for renal dysfunction, the tacrolimus level should be followed more closely per institutional practice
2. The tacrolimus dose should be adjusted to maintain a suggested tacrolimus level of 5-15 ng/mL.
3. To avoid contamination, all serum tacrolimus levels should be drawn from the central catheter port opposite the lumen used to infuse tacrolimus or peripherally.
4. Dose adjustments are recommended if tacrolimus levels are outside the therapeutic range, or if there is evidence of toxicity that may be related to tacrolimus.
5. Although elevated tacrolimus blood levels are more frequently associated with toxicity (especially renal or hepatic), ***"therapeutic" tacrolimus blood levels may also be associated with toxicity*** (e.g., significant tremors). Therefore, dose decreases are recommended for significant organ toxicities that manifest despite a "therapeutic" tacrolimus level.
6. Patients with severe intolerance of tacrolimus may be placed on cyclosporine (trough level of 200-400 ng/mL).

2.5.3.1.3 Conversion from IV to Oral Dosing

1. The patient may be changed to oral dosing per institutional practice in order to maintain a suggested tacrolimus level of 5-15 ng/mL.
2. ***Children*** generally require a larger total daily dose of tacrolimus by weight than adults. For children < 6 years of age, every 8-hour administration may be required to maintain desirable serum trough levels. For those patients in whom adequate serum trough levels cannot be maintained using intermittent oral or IV dosing, continuous IV infusion may be warranted.

2.5.3.1.4 Tacrolimus Taper

1. In the absence of GVHD, we recommend tapering tacrolimus starting at day +180. The dose should be tapered per institutional practice with a suggested taper of 10% of the day +180 dose each week.
2. In the presence of GVHD, or if the patient is receiving corticosteroids or other therapy for GVHD, we recommend that tacrolimus should NOT be tapered at day +180. We recommend that tacrolimus dosing should be maintained to target therapeutic levels until GVHD has been successfully treated per institutional practice.
3. Tacrolimus may be continued beyond day +180 per the treating physician's discretion if there is mixed donor/host chimerism. Of note, it is common to see mixed T-cell chimerism early post-HCT due to ATG. Historically, the donor T-cell chimerism will rise over the first year.

2.5.3.1.5 Drug Interactions and Recommended Tacrolimus Dose-Modifications:

1. ***Review of concomitant medications for potential interactions that may significantly alter serum tacrolimus levels is essential*** because tacrolimus undergoes extensive metabolism by the hepatic and intestinal cytochrome P-450 system which may impact toxicity and efficacy of tacrolimus.
2. Subjects should ***avoid beverages containing the enzyme bergamottin*** (grapefruit juice, Sunny Delight, Fresca, and Squirt) when taking tacrolimus.
3. Tacrolimus is extensively metabolized by the cytochrome P-450 (CYP3A4) system and metabolized products are excreted in the urine.
    a. Drugs that may increase tacrolimus levels include tri-azole drugs (especially voriconazole, isavuconazonium sulfate, and posaconazole), calcium channel blockers, cimetidine and omeprazole, metoclopramide, macrolide antibiotics, quinupristin/dalfopristin, danazol, ethinyl estradiol, methylprednisolone, and HIV protease inhibitors.
    b. Drugs that may decrease tacrolimus levels include some anticonvulsants (phenobarbital, phenytoin, carbamazepine), caspofungin, rifampin, and St. John's wort.
4. Recommended Tacrolimus dose-modification in setting of azole therapy: Triazole antifungal medications are expected to increase tacrolimus levels, therefore, dosages of tacrolimus should be adjusted accordingly using the guidelines recommended in **Tables 4 and 5**.

Table 4: Recommended pre-emptive dose reduction of tacrolimus when azoles are initiatedat steady state levels of tacrolimus	
Antifungal Medication	**Tacrolimus Dose ↓**
Voriconazole	67%
Posaconazole	67%
Itraconazole	50%
Table 4: Recommended pre-emptive dose reduction of tacrolimus when azoles are initiatedat steady state levels of tacrolimus	
Isavuconazole	25%-50%
Fluconazole	25%

Table 5: Anticipated tacrolimus dose increase when azoles are stopped during concomitant tacrolimus therapy	
Antifungal Medication	**Tacrolimus Dose ↑**
Voriconazole	3-fold
Posaconazole	3-fold
Isavuconazole	3-fold
Itraconazole	2-fold
Fluconazole	1.3-fold
COMMENT: Dose increase may not be necessary for 5-10 days	
Note: Although tacrolimus doses may need to be substantially increased when azole therapy is stopped, the azole mediated inhibition of cytochrome CYP3A4 (and other) and P-glycoprotein may take 5-10 days to abate and therefore immediate dose increases are not advised. Rather, tacrolimus dose increases should be cautious and more frequent monitoring of tacrolimus levelsis appropriate.	

2.5.3.2 Methotrexate

2.5.3.2.1 Methotrexate Dose/Administration

Methotrexate (MTX) should be administered as an IV push at a dose of 15 mg/m2 on day +1 after transplant and at 10 mg/m2 on days +3, +6 and +11. Calculation of m2 will be per (**Appendix D**). Actual body weight is to be used to calculate BSA for MTX. However, for patients > 120% of ideal weight, BSA will be calculated using adjusted weight.

The first dose of MTX should be given approximately, but no sooner than, 24 hours after completion of the initial stem cell infusion.

MTX should be dose reduced, given with leucovorin rescue, or held for complications such as severe mucositis per institutional practice.

2.5.4 GVHD Treatment

In the event of the development of either acute or chronic GVHD, therapy will be per institutional practice.

2.5.5 Supportive Care

All supportive care will be given in keeping with institutional practice.

2.5.5.1 Infectious Disease Prophylaxis

Patients will receive infection prophylaxis according to institutional practice. Infection prophylaxis will include, but is not limited to, agents or strategies (e.g., PCR screening and preemptive therapy) to reduce the risk of bacterial, herpes simplex, CMV, HHV-6, EBV, Pneumocystis jirovecii, and fungal infections.

2.5.5.2 Infectious Disease Monitoring

1. **CMV PCR (peripheral blood):** Day 0 through day +100 (minimum weekly or more often if clinical concerns) and then it is recommended to continue weekly or every otherweek monitoring of CMV PCR through day +180 due to receipt of serotherapy. Additional monitoring should be per institutional practice for patients with a history of CMV

reactivation or CMV disease post-transplant or for those who remain on immune suppression. Use of letermovir is allowed. Any reactivation and/or CMV disease will be captured in this study.

2. **EBV PCR (peripheral blood):** EBV PCR: Day 0 through day +100 (minimum weekly or more often if clinical concerns) and then it is recommended to continue weekly or every other week monitoring of EBV PCR through day +180 due to receipt of serotherapy. Additional monitoring should be per institutional practice for patients with ahistory of EBV reactivation or PTLD post-transplant. Treatment of EBV reactivation or EBV-related post-HCT lymphoproliferative disease (PTLD) will be per institutional practice.
3. Monitoring of adenovirus, HHV6 and other viruses will be per institutional practice.

Grade 2 and 3 infections will be reported according to the BMT CTN Technical guidelines.

2.5.5.3 Intravenous Immune Globulin (IVIG)

IVIG administration will be according to institutional practice.

2.5.5.4 Growth Factors

Filgrastim (G-CSF) or biosimilar equivalent may be given per institutional practice.

2.5.5.5 Blood Products

Transfusion thresholds for blood product support will be consistent with institutional practice. All blood products will be irradiated.

2.5.5.6 Management of Graft Failure

Graft failure will be managed as per institutional guidelines.

2.5.5.7 Supportive Care for Treosulfan

In order to prevent skin toxicity especially in diaper bearing children it is recommended to wash the skin which is in contact with urine daily or take brief showers during and 48 hours after Treosulfan. Approximately 30–40% of the total dose of Treosulfan is excreted unchanged in urine and is activated to cytotoxic epoxide structures in an alkaline milieu. Since diaper rashes and perineal ulcers can occur in infants after Treosulfan conditioning, frequent diaper changes are recommended.

2.5.5.8 Management of Thymoglobulin Intolerance

Patients experiencing a new, severe, or life-threatening reaction to ATG and therefore unable or unwilling to receive the full planned cumulative dose will continue to be evaluated for the study, but their conditioning regimen may then be altered as per institutional preference or practice with documentation of the deviation.

2.5.6 Risks and Toxicities

2.5.6.1 General

Except for Treosulfan, the agents being used in the study have been used extensively in the HCT setting and have well defined toxicity profiles. In addition, there are many expected toxicities of allogeneic HCT. The following are examples of toxicities that are serious but not unexpected: Grade 4 cytopenias; neutropenic fever and sepsis; bacterial, fungal, or viral (including CMV, EBV, adenovirus, BK virus among others) infection; severe mucositis; severe GVHD; hepatic SOS; pulmonary toxicities; hemorrhagic cystitis; bleeding without hemodynamic compromise.

Additional potential side effects associated with the transplant in general include constitutional (fever/chills, malaise, fatigue, headache, pain, weakness), cardiovascular (edema, cardiac dysrhythmia, hypertension, thrombosis, cardiac failure), dermatologic (alopecia, rash, pruritus, skin depigmentation or hypopigmentation, photosensitivity), gastrointestinal (anorexia, nausea, vomiting, diarrhea, mucositis, dry mouth), hematologic/Immunologic (myelosuppression, immunosuppression, infections/sepsis, hemorrhage, acute and chronic GHVD, graft failure), hepatic (elevation of liver transaminases and/or bilirubin, SOS, hepatic failure), metabolic (electrolyte abnormalities, hyperglycemia), neurologic (dizziness, confusion, anxiety, depression, rarely seizures, encephalopathy, demyelination), pulmonary (cough, dyspnea, interstitial pneumonitis), and renal/bladder (elevation of BUN and/or creatinine, hemorrhagic cystitis, renal failure).

2.5.6.2 Drug Information

2.5.6.2.1 Treosulfan

Treosulfan will be supplied to investigators by Medac GmbH, Hamburg Germany under an IND held by Fred Hutchinson Cancer Center. The drug will be delivered to each participatingcenter's investigational drug pharmacy as a clinical investigational product with a study-specific label on the glass vial, as approved by the FDA.

Information regarding the physical, chemical, and pharmaceutical properties for Treosulfan, as well as formulations, storage and handling, and reconstitution recommendations can be found summarized in **Table 6** below as well as in the investigator brochure.

<table>
<tr><th colspan="2">Table 6: Investigational Product Properties, Formulation, Storage, Handling, and Reconstitution</th></tr>
<tr><td colspan="2">Dosage form/Presentations:</td></tr>
<tr><td></td><td>Powder/Lyophilisate for solution for infusion</td></tr>
<tr><td></td><td>1 g Treosulfan per vial</td></tr>
<tr><td></td><td>5 g Treosulfan per vial</td></tr>
<tr><td></td><td>Treosulfan is available as a powder or lyophilizate presentation. These 2 presentations are a result of the manufacturing process at different manufacturing sites. It has been confirmed during development that both presentations can be used interchangeably. Therefore, the available presentation has no influence on the safety and efficacy of the product, but only results in a different optical appearance of the drug product. For the 1 g Treosulfan strength, the vial size of the lyophilized product is 30 mL while the vial size of the powder product is 100 mL. This is due to technical reasons. The content of the product is identical.</td></tr>
<tr><td colspan="2">Recommended Solvents:</td></tr>
<tr><td></td><td>Sterile 0.45% sodium chloride solution;</td></tr>
<tr><td></td><td>Sterile 0.9% sodium chloride solution;</td></tr>
<tr><td></td><td>Sterile 5% glucose solution;</td></tr>
<tr><td></td><td>Sterile water for injection</td></tr>
<tr><td></td><td>Final Treosulfan concentration in the reconstituted solution: 50 mg/mL</td></tr>
<tr><td colspan="2">Incompatibilities:</td></tr>
<tr><td></td><td>In the absence of compatibility studies, the drug product must not be mixed with other medicinal products.</td></tr>
<tr><td></td><td>Treosulfan should be stored at room temperature (unopened vial). The expiration date is stated on the vial and the outer packaging and is defined in line with the approved shelf life in the respective investigational new drug application/investigational medicinal product dossier.</td></tr>
<tr><td></td><td>After reconstitution with a recommended solvent, chemical and physical stability has been demonstrated for 3 days at room temperature.</td></tr>
<tr><td></td><td>From a microbiological point of view, the product should be used immediately after reconstitution unless reconstitution has taken place in controlled and validated aseptic conditions. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and may not be longer than 72 hours at room temperature.</td></tr>
<tr><td colspan="2">Storage and Handling:</td></tr>
<tr><td></td><td>If not used immediately store reconstituted solution at room temperature. Do not use if the solution contains a precipitate.</td></tr>
<tr><td></td><td>Do not refrigerate, as this might cause a precipitate</td></tr>
<tr><td></td><td>See investigators Brochure (IB) for additional details regarding special precautions for disposal and handling of Treosulfan.</td></tr>
<tr><td colspan="2">Instructions for reconstitution of the IMP:</td></tr>
<tr><td></td><td>Calculate the dose, the total volume of reconstituted Treosulfan solution required, and the number of Treosulfan vials needed.</td></tr>
<tr><td></td><td>Reconstitute each vial with 0.45% sodium chloride injection, 0.9% sodium chloride injection, 5% glucose injection, or sterile water for injection in its original glass container using volumes described in the table below to obtain a final concentration of approximately 0.05 g/mL of Treosulfan.
<table>
<tr><th colspan="2">Reconstitution Solution Volume</th></tr>
<tr><th>Strength</th><th>Volume</th></tr>
<tr><td>1 g/vial</td><td>20 mL</td></tr>
<tr><td>5 g/vial</td><td>100 mL</td></tr>
</table></td></tr>
<tr><td></td><td>Shake the vials(s) to dissolve.</td></tr>
<tr><td></td><td>Determine the volume of 0.05 g/mL reconstituted solution needed based on the required dose. Reconstitution solutions of Treosulfan may be combined into a larger glass vial, polyvinyl chloride, ethylene vinyl acetate, polyolefin, or polyethylene bag.</td></tr>
</table>

Table 6: Investigational Product Properties, Formulation, Storage, Handling, and Reconstitution	
	To avoid solubility issues, prolonged standing time or slight warming of the reconstituted solution (hand warm) may be useful. Note, a warmer or water bath 25-30ºC (not higher) may also be used to warm the 0.45% sodium chloride injection, 0.9% sodium chloride injection, 5% glucose injection, or sterile water for injection (personal communication Medac; also in prior IBs) to be used for reconstitution.
	Inspect the reconstituted solution for discoloration and particulate matter. The reconstituted solution contains approximately 0.05 g/mL of Treosulfan and appears as a clear colorless solution. Solutions showing any sign of precipitation should not be used.
	For preparation of 0.45% sodium chloride solution equivalent volumes of 0.9% sodium chloride solution and water for injections can be mixed.

Voriconazole, posaconazole, itraconazole, and isavuconazole should be discontinued 72 hours prior to the start of conditioning and should not be administered during conditioning due to risk for drug-drug interaction/toxicities. Voriconazole, posaconazole, itraconazole, and isavuconazole may be administered if needed following conditioning.

Treosulfan toxicities are outlined in **Tables 7a (Children) and 7b (Adults)**.

Table 7a: Treosulfan Side Effects in Children

Very Common (10% or Greater)	Common (1% - 9%)	Uncommon/rare/unknown frequency
Abdominal pain	Abnormal creatinine (kidney function test)	Bleeding in the eye
Abnormal liver tests	Anal inflammation	Blistering of the skin
Anemia (low red blood cell count which can lead to fatigue)	Chills	Burning or prickling sensation on the skin
Diarrhea	Darkening of the skin	Capillary leak syndrome (tissue swelling) which can lead to dangerously low blood pressure
Fever	Difficulty swallowing	Cerebral hemorrhage (bleeding in the brain)
Hair loss	Headache	Constipation
Hepatotoxicity – damage to the liver including abnormal liver tests (not related to venoocclusive disease/sinusoidal obstruction syndrome)	Hives	Decreased appetite
Infections (bacterial, viral, fungal)	Mouth or throat pain	Diaper rash
Itching	Nosebleeds	Dry eyes
Low platelet counts which increases the risk of bleeding	Rash	Edema (swelling due to retention of fluids)
Low white blood cell count which increases the risk of infection	Redness of skin	Enlarged liver
Mucositis/stomatitis (painful inflammation/sores of the lips and lining of the mouth which may cause difficulties eating)	Severe rash with sores and/or peeling of the skin	Excess fluid in body cavity that surrounds the lung
Nausea	Skin pain	Feeling weak or tired
Vomiting (throwing up)		Hand-foot syndrome (redness, swelling, and pain on the palms of the hands and/or soles of the feet)
		Hematuria (blood in urine)
		High blood pressure
		Hypoxia (decrease in the amount of oxygen reaching the tissues)
		Indigestion
		Inflammation of the bladder

Very Common (10% or Greater)	Common (1% - 9%)	Uncommon/rare/unknown frequency
		Less acid than normal in the blood
		Low blood pressure
		Lung failure or pneumonia requiring oxygen therapy or mechanical ventilation
		Pain
		Pain in extremities
		Penile pain
		Pericardial effusion (fluid around the heart)
		Proctitis (rectal inflammation)
		Pulmonary hemorrhage (bleeding within the lungs)
		Seizure
		Severe inflammation of the colon
		Severe loss of kidney function which may require artificial kidney machine
		Skin ulcer
		Swelling of the scrotum
		Treatment-related secondary cancer
		Venoocclusive Disease/Sinusoidal obstruction syndrome (injury to the liver that can result in enlarged liver, liver pain, abdominal fluid, and abnormal liver tests)

Table 4: Treosulfan Side Effects in Adults			
Very Common (10% or Greater)	**Common (1% - 9%)**	**Uncommon (0.1% - 0.9%)**	**Rare/unknown frequency**
Abnormal liver tests	Abdominal/ esophageal pain	Abdominal bloating	Abnormal enzyme test – increased lactate dehydrogenase (LDH)
Anemia (low red blood cell count which can lead to fatigue)	Abnormal heartbeat	Bruising (small red or purple spots on the skin caused by bleeding into the skin)	Abnormal kidney function test
Diarrhea	Back pain	Chest pain	Acidosis (more acid than normal in the blood)
Feeling weak or tired	Bone, joint, or muscle pain	Confusion	Allergic reaction at the site of the drug injection
Fever	Chills	Death of skin tissue	Anal inflammation
Hair loss	Constipation	Dry mouth	Blood clot
Infections (bacterial, viral, fungal)	Decreased appetite	Dry skin	Burning or prickling sensation on the skin
Low platelet counts which increases the risk of bleeding	Difficulty swallowing	Excess fluid in the body cavity that surrounds the lungs	Cardiac arrest
Low white blood cell count which increases the risk of infection	Dizziness (feeling faint, woozy, weak or unsteady)	Glucose intolerance (high or low blood sugar)	Cough
Mucositis/stomatitis (painful inflammation/sores of the lips and/or lining of the mouth that may cause difficulties eating)	Hand-foot syndrome (redness, swelling, and pain on the palms of the hands and/or soles of the feet)	Hiccups	Darkening of the skin
Nausea	Headache	Hyperhidrosis (excessive sweating)	Difficulty speaking
Vomiting (throwing up)	Hematuria (blood in urine)	Liver failure	Dry eyes
	High blood pressure	Lung infection including pneumonia that may require mechanical ventilation	Dysphonia (vocal changes)
	Hypersensitivity (overactive immune system which can	Mouth hemorrhage (excessive bleeding from the mouth)	Enlarged liver

Table 4: Treosulfan Side Effects in Adults			
Very Common (10% or Greater)	**Common (1% - 9%)**	**Uncommon (0.1% - 0.9%)**	**Rare/unknown frequency**
	range from mild to severe)		
	Indigestion	Numbness or pain in hands and feet	Encephalopathy (brain disease that can include temporary or permanent damage)
	Insomnia (trouble sleeping)	Pain	Fainting
	Itching	Pain with urination / urinary tract pain	Feeling cold
	Low blood pressure	Pulmonary Hemorrhage (bleeding within the lungs)	Gastric or intestinal bleeding which can be severe
	Mouth pain	Severe brain bleeding	Heart attack
	Nosebleeds	Severe skin rash with sores and/or peeling of skin	Heart failure
	Pain in extremities	Throat, lung, or mouth inflammation	Hepatotoxicity – damage to the liver including abnormal liver tests (not related to venoocclusive disease / sinusoidal obstruction syndrome)
	Rash	Throat pain	Hypoxia (decrease in the amount of oxygen reaching the tissues)
	Redness of skin	Treatment-related secondary cancer	Inflammation of the bladder
	Sepsis, septic shock (overwhelming blood infection, can be fatal)	Venoocclusive disease / Sinusoidal obstruction syndrome (injury to the liver that can result in enlarged liver, liver pain, abdominal fluid and abnormal liver tests)	Liver pain
	Severe loss of kidney function which may require artificial kidney machine	Vertigo (spinning feeling)	Muscle weakness

Table 4: Treosulfan Side Effects in Adults			
Very Common (10% or Greater)	**Common (1% - 9%)**	**Uncommon (0.1% - 0.9%)**	**Rare/unknown frequency**
	Stomach inflammation		Restlessness or jerking
	Sudden reddening of the face and/or neck		Severe bleeding
	Swelling		Severe inflammation of the colon
	Trouble breathing		Swelling around the heart
	Weight loss or gain		

2.5.6.2.2 Fludarabine

Fludarabine phosphate is commercially available.

Fludarabine (Fludara®) is a purine analog that inhibits lymphocyte proliferation, promotes lymphocyte apoptosis, and is effective in the treatment of lymphoid and myeloid malignancies.[63-65] Fludarabine is phosphorylated intracellularly in several steps to its active form 2-fluoroadenosine arabinoside triphosphate, which acts by inhibiting DNA synthesis.[63,64] Fludarabine induces immunosuppression and long-lasting lymphopenia, reducing the incidence of GVHD and facilitating engraftment along histocompatibility barriers when used in combination with other chemotherapeutic agents or low-dose TBI.[64,66] Toxicities related to fludarabine are outlined in **Table 8**.

Table 8: Fludarabine Side Effects		
Common, some may be serious (>20%)	**Occasional, some may be serious (4 - <20%)**	**Rare and serious (3% or fewer)**
• Anemia (low red blood cells) which may require blood transfusions (may lead to tiredness or shortness of breath) • Cough • Feeling of being tired or irritable • Infection, especially when white blood cell count is low • Low platelet counts, which may cause bruising or bleeding • Pain	• Confusion • Chills • Damage to organs (brain, lungs, others) which may cause tiredness, changes in thinking or shortness of breath • Diarrhea (loose stools) • Feeling of "pins and needles" in arms and legs • Nausea, vomiting (throwing up), loss of appetite • Sores in mouth which may cause difficulty swallowing	• Blood in urine • Changes in vision • Coma • Kidney damage, which may require an artificial kidney machine • Liver damage • Seizures

2.5.6.2.3 Thymoglobulin® (Anti-Thymocyte Globulin; Rabbit ATG)

Thymoglobulin® is commercially available.

Thymoglobulin® (Anti-Human Thymocyte Globulin; ATG; Sangstat Medical Corp., Fremont, CA) is an immunosuppressant which alters the function of or eliminates T-cells and NK cells.

Rabbit anti-thymocyte globulin (Thymoglobulin®) is a polyclonal immunoglobulin mixture (IgM/IgG) raised in rabbits against human T thymocytes which reduces all circulating lymphocytes or lymphocyte subsets. ATG is used in preparation for HCT, as immunosuppressive therapy in aplastic anemia, and for GVHD treatment.

Thymoglobulin® is provided in 25mg vials by Sangstat. Each thymoglobulin® vial should be reconstituted with 5ml sterile water to a concentration of 5mg/ml. Further dilute dose in D5W or NS to a final concentration of 0.5 mg/mL. Diluted drug should be used immediately.

Thymoglobulin® should be administered alone and not run piggyback with any other solution. A 0.22 micron in-line filter should be used during administration via central line or high flow vein. The first and second dose of Thymoglobulin® should be infused over 6 hours, and the subsequent dose may be infused over 4 hours if there are no clinical concerns. Toxicities related to thymoglobulin are outlined in **Table 9**.

Table 9: Thymoglobulin® (rATG) Side Effects

Likely (May happen in more than 20% of patients)	Less Likely (May happen in 20% or fewer patients, but more than 2%)	Rare, but Serious (May happen in 2% or fewer patients)
• General pain, could also include joint and muscle pain • Headache • High or low potassium levels in the blood • High blood pressure • Fast heart rate • Infection, which may cause fever and chills • Low red blood cell counts, which may cause tiredness or may require blood transfusions • Low white blood cells, which may cause increased infections and slower wound healing • Low platelet levels, which may cause bruising, bleeding • Nausea, vomiting, diarrhea, constipation, stomach pain	• Acid/base imbalances in the blood • Cough • Feeling anxious • Feeling tired • High fat levels in the blood • High phosphate levels in the blood • Rash, itching • Reaction to the medication infusion, which may include itching, fever, chills, low blood pressure and problems breathing • Sweating • Swelling of the body • Post-transplant lymphoproliferative disease (PTLD) often caused by Epstein-Barr Virus (EBV). This can lead to fatal lymphoma. • Problems sleeping	• Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat

2.5.6.2.4 Tacrolimus

Tacrolimus is a calcineurin inhibitor used for immunosuppression. Toxicities related to tacrolimus are outlined in **Table 10**.

Table 10: Tacrolimus Toxicities

Common, some may be serious (>20%)	Occasional, some may be serious (4 - <20%)	Rare and serious (3% or fewer)
• Abnormal body movement, including tremors • Change of laboratory values that may need corrected: high sugar, high lipids, altered electrolytes • Constipation, diarrhea, nausea, vomiting, reflux, lack of desire to eat • Difficulty sleeping • Dizziness • Feeling of "pins and needles" in arms and legs • Headache • High blood pressure which may cause dizziness, chest pain • Itching, rash • Kidney damage which may cause swelling, may require an artificial kidney machine • Low platelet levels, which may cause bruising, bleeding • Liver damage • Low red blood cell counts, which may cause tiredness, or may require blood transfusions • Low white blood cell counts, which may lead to infection • Swelling of the body	• A new cancer resulting from treatment of earlier cancer • A tear or a hole in the bowels which may cause belly pain or that may require surgery • Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Change in the heart rhythm, abnormal heartbeat, or heart stops beating • Damage to lungs, which may cause shortness of breath, fluid around lungs • Damage to brain, which may cause headache, seizure, blindness • Heart attack or failure which may cause chest pain, swelling of ankles, and tiredness	• Damage to small blood vessels with resulting small blood clots and possible organ damage.

Occasionally the kidney damage is severe enough to require the use of intravenous fluids or even an artificial kidney machine. If the patient's kidney function is poor prior to transplant, there is an increased risk that the patient may develop kidney failure necessitating an artificial kidney machine, possibly permanently.

2.5.6.2.5 Methotrexate

Methotrexate (MTX) is an antimetabolite used for immunosuppression. Toxicities related to methotrexate are outlined in **Table 11**.

Table 11: Methotrexate Side Effects

Common, some may be serious (>20%)	Occasional, some may be serious (4 - <20%)	Rare and serious (3% or fewer)
• Increased risk of sunburn, rash	• Anemia from low red blood cell count which may cause tiredness, or may require transfusion	• A new cancer resulting from treatment of an earlier cancer
• Nausea, vomiting, loss of appetite • Sores in mouth which may cause difficulty swallowing	• Blood clot which may cause swelling, pain, shortness of breath • Bruising, bleeding from low platelet count • Diarrhea, sores in the gastrointestinal tract • Fluid around heart • Hair loss • Hepatitis or damage to the liver which may cause yellowing of eyes and skin, generalized swelling • Infection, especially when white blood cell count is low • Kidney damage	• Internal bleeding which may cause stomach pain, black tarry stool, blood in vomit • Scarring of the lungs which may cause shortness of breath, confusion • Severe skin rash with blisters and peeling which can involve mouth and other parts of the body

In the setting of renal or hepatic dysfunction, where there is impaired clearance of the medication, methotrexate may contribute to delayed neutrophil engraftment. Methotrexate may also cause an elevation in serum transaminase.

2.6 Study Conduct

This study will be conducted in accordance with the protocol, the BMT CTN MOP (located on the BMT CTN Website), and the following:

a. Consensus ethical principles derived from international guidelines including the Declaration of Helsinki
b. Applicable ICH Good Clinical Practice (GCP) Guidelines
c. Applicable laws and regulations

The National Marrow Donor Program (NMDP) single Institutional Review Board (IRB) of Record will oversee this study and conduct the study-specific reviews as required by federal regulations and per the NMDP IRB Standard Operating Procedures (SOPs).

Site personnel will enter data in the electronic case report form (eCRF) in Advantage eClinical as described in the BMT CTN 1904 Forms Guide. Source documentation should be made available for monitoring visits, audits, and regulatory inspections as described in the BMT CTN MOP.

Participating Principal Investigators (PIs) bear ultimate responsibility for training of site staff as

well as the scientific, technical, and administrative aspects of conduct of the protocol, even when certain tasks have been delegated to coinvestigators, sub-investigators, or staff. The PIs have a responsibility to protect the rights and welfare of patients and comply with all requirements regarding the clinical obligations and all other pertinent requirements in 21 CFR part 312. In addition to following applicable federal, state, and local regulations, investigators are expected to follow ethical principles and standards and receive training in GCP every three years and human subjects training within the past 3 years and thereafter as per institutional requirements.

CHAPTER 3

3 STUDY ENDPOINTS AND DEFINITIONS

3.1 Primary Endpoint

3.1.1 Graft-Versus Host-Disease (GVHD), Event-Free-Survival (EFS)

The primary endpoint is the incidence of 1-year GVHD free, EFS (GEFS). An event is defined as death due to any cause, graft rejection/failure, or 2nd HCT whichever occurs first. Grade III-IV acute GVHD and chronic GVHD (using NIH consensus criteria) requiring systemic immune suppression will be considered in this estimate.[61,62]

3.2 Secondary Endpoints

3.2.1 Overall Survival

Overall survival will be estimated at day 100, 6 months, and 1 year after HCT.

3.2.2 Event-Free Survival

Event-free survival will be estimated at 12 months after HCT. Grade III-IV acute GVHD and chronic GVHD requiring systemic immune suppression will be considered in this estimate.[61,62] An event is defined as death due to any cause, primary or secondary graft failure/rejection, or 2nd HCT whichever occurs first.

Primary Graft Failure/Rejection: defined as never achieving ANC ≥ 500/μL or never achieving ≥ 5% donor myeloid chimerism assessed by peripheral blood chimerism assays by day +42 post-HCT. Second infusion of hematopoietic cells is also considered indicative of primary graft failure by day +42 post-HCT.

Secondary Graft Failure/Rejection: defined as < 5% donor myeloid chimerism in peripheral blood beyond day +42 post-HCT in patients with prior documentation of hematopoietic recovery with ≥ 5% donor cells by day +42 post-HCT. Second infusion of hematopoietic cells is also considered indicative of secondary graft failure.

3.2.3 Hematologic Recovery

Hematologic recovery will be assessed according to neutrophil and platelet counts recovery after transplant. Neutrophil recovery will be estimated at day 28 and platelet recovery at day 100 after HCT.

Neutrophil recovery is defined as achieving an absolute neutrophil count (ANC) ≥ to 500/mm^3 for 3 consecutive measurements on 3 different days. The first of the three days will be designated the day of neutrophil recovery. The competing event is death without neutrophil recovery.
Platelet recovery is defined as the first day of a minimum of 3 days that the patient has a sustained platelet count ≥ 20,000/mm^3 with no platelet transfusions in the preceding 7 days. The first day of sustained platelet count above these thresholds will be designated the day of platelet engraftment.

3.2.4 Donor Chimerism (CD3 and Myeloid)

Donor cell engraftment will be assessed with donor/recipient chimerism studies at day 28, 100, 180, and 1 year after HCT. Chimerism will be evaluated in fractionated blood for CD3 T-cell and myeloid (CD33 or CD15). Peripheral blood myeloid donor chimerism will be used to define the donor/recipient chimerism status.

Full donor chimerism is defined as ≥95% donor cells in the myeloid fraction.

Mixed donor chimerism/engraftment is defined as the presence of 5-94.99% donor cells in the myeloid fraction.

Graft rejection is defined as <5% donor cells in the myeloid fraction.

3.2.5 Acute GVHD

The cumulative incidences of acute grade II-IV and III-IV GVHD at day 100 and day 180 after HCT will be determined. Acute GVHD by day 100 and day 180 will be graded according to the BMT CTN Technical guidelines on Acute GVHD and based on the Mount Sinai Acute GVHD International Consortium (MAGIC) criteria (**Appendix F**).[61] The time of onset of acute grades II-IV and III-IVGVHD will be recorded, as well as the maximum grade achieved at day 100 and day 180.

3.2.6 Chronic GVHD

The cumulative incidence of chronic GVHD (using NIH consensus criteria) requiring systemic immune suppression at 1 year after HCT will be determined. Data will be collected directly from providers and chart review as defined by the NIH Consensus Conference Criteria (**Appendix G)**.[62] Eight organs will be scored on a 0-3 scale to reflect the degree of chronic GVHD involvement. Liver and pulmonary function test results and use of systemic therapy for treatment of chronic GVHD will also be recorded. These data will allow calculation of the NIH global severity scores of mild, moderate, and severe chronic GVHD, which has been associated with transplant related mortality and overall survival. Assessment of chronic GVHD will occur up to 1 year after transplant.

3.2.7 Grade 3-5 Toxicities

Grade 3-5 toxicities by day 30 and day 100 after HCT, according to the BMT CTN MOP, will be collected.

3.2.8 Systemic Infections

All microbiologically documented infections or significant infections requiring antibiotic/antifungal therapy occurring up to 6 months after HCT will be reported by disease type (bacterial, fungal, viral, etc.), date of onset, and severity. For definitions see the BMT CTN Technical guidelineslocated on the BMT CTN Website.

3.2.9 EBV Reactivation

The incidence of EBV reactivation requiring therapy in the first 180 days after HCT, and of EBV-associated lymphoproliferative disorder in the first 180 days after HCT will be evaluated.

3.2.10 CMV Reactivation

The incidence of CMV reactivation requiring therapy in the first 180 days after HCT will be evaluated.

3.3 Exploratory Endpoints

3.3.1 Pharmacokinetic Studies

Treosulfan pharmacokinetic studies will be performed from peripheral blood samples if consent is obtained from subjects/guardians. This is an optional research study (**Appendix E**).

3.3.2 Biological Studies

Biological studies will be performed if consent is obtained from subjects/guardians. Prior to the initiation of the preparative regimen, blood and marrow samples (if obtained clinically a research specimen will be collected; cells, DNA, serum) will be banked. If samples have been donated and subject does not continue to transplant, samples will be stored for future research. Fibroblast cultures will be established from marrow aspirates as a source of germline DNA. Studies will include:

1. Assess clonal hematopoiesis before and after HCT in pediatric BMFD.

   Rationale: Clonal hematopoiesis with acquisition of mutations associated with myeloid malignancies are common in children with BMFD. The host marrow niche may contribute to clonal hematopoiesis but the effect of HCT on clonal hematopoiesis has not been studied in patients.

   Methods:

   - Deep error-corrected targeted sequencing of genes mutated in myeloidmalignancies and bone marrow failure.
   - Assess somatic mutations (type, number, mutational spectrum) and variant allelefrequencies before and after HCT.

2. Measure cytokine levels before and after HCT.

   Rationale: Inflammation contributes to the pathogenesis of bone marrow failure and may also contribute to clonal evolution.

   Methods:

   - Marrow plasma cytokine levels, particularly inflammatory cytokines, will bemeasured before and after HCT.

These biological studies will be correlated with clinical outcomes for study subjects (**Appendix E**). This is an optional research study.

3.3.3 Mutational Testing

Mutational testing will be performed for patients who lack a known genetic mutation responsible for their bone marrow failure phenotype, if consent is obtained from subjects/guardians. Prior to the initiation of the preparative regimen, peripheral blood will be obtained for whole exome/genome sequencing (**Appendix E**). This is an optional research study.

3.3.4 Health Related Quality of Life

Health-related quality of life using the PROMIS instruments will be performed at baseline, day +180 and day +365 post-HCT, if consent is obtained by subjects/guardians. Patients aged 8-17 at the time of assessment will complete pediatric instruments. Proxy reports (e.g., parents, guardians) will be collected for patients aged 5 to 8. The PROMIS instruments are available in Spanish and English. Patients who do not speak Spanish or English will not complete the health-related quality of life surveys (**Appendix H**). This is an optional research study.

CHAPTER 4

4 PATIENT ENROLLMENT AND STUDY EVALUATIONS

4.1 Disease and Donor Screening Procedures

Patients are identified and recruited by their physician, usually a hematologist and are referred to an HCT physician for further consultation. This consultation includes a detailed review of the trial design, the anticipated benefits and complications, and compliance including availability at 1-year post-HCT for trial assessments.

The order of events for patient enrollment is as follows:

1. Interested patients will sign an NMDP IRB-approved study consent form which will include consent for screening assessments to determine if the patient's underlying disease is eligible for this study.
2. The patient will be entered intoAdvantage eClinical Segment 0.
    a. If required per inclusion criteria, the patient will be reviewed by the BMT CTN 1904 protocol Eligibility Review Committee (ERC) for determination of disease eligibility for this clinical trial (**See Appendix B**).
    b. If the potential patient is deemed eligible by disease by the BMT CTN 1904 protocol eligibility review committee, the potential patient will then proceed forward with local assessment of eligibility criteria.
3. If ERC review is not required per inclusion criteria, the potential patient will thenproceed forward with local assessment of eligibility criteria.
4. The patient will be enrolled into Segment A in Advantage eClinical, a web-based data collection system.

4.2 Enrollment Procedures

Patients will be registered using the BMT CTN Electronic Data Capture System (Advantage eClinical). The following procedures should be followed:

1. An authorized user at the transplant center completes the Demographics Form and Segment 0 Enrollment Form in Advantage eClinical, at which point a study number will be generated.
    a. Patients requiring protocol ERC review per inclusion criteria:
        i. Enrollment into Segment 0 must be completed prior to submission to the protocol ERC. Once enrolled into Segment 0, the patient's medical recordsfor the ERC can be updated to the system.
        ii. Following ERC review, if the patient is found to be eligible for the study, the authorized user will complete any required Segment 0 forms and then be able to enroll the patient on Segment A using the Segment A EnrollmentForm.
    b. If not undergoing an ERC review, the authorized user will complete any required Segment 0 forms in Advantage eClinical and then if the patient is still eligible for the study, the authorized user will be able to enroll the patient on Segment A usingthe Segment A Enrollment Form.

2. The patient is not officially enrolled on study and may not begin study treatment until they are enrolled on Segment A. If the patient is found to be not eligible for the study, the site will indicate this in the data system.
3. Patients should not be enrolled into Segment A more than 14 days prior to the planned initiation of conditioning. If initiation of conditioning has not started within 14 days of enrollment, the Protocol Coordinator and Protocol Chairs and Officer must be notified.
4. A visit schedule based on transplant date is displayed in the Forms Grid.

4.3 Study Monitoring

4.3.1 Follow-Up Schedule

The follow-up schedule for study visits is outlined in **Table 12**. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User's Guide.

Table 12: Follow-Up Schedule

Study Visit	Target Day Post-HCT
Day of Transplant	Day 0*
Day 2	Day 2
1 week	7 ± 3 days
2 weeks	14 ± 3 days
3 weeks	21 ± 3 days
4 weeks	28 ± 3 days
5 weeks	35 ± 3 days
6 weeks	42 ± 3 days
7 weeks	49 ± 3 days
8 weeks	56 ± 3 days
9 weeks	63 ± 3 days
10 weeks	70 ± 3 days
11 weeks	77 ± 3 days
12 weeks	84 ± 3 days
13 weeks	91 ± 7 days
100 days	100 ± 7 days
6 months	180 ± 28 days
12 months	365 ± 28 days
*Additional rest days may be added given delays that may occur with stem cell collection, transportation, processing. The actual day the stem cell infusion finishes will remain "Day 0".	

4.3.2 Patient Assessments

Table 13 summarized patient clinical assessments over the course of the study. See **Table 12** for the study day and windows for clinical assessments.

Table 13: Patient Clinical Assessments/Testing

		Day pre or post-transplant																		
Patient Clinical Assessments/Testing	**Baseline**	**-6 &-5**	**0**	**1**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**91**	**100**	**180**	**365**
History, Physical Exam, Weight, and Height[1]	●		●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Karnofsky/Lansky Performance Status[1]	●																			●
Genetic testing[2]	●																			
Diepoxybutane (DEB) or Mitomycin C (MMC)[3]	●																			
Telomere testing by flow-FISH[4]	●																			
PNH testing[5]	●																			
HLA Typing[6] & Anti-Donor Antibody Testing[1]	●																			
CBC with differential and platelet count[1,7]	●[7e]	●[7d]	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●[7c]	●	●[7e]
Blood chemistries[1,8] and liver function studies[1,9]	●	●[8d]	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●[8c]	●	●
ABO/Rh[1]	●																			
PT/PTT/INR[1]	●																			
Serum ferritin & % iron saturation[1]	●																			●
Serum Immunoglobulins: IgG, IgM, and IgA[1]	●																●		●	●
Pregnancy test (females of childbearing potential)[1]	●																			
Infectious disease testing per institutional practice. Recommend CMV, HSV, VZV, toxoplasmosis, hepatitis B & C, HIV 1&2, HTLV 1&2, and syphilis[1]	●																			
CMV & EBV DNA Quantitative PCR Testing[10]			●		●	●	●	●	●	●	●	●	●	●	●	●	●	●[10a & 10b]	●	
Peripheral Blood Chimerism (Myeloid and CD3)[11]	●							●		●								●	●	●
Tacrolimus levels[12]				●	●	●	●	●	●	●	●	●	●	●	●	●	●	●[12b]	●	
Lymphocyte subsets (absolute CD3, CD4, CD19, and CD56)																		●	●	●

Table 13: Patient Clinical Assessments/Testing																				
CXR or chest CT[13]	●																			
		Day pre or post-transplant																		
Patient Clinical Assessments/Testing	**Baseline**	**-6 &-5**	**0**	**1**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**91**	**100**	**180**	**365**
EKG[1,] & Echocardiogram/MUGA[14]	●																			
Pulmonary Function Tests[1,15]	●																			●
Assessment for hepatic or cardiac iron overload[16]	●																			●
Bone marrow evaluation[17]	●																			●
Acute +/- Chronic GVHD assessments[18]					●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Toxicity assessments[19]								●				●						●	●	●
Treosulfan PK Samples on peripheral blood[20]		●																		
Biology Studies[21]	●[21]																			●[21]
Mutational Testing[22]	●																			
Heath related quality of life (PROMIS) [23]	●																		●	●

1. Within 30 days prior to enrollment. In addition, for patients enrolled on the Treosulfan PK studies, the patient's height and weight must be repeated if not already obtained within 3 days of the start of Treosulfan.
2. Genetic testing is required on all patients prior to enrollment (excluding patients with PNH). This testing does not need to be repeated pre-transplant if it has been previously done.
3. Diepoxybutane (DEB) or Mitomycin C (MMC) testing on peripheral blood or skin fibroblasts for Fanconi Anemia is required for all patients who lack a genetic mutation (excluding patients with PNH). This testing does not need to be repeated pre-transplant if it has been previously done.
4. Telomere testing by flow-FISH is required for all patients who lack a genetic mutation (excluding patients with PNH). This testing does not need to be repeated pre-transplant if it hasbeen previously done.
5. PNH testing (for PNH patients only): Per institutional practice. Repeat PNH testing within 30 days prior to enrollment.
6. HLA typing may be completed at any time prior to enrollment.
7. CBC with differential and platelet count
    a. Day 0 until count recovery/engraftment (defined as an ANC > 0.5 x 10^9/L x 3 consecutive measurements): Minimum three times per week.
    b. Count recovery/engraftment through day +100: Minimum weekly
    c. Day 101 through day +365: Minimum monthly
    d. For patients enrolled on the Treosulfan PK studies, a CBC is required in the morning on day -6 and -5 prior to the start of the Treosulfan infusion. This can be done in conjunction with standard of care tests.
    e. For patients enrolled in the Biological Studies, a CBC is required to be done on the same day as the sample collection. This can be done in conjunction with standard of care tests.

Table 13: Patient Clinical Assessments/Testing

8. Blood chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, magnesium, phosphorus
    a. Day 0 though day +28: Minimum twice weekly.
    b. Day +29 through day +100: Minimum weekly
    c. Day +101 through day +365: Minimum monthly
    d. For patients enrolled on the Treosulfan PK studies, a serum creatinine is required in the morning on day -6 and -5 prior to the start of the Treosulfan infusion. This can be done in conjunctionwith standard of care tests.
9. Liver function studies: Total and direct bilirubin, alkaline phosphatase, AST, and ALT
    a. Day 0 through day +28: Minimum twice weekly
    b. Day +29 through day +100: Minimum weekly
    c. Day +101 through day +365: Minimum monthly
10. Peripheral blood CMV & EBV PCR monitoring
    a. CMV PCR (peripheral blood): Day 0 through day +100 (minimum weekly or more often if clinical concerns) and then it is recommended to continue weekly or every other week monitoring of CMV PCR through day +180 due to receipt of serotherapy. Additional monitoring should be per institutional practice for patients with a history of CMV reactivation or CMV disease post-transplant or for those who remain on immune suppression.
    b. EBV PCR (peripheral blood): Day 0 through day +100 (minimum weekly or more often if clinical concerns) and then it is recommended to continue weekly or every other week monitoring of EBV PCR through day +180 due to receipt of serotherapy. Additional monitoring should be per institutional practice for patients with a history of EBV reactivation or PTLD post-transplant.
11. Peripheral blood chimerism studies (myeloid and CD3 T-cell)
    a. Peripheral blood samples for chimerism analysis by molecular methods (patient and donor) at baseline (any time prior to study enrollment)
    b. Day +28 (day +28 chimerism should only be evaluated if the patient has an ANC $\geq$ 0.5 x 10^9/L
    c. Day +42 (day +42 chimerism should only be evaluated if chimerism studies were not performed at day +28)
    d. Day +100, +180, and +365
    e. For patients with mixed chimerism, follow up chimerism studies per institutional practice is recommended to document sustained engraftment or graft failure
12. Tacrolimus levels:
    a. Day +1 through day +28: Minimum twice weekly
    b. Day +29 through day +100: Minimum weekly
    c. Day +101 through day +180 or initiation of the tacrolimus taper whichever is later: minimum every other week then monthly per institutional practice
    d. If there are concerns for renal dysfunction, the tacrolimus level should be followed more closely per institutional practice
13. CXR or Chest CT as clinically indicated or per institutional practice. It is recommended that patients with a history of neutropenia or underlying immunodeficiency have a CT scan of the chest, abdomen, and pelvis as well as the sinuses (if age appropriate) due to increased risk for infection including fungal disease
14. Echocardiogram/MUGA: Echocardiogram or MUGA for assessment of left ventricular ejection fraction (LV EF) or, for patients unable to obtain LV EF, LV shortening fraction (LVSF; within 3 months of study enrollment)
15. Pulmonary function testing (PFT): to include FEV1, FVC, FEV1/FVC, TLC, RV, and DLCO (corrected for hemoglobin). For patients who are unable to perform PFTs due to age or developmental delay, pulse oximetry is an acceptable alternative.
16. Assessment for hepatic or cardiac iron overload:

Table 13: Patient Clinical Assessments/Testing

a. Formal assessment of hepatic and/or cardiac iron overload within 6 months of study enrollment using a validated methodology such as T2* MRI (liver and heart), ferriscan (liver), or biopsy (liver) per institutional practice.
   i. For patients ≥ 18 years with a history of significant transfusions defined as ≥8 packed red blood cell transfusions per year for ≥1 year or have received ≥20 packed red blood cell transfusions (lifetime cumulative) will require formal hepatic and cardiac iron measurement. In addition, patients with a prior history of hepatic or cardiac iron overload will also require formal assessment for hepatic and cardiac iron overload.
   ii. For patients < 18 years old with a history of significant transfusions defined as ≥8 packed red blood cell transfusions per year for ≥1 year or have received ≥20 packed red blood cell transfusions (lifetime cumulative) will require formal hepatic iron measurement. In addition, patients with a prior history of hepatic iron overload will also require formal assessment for hepatic iron overload.

b. Day +365: Follow-up assessment for hepatic and potentially cardiac iron overload is recommended for patients with a history of iron overload pre-HCT or those considered at risk for iron overload post-HCT per institutional practice.

17. Bone marrow aspirate and biopsy for pathology, flow cytometry, cytogenetics, and MDS FISH panel. The MDS FISH panel should evaluate for deletion 5q/monosomy 5, monosomy 7/deletion 7q, trisomy 8, and deletion 20q
    a. Baseline (within 60 days prior to enrollment)
    b. Day +365: Per the discretion of the treating physician a bone marrow evaluation around 1-year post-HCT.
18. GVHD assessments: Patients should be assessed weekly until Day +100 post-HCT for GVHD. After Day +100, patients will be assessed at each follow-up visit through 12 months for the presence of GVHD.
19. Toxicity assessments: At day +28, +56, +100, +180, and +365
20. Treosulfan Pharmacokinetic Studies (Optional): Treosulfan pharmacokinetic sampling and analysis on peripheral blood samples on days -6 and -5. The same peripheral blood sample will be used for both Treosulfan and Treosulfan monoepoxide quantitation (samples will be split in PK Lab during analysis process). This will only be done in subjects/guardians who have provided consent. (see **Appendix E for additional details**)
21. Biological Studies (Optional): Biological studies will be performed on peripheral blood and bone marrow (if a clinical marrow occurs) samples at baseline (within 60 days) and at Day +365 post-HCT, if consent isobtained from subjects/guardians (see **Appendix E for additional details**).
22. Mutational Testing (Optional): Mutation studies will be performed in patients who lack a known genetic mutation responsible for their bone marrow failure phenotype at baseline, if consent is obtained from subjects/guardians (excluding patients with PNH; see **Appendix E for additional details**).
23. Health related quality of life (Optional): Health related quality of life using the PROMIS instruments will be performed at baseline, day +180 and day +365 post-HCT in English and Spanish speaking patients only if consent is obtained from subjects/guardians.

4.3.3 Pre-HCT Evaluations

The following observations must be performed within 30 days (unless noted otherwise) prior to enrollment. Subjects must also continue to meet eligibility criteria at the start of conditioning:

1. History, physical examination, height, and weight. The recommended history is to include the age at diagnosis, race/ethnicity, gender, and indications for HCT. In addition, for patients enrolled on the Treosulfan PK studies, the patient's height and weight must be repeated if not previously obtained within 3 days of the start of Treosulfan.
2. Karnofsky or Lansky performance status.
3. Disease Assessments including Genetic Testing:
    a. Patients should have genetic testing to document their underlying BMFD (at any time prior to screening assessment for disease eligibility; this excludes patients with PNH).
    b. Patients who lack a genetic mutation (excluding patients with PNH) must have negative testing for Fanconi anemia via Diepoxybutane (DEB) or Mitomycin C (MMC) testing on peripheral blood or skin fibroblasts (at any time prior to screening assessment for disease eligibility).
    c. Patients who lack a genetic mutation (excluding patients with PNH) must have telomere testing by flow-FISH (at any time prior to screeningassessment for disease eligibility).
    d. For patients with PNH: flow cytometry for PNH per institutional practice. Repeat PNH testing within 30 days prior to enrollment.
4. HLA typing (patient and donor) and anti-donor antibody testing (if not already performed). HLA typing may be done more than 30 days prior to enrollment; however anti-donor antibody testing should be performed within 30 days of study enrollment.
5. Additional Laboratory Evaluations:
    a. CBC with differential and platelet count
        i. For patients enrolled on the Treosulfan PK studies, a CBC is required in the morning on days -6 and -5 prior to the start of the Treosulfan infusion. This can be done in conjunction with standard of care tests.
        ii. For patients enrolled in the Biological Studies, a CBC is required to be done on the same day as the sample collection. This can be done in conjunction with standard of care tests.
    b. Patient/Donor ABO/Rh
    c. BUN and Creatinine
        i. For patients enrolled on the Treosulfan PK studies, a serum creatinine is required in the morning on days -6 and -5 prior to the start of the Treosulfan infusion. This can be done in conjunction with standard of care tests.
    d. Electrolytes: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus
    e. Liver function studies: total bilirubin, direct bilirubin, ALT, AST, and alkalinephosphatase
    f. PT, PTT, INR

g. Serum ferritin and % iron saturation
h. Quantitative immunoglobulins (IgG, IgA, and IgM) per institutional practice
i. Serum pregnancy test for females of childbearing potential
j. Baseline peripheral blood samples for chimerism analysis by molecular methods(patient and donor) at any time prior to study enrollment

6. Infectious Disease Evaluations per institutional practice. Recommend including:
    a. CMV, HSV, VZV, and toxoplasma per institutional practice
    b. Hepatitis B and C, HIV 1&2, HTLV 1&2, and syphilis per institutional practice
7. Radiological Evaluations:
    a. CXR or chest CT as clinically indicated or per institutional practice. It is recommended that patients with a history of neutropenia or underlying immunodeficiency have a CT scan of the chest, abdomen and pelvis as well as the sinuses (if age appropriate) as part of their pre-transplant work-up due to increased risk for infection including fungal disease.
    b. Cardiac
        i. EKG
        ii. Echocardiogram or MUGA for assessment of left ventricular ejection fraction (LVEF) or, for patients unable to obtain a LV EF, LV shortening fraction.
    c. Pulmonary: pulmonary function testing to include FEV1, FVC, FEV1/FVC, TLC, RV, and DLCO (corrected for Hemoglobin). For patients who unable to perform PFTs due to age or developmental ability, pulse oximetry is an acceptable alternative.
    d. Formal assessment of hepatic and/or cardiac iron overload within 6 months of study enrollment using a validated methodology such as T2* MRI (hepatic or cardiac), ferriscan (hepatic), or biopsy (hepatic) per institutional practice.
        i. For patients ≥ 18 years with a history of significant transfusions defined as ≥8 packed red blood cell transfusions per year for ≥1 year or have received ≥20 packed red blood cell transfusions (lifetime cumulative) will require formal hepatic and cardiac iron measurement. In addition, patients with a prior history of hepatic or cardiac iron overload will also require formal assessment for hepatic and cardiac iron overload.
        ii. For patients < 18 years old with a history of significant transfusions defined as ≥8 packed red blood cell transfusions per year for ≥1 year or have received ≥20 packed red blood cell transfusions (lifetime cumulative) will require formal hepatic iron measurement. In addition, patients with a prior history of hepatic iron overload will also require formal assessment for hepatic iron overload.
8. Bone marrow Evaluation: A bone marrow aspirate/biopsy for pathology, flow cytometry, cytogenetics and MDS FISH panel within 60 days prior to study enrollment. The MDS FISH panel should evaluate for deletion 5q/monosomy 5, monosomy 7/deletion 7q,trisomy 8, and deletion 20q.
9. Research Studies (Optional):

1. Treosulfan Pharmacokinetic Studies (Optional): Treosulfan pharmacokinetic sampling and analysis on peripheral blood samples will be done on days -6 and -5. The same sample will be used for both Treosulfan and Treosulfan monoepoxide quantitation (samples will be split in PK Lab during analysis process). PK samples must be drawn from a different lumen/venous access than the drug infusion. If this is not possible and blood sampling needs to occur from the same lumen for Treosulfan infusion, document on the Treosulfan PK worksheet.

   The Treosulfan PK studies will be performed in approximately 5 patients for each of the following age groups: 1) 1 year to < 2 years, 2) 2 years to <4 years, 3) 4 years to <12 years and 4) ≥ 12 years (total patients: approximately 20). CBC with differentials and a serum creatinine is required in the morning on day -6 and -5 prior to the start of the Treosulfan infusion. This can be done in conjunction with standard of care tests (**Table 14 and Appendix E**).

Table 14: PK Sample Collection Schedule for Day -6 and Day -5 Treosulfan Infusions

Time Point	Collection Window	Blood Volume
Pre-infusion	Within 5 minutes before starting Treosulfan infusion	1 mL
End-of-infusion (EOI)	EOI + 5 minutes	1 mL
EOI + 20 minutes	+/- 5 minutes	1 mL
3.5 hours after start of infusion	+/- 10 minutes	1 mL
6 hours after start of infusion	+/- 10 minutes	1 mL
9 hours after start of infusion	+/- 10 minutes	1 mL

2. Biological Studies (Optional): Baseline assessment

   Blood and marrow samples will be obtained within 60 days prior to enrollment for somatic genomic analyses, T-cell phenotypes, and cytokine measurement. Those providing consent will have blood (3mL-5mL EDTA tube and 5mL in sodium heparin tube) and marrow samples (3mL-5mL in EDTA tube and 5mL in sodium heparin tube) sent to the central laboratory for these studies. A CBC with differentials is required to be done on the same day as the sample collection. This can be done in conjunction with standard of care tests. (**Appendix E**) If samples have been donated and subject does not continue to transplant, samples will be stored for future research.

3. Mutational Testing (Optional) – Baseline assessment

   Individuals who have participated in previous genetic studies and who have no identified mutation will be eligible to participate in this whole exome/genome sequencing study (excluding patients with PNH). Those interested in participation will be asked to provide peripheral blood (10 or 20 ml, based on subject's weight) in EDTA (purple top) tubes for DNA extraction after informed consent for whole exome/genome sequencing is obtained. (**Appendix E**)

4. Health Related Quality of Life (Optional): Baseline assessment

   At the time a patient is identified as participating in the study, the CIBMTR Survey Research Group (SRG) is notified and then adds that patient to CIBMTR's electronic Patient Reported Outcomes (ePRO) system for long-term QOL tracking. Pre-transplant (baseline) QOL data will be collected by the center or CIBMTR electronically or on paper forms within 30 days prior to HCT. If conditioning is delayed, the QOL surveys should be repeated so the QOL surveys are completed within 30 days prior to HCT. Electronically collected baseline QOL surveys will be directly entered in the ePRO system by the patient or proxy. The center will securely email, or fax baseline QOL instruments completed on paper to the SRG for entry into the ePRO system. (**Appendix H**)

4.3.4 Post-HCT Evaluations – The Following Studies are Recommended

1. History and physical exam to assess GVHD and other morbidities weekly until Day +100, then at Day +180 and Day +365. GVHD evaluation and grading to be in keeping with the BMT CTN Technical guidelines. For scheduling purposes, a target day range has been provided in **Table 12.**
2. Karnofsky or Lansky performance status at Day +365.
3. Laboratory Evaluations – the following laboratory evaluations are required with the minimum frequency of monitoring; however, more frequent monitoring per institutional practice is allowed:
   a. CBC with differential
      i. Day 0 until count recovery/engraftment (defined as an ANC > 0.5 x 10^9/Lx 3 consecutive measurements): Minimum three times per week.
      ii. Count Recovery/Engraftment through day +100: Minimum weekly
      iii. Day +101 trough day +365: Minimum monthly
      iv. For patients enrolled on the Treosulfan PK studies, a CBC is required in the morning on days -6 and -5 prior to the start of the Treosulfan infusion.
   b. Blood Chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine,calcium, magnesium, phosphorus:
      i. Day 0 through Day +28: Minimum twice a week.
      ii. Day +29 through day +100: Minimum weekly
      iii. Day +101 through day +365: Minimum monthly
      iv. For patients enrolled on the Treosulfan PK studies, a serum creatinine is required in the morning on days -6 and -5 prior to the start of the Treosulfan infusion.
   c. Liver function studies: Total and direct bilirubin, alkaline phosphatase, AST, and ALT:
      i. Day 0 through Day +28: Minimum twice a week
      ii. Day +29 through day +100: Minimum weekly
      iii. Day +101 through day +365: Minimum monthly

d. Tacrolimus levels:
    i. Day +1 through day +28: Minimum twice weekly
    ii. Day +29 through day+100: Minimum weekly
    iii. Day +101 through day+180 or initiation of tacrolimus taper whichever is later:minimum every other week then monthly per institutional practice.
    iv. If there are concerns for renal dysfunction, the tacrolimus level should be followed more closely per institutional practice

e. Chimerism studies on peripheral blood: Myeloid and CD3
    i. Day +28 (Day +28 chimerism should only be evaluated if the patient has anANC $\geq$ 0.5 x 10^9/L)
    ii. Day +42 (Day +42 chimerism should only be evaluated if chimerism studieswere not performed at day +28)
    iii. Day +100, Day +180, Day +365
    iv. For patients with mixed chimerism, follow up chimerism studies per institutional practice is recommended to document sustained engraftment or graft failure.

f. Peripheral blood CMV and EBV PCR monitoring:
    i. CMV PCR: Day 0 though day +100 (minimum weekly) and then it is recommended to continue weekly or every other week monitoring of CMV by PCR in the peripheral blood through day 180 due to receipt of serotherapy. Additional monitoring should be per institutional practice for patients with a history of CMV reactivation/disease within the first 100 days or for those who remain on immune suppression.
    ii. EBV PCR: Day 0 though day +100 (minimum weekly) and then it is recommended to continue weekly or every other week monitoring of EBV by PCR in the peripheral blood through day 180 due to receipt of serotherapy. Additional monitoring should be per institutional practice for patients with a history of EBV reactivation post-transplant.

g. Serum ferritin and % iron saturation: Day +365

h. Absolute lymphocyte numbers by flow cytometry for lymphocyte subpopulationsto include CD3, CD4, CD19, and CD56 at day +100, +180 and +365.

i. Serum quantification of IgG, IgM, and IgA: Minimum at day +90, 180, and +365.

4. Bone marrow evaluation including pathology, flow cytometry, cytogenetics, and MDS FISH panel per the discretion of the treating physician at day +365. The MDS FISH panel should evaluate for deletion 5q/monosomy 5, monosomy 7/deletion 7q, trisomy 8, and deletion 20q.

5. Radiological Evaluations:
    a. Pulmonary function testing: FEV1, FVC, FEV1/FVC, TLC, RV, and DLCO (corrected for Hemoglobin) around day +365 in those patients who can perform PFTs.
    b. T2* MRI of the liver and heart is recommended around day +365 in those patients with a history of iron overload pre-HCT or those considered at risk for iron overload post-HCT per institutional practice.

6. Toxicity assessments at Day +28, +56, +100, +180, and +365.
7. GVHD assessments: Patients should be assessed weekly until Day +100 post-transplant for GVHD. After Day +100, patients will be assessed at each follow-up visit through 12 months for the presence of GVHD.
8. Research Studies:

    a. Biological Studies (optional): At day +365 post-HCT.
    Blood and marrow samples will be obtained at one-year post-HCT for somatic genomic analyses, T-cell phenotypes, and cytokine measurement. Consent will be obtained by phone. Those providing consent will have blood (3mL-5mL EDTA tube and 5mL in sodium heparin tube) and marrow samples (3mL-5mL in EDTA tube and 5mL in sodium heparin tube) sent to the central laboratory for these studies. If samples have been donated and subject does not continue to transplant, samples will be stored for future research.

    b. Health Related Quality of Life (Optional): At day +180 and day +365 post-HCT

    The SRG will administer the 180 day and 1-year QOL instruments online, or on paper if requested by the patient. They will first confirm the patients' status with the transplant center because reporting of deaths may lag. They will then contact the patient via email, phone, or mail to collect the QOL information online or on paper.

    At the conclusion of each QOL administration, patients will be reminded of the next date of contact. The SRG will notify the transplant center if a patient's contact information has changed, if they find through follow-up that the patient has died or that the patient wishes to be removed from future QOL surveys.

4.3.5 Case Report Forms

Criteria for Forms Submission: Criteria for timeliness of submission for all study forms are detailed in the Forms Guide. Forms that are not entered into Advantage eClinical within the specified time will be considered delinquent. A missing form will continue to be requested either until the form is entered into Advantage eClinical® and integrated into the Data Coordinating Center's (DCC) master database, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Forms Guide.

Reporting Patient Deaths: Recipient death information must be entered into Advantage eClinical® within 24 business hours of knowledge of the patient's death. If the cause of death is unknown at that time, it does not need to be recorded at that time. However, once the cause of death is determined, the Death Form must be updated in Advantage eClinical®.

Center for International Blood and Marrow Transplant Research (CIBMTR) Data Reporting: Centers participating in BMT CTN trials must register pre- and post-HCT outcomes on all consecutive HCTs done at their institution during their time of participation to the CIBMTR. Registration is done using procedures and forms of the Stem Cell Transplant OutcomesDatabase (SCTOD). (Note: Federal legislation requires submission of these forms for all US allotransplant recipients.)

BMT CTN 1904 enrollment must be indicated on the SCTOD pre-transplant registration form. Additionally, CIBMTR pre- and post- transplant Comprehensive Report Forms must also be submitted for all patients enrolled on this trial. CIBMTR forms will be submitted directly to the CIBMTR through routine CIBMTR mechanisms.

Weekly GVHD Monitoring: GVHD should be monitored in accordance with BMT CTN guidelines as specified in the Manual of Procedures. Patients should be assessed weekly until Day +100 post-transplant for GVHD. After Day +100, patients will be assessed at each follow-up visit through 12 months for the presence of GVHD. For scheduling, a target day range has been provided in **Table 11.**

Internal Data Monitoring

It is the responsibility of the site PI to ensure the validity of all patient data as well as the safety of all patients.

Data Monitoring and Auditing

The Data Safety and Monitoring Board (DSMB) for the BMT CTN will convene as per standard procedure to review serious toxicities and adverse events for the purpose of determining whether the trial should be modified or stopped. Triggers for referral to the DSMB are described in the Stopping Rules Criteria of **Section 5.3.** If the monitoring of the safety endpoints described in **Section 5.3** results in a trigger for consultation with the DSMB, the DSMB may make recommendations about whether or not to continue accrual.

Records

Any publication or presentation will refer to patients by a unique patient number and not by name to assure patient confidentiality.

4.3.6 Adverse Event Data

4.3.6.1 Definitions

Adverse Event (AE): An AE is any untoward medical occurrence in a patient administered an investigational product or has undergone study procedures and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease that is temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

An abnormality identified during a medical test (e.g., laboratory parameter, vital sign, ECG data, physical exam) should be defined as an AE only if the abnormality meets 1 of the following criteria:

1. Induces clinical signs or symptoms.
2. Requires active intervention.
3. Requires interruption or discontinuation of study medication.
4. The abnormality or investigational value is clinically significant in the opinion of the investigator.

Expectedness: An adverse event can be Expected or Unexpected

- **Expected adverse events** are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the

package inserts or is included in the informed consent document as a potential risk.

- **Unexpected adverse events** are those that vary in nature, intensity, or frequency from information in the current adverse event list, the Investigator's Brochure, the package insert, or when it is not included in the informed consent document as a potential risk.

Serious Adverse Event: A serious adverse event (SAE), as defined by per 21 CFR 312.32, is any adverse event that results in one of the following outcomes, regardless of causality and expectedness:

- **Results in death**
- **Is life-threatening.** Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- **Requires or prolongs inpatient hospitalization** (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- **Results in persistent or significant disability/incapacity.** Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- **Is a congenital anomaly or birth defect; or**
- **Is an important medical event** when, based upon appropriate medical judgment, it may jeopardize the patient and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Medical and scientific judgment should be exercised in deciding whether expected reporting is also appropriate in situations other than those listed above. For example, important medical events may not be immediately life threatening or result in death or hospitalization but may jeopardize

the subject or may require intervention to prevent one of the outcomes listed in the definition above (e.g., suspected transmission of an infectious agent by a medicinal product is considered a Serious Adverse Event). Any event is considered a Serious Adverse Event if it is associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

4.3.6.2 Classification of Adverse Events by Severity

The severity refers to the intensity of the reported event. The Investigator must categorize the severity of each SAE according to the National Cancer Institute (NCI) CTCAE Version 5.0. For any term that is not specifically listed in the CTCAE scale, intensity will be assigned a grade of one through five using the following CTCAE guidelines:

5. Grade 1: Mild; asymptomatic or mild symptoms, clinical or diagnostic observations only; intervention not indicated
6. Grade 2: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living
7. Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
8. Grade 4: Life-threatening consequences; urgent intervention indicated
9. Grade 5: Death related to AE

4.3.6.3 Attribution of the Adverse Event in Relation to the Study Drug - Treosulfan

The relationship of each reported event to the study treatment (Treosulfan) will be assessed by the Investigator; after careful consideration of all relevant factors such as (but not limited to) the underlying study indication, coexisting disease, concomitant medication, relevant history, pattern of the SAE, temporal relationship to any study treatment interventions and de-challenge or re-challenge according to the following guidelines:

> **Possibly, Probably, or Definitely Related:** there is a reasonable possibility that the study treatment caused the event. A relationship of possibly, probably, or definitely related to the investigational product is considered related for the purposes of regulatory authority reporting.
>
> **Unlikely, or Not Related:** There is no reasonable possibility that the investigational product caused the event. An unlikely or not related relationship to the investigational product is considered unrelated for the purposes of regulatory authority reporting.

4.3.6.4 Required Adverse Event Reporting Requirements

Adverse event reporting will be consistent with BMT CTN procedures (BMT CTN Administrative Manual of Procedures, Chapter 6). It is BMT CTN policy that AEs meeting reporting criteria must be reported even if the investigator is unsure whether a relationship exists between the adverse event and the use of study treatment.

Unexpected, serious adverse events (SAEs) will be reported through an expedited AE reporting system via Advantage eClinical. **Unexpected, life-threatening and fatal SAEs must be reported within 24 hours of knowledge of the event. All other unexpected SAEs must be reported within three business days of knowledge of the event.** Events entered in Advantage eClinical will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.

Expected AEs will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 at regular intervals as defined on the Form Submission Schedule, including calendar-driven case report forms (e.g., Toxicity and GVHD) or event-driven case report forms (e.g., Relapse/Progression, Infection, and Death). **Any expected life-threatening SAE not collected on another study form must be reported through the expedited AE reporting system via Advantage eClinical.**

The Data and Safety Monitoring Board will receive reports of all unexpected SAEs and pregnancies upon review by the BMT CTN Medical Monitor. Summary reports for all reported SAEs will be reviewed by the DSMB on a semi-annual basis.

4.3.6.5 Procedure in Case of Pregnancy

If a female patient becomes pregnant during the study dosing period or within 90 days from the Treosulfan infusion, the investigator should report within 24 hours through an expedited AE reporting system via Advantage eClinical. The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated conception date, pregnancy result, neonatal data and other related information will be requested.

The investigator will follow the medical status of the mother, as well as the fetus, as if the pregnancy is an SAE and will report the outcome. Additional information regarding the outcome of a pregnancy (which is categorized as an SAE) is mentioned below.

- "Spontaneous abortion" includes miscarriage, abortion and missed abortion
- Death of an infant within 1 month after birth should be reported as an SAE regardless of its relationship with the study drug
- If an infant die more than 1 month after the birth, it should be reported if a relationship between the death and intrauterine exposure to the study drug is judged as "possible" by the investigator
- In the case of a delivery of a living newborn, the "normality" of the infant is evaluated at the birth
- Unless a congenital anomaly is identified prior to spontaneous abortion or miscarriage, the embryo or fetus should be assessed for congenital defects by visual examination

Information will be collected at the time of delivery/birth and 6 months and 12 months after birth. If the patient completes the final study follow-up prior to 12 months after birth, a final status should be reported following the final study visit.

4.3.6.6 Reporting Requirements to the FDA

The sponsor (Fred Hutchinson Cancer Center) assumes responsibility for IND safety reporting to the FDA and participating investigators, in accordance with regulations under 21 CFR 312.32. The following process will be followed:

Each reportable SAE received from the transplant centers will be evaluated by the BMT CTN Medical Monitor, who will assess the seriousness, expectedness, and the relationship to the investigational product. Any event determined to be a suspected unexpected serious adverse reaction (SUSAR) will be reported to the FDA as an IND safety report in accordance with regulations under 21 CFR 312.32 by the Sponsor or designee. Investigators involved in the study will be provided with an Investigational Safety Letter (ISL) notifying them of an event that meets FDA IND reporting criteria.

- SAEs will be reported by the study site PIs to the IND Sponsor and the BMT CTN Medical Monitor via Advantage eClinical per the reporting timelines.
- SAEs received will first be evaluated by the BMT CTN Medical Monitor, who will make a determination of whether the SAE constitutes a SUSAR.
- SAE determined to be a SUSAR will be reported by BMT CTN to the Fred Hutch Medical Monitor.
- SAEs identified by the BMT CTN Medical Monitor and/or Fred Hutch as SUSARs will be reported by Fred Hutch to FDA in an IND safety report in accordance with regulations under 21 CFR 312.32.
- The Fred Hutch Medical Monitor and BMT CTN Medical Monitor will review cumulative SAE data at regular intervals to monitor for new safety signals or events meeting IND safety reporting criteria.
- To make their determination of whether an event is a SUSAR, the BMT CTN and Fred Hutch Medical Monitors will assess the seriousness and expectedness of the event, as well as relationship to participation in the study.
- Fred Hutch or its designee will provide all study site PIs with a safety letter notifying them of an event that meets FDA IND Safety Reporting criteria. Study site PIs will be requested to provide written notification of safety reports to the IRB of record as soon as is practical, consistent with IRB requirements.

CHAPTER 5

5 STATISTICAL CONSIDERATIONS

5.1 Study Design

This is a prospective, multicenter phase II study designed to evaluate the outcomes of patients with BMFD undergoing HCT using Treosulfan-based conditioning. The primary endpoint is GVHD-free, Event-free survival (GEFS) at 1 year. The target enrollment is 40 patients transplanted using a Treosulfan-based conditioning.

5.1.1 Accrual

It is estimated that 48 months of accrual will be necessary to enroll the targeted sample size. Accrual will be reported by race, ethnicity, gender, and age.

5.1.2 Study Duration

Patients will be followed on trial for 12 months post-HCT for primary, secondary, and exploratory endpoints. Subsequent longer-term follow up on select endpoints will be available through the CIBMTR.

5.1.3 Randomization and Blinding

This is a single arm trial with no randomization and no blinding.

5.1.4 Primary Endpoint

The primary endpoint of the trial is GVHD-free, EFS (GEFS) at 1-year post-HCT using Treosulfan-based conditioning. Events contributing to this endpoint are specified in Section 3.1. The primary objective of this trial is to estimate the 1-year GEFS probability, using the Kaplan Meier method, along with 95% confidence intervals.

5.2 Sample Size and Power Considerations

Sample size justification is based on targeting acceptable confidence interval widths for the 1-year GEFS estimate. With n=40 patients, sample confidence intervals and their widths are provided in **Table 15** as a function of the observed GEFS, assuming complete follow up and based on exact Clopper-Pearson binomial confidence intervals. If GEFS is at least 85%, the lower confidence limit will rule out GEFS probabilities of 70% or less. In practice, if there is censoring of the primary endpoint prior to 1 year, Kaplan-Meier estimates will be used along with 95% confidence intervals. But we expect minimal to no censoring of the primary endpoint through 1 year, so the confidence intervals and their widths below should provide a good approximation.

Table 15: Sample 95% confidence intervals with n=40 as a function of observed GEFS.

GEFS	95%	90%	85%	80%
95% CI	83-99%	76-97%	70-94%	64-91%

5.3 Interim Analysis and Stopping Guidelines

There will be no planned interim analyses for efficacy or futility for this trial, while safety will be monitored using stopping guidelines as described below.

Monitoring of two key safety endpoints (overall mortality by 100 days post-HCT, graft failure by 100 days post-HCT) will be conducted, and if either rate significantly exceeds pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures. These stopping guidelines serve as triggers for consultation with the DSMB for additional review and are not formal "stopping rules" that mandate automatic closure of study enrollment. The number of enrolled patients that fail to receive transplant using Treosulfan and the occurrence of toxicity, adverse events, and other safety endpoints will be monitored and reported to the DSMB annually, at a minimum; in the event that any safety concerns arise, these data will be conveyed to the DSMB expeditiously.

Monitoring rule for overall mortality by 100 days post-HCT:

Monitoring will be performed beginning when at least 3 patients are evaluable for the monitoring rule (died or been followed for at least 100 days post-HCT), until enrollment is closed. At least three deaths must be observed, along with crossing of a stopping boundary as described below, in order to trigger referral to the DSMB for further review. The expected probability of mortality within 100 days is expected to be no more than 15%. The null hypothesis that the 100-day mortality rate is less than or equal to 15% is tested. An extension of the sequential probability ratio test (SPRT) for censored exponential data will be used for monitoring, as described in greaterdetail below and in **Appendix I.** This SPRT is based on testing the null hypothesis that the 100- day mortality rate is less than or equal to 15%. The sequential testing procedure conserves type Ierror at 5% across all of the assessments and can be represented graphically. At each interim analysis, the total time on study (e.g., in months or years, x axis) is plotted against the total numberof endpoints (e.g., patients experiencing death, y axis). The continuation region of the SPRT is defined by two parallel lines. Only the upper boundary will be used for monitoring to protect against excessive 100-day mortality. If the graph falls above the upper boundary, the SPRT rejects the null hypothesis, and concludes that there are more events than predicted by the observed time on study. Otherwise, the SPRT continues until enrollment is closed.

This procedure assumes a censored exponential distribution for the time until death during the first 100 days, and censor's follow-up time after 100 days. Only deaths that occur on or before the patient has been followed for 100 days are counted. Total time on study is computed as time from transplant to death, or to 100 days, whichever comes first, summed for all patients on study.

The usual measures of performance of an SPRT are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$ and of accepting H_1 when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta_i)$. The test to be used in this protocol were developed from an SPRT contrasting 15% versus 35% 100-day rate of mortality, resulting in decision boundaries with a common slope of 0.084 and an upper intercept of 2.424, with nominal type I and II errors of 8% and 15%, respectively.

The actual operating characteristics of the truncated tests, shown in **Table 16**, were determined in a simulation study that assumed uniform accrual of 40 patients over a four-year time period, exponential time to failure after HCT, and implemented monthly monitoring of the SPRT. In

practical implementation, however, the SPRT is monitored monthly and also reviewed each time a new event occurs to identify any crossing of a toxicity boundary in between monthly assessments.

TABLE 16: OPERATING CHARACTERISTICS OF SEQUENTIAL TESTING PROCEDURE FOR DAY 100 MORTALITY FROM A SIMULATION STUDY WITH 10,000 REPLICATIONS			
True 100-Day Rate	15%	25%	35%
Probability Reject Null	0.052	0.427	0.855
Mean Month Stopped	49.7	39.7	25.3
Mean # Endpoints in 100 Days	5.8	7.8	7.0
Mean # Patients Enrolled	38.8	31.7	21.0

For example, the testing procedure for 100-day mortality rejects the null hypothesis in favor of the alternative 5.2% of the time when the true 100-day mortality rate is 15%, and 85.5% of the time when the rate is 35%. This corresponds to a type I error rate of $\alpha = 0.052$ and a type II error rate of $\beta = 0.145$. When the true 100-day mortality rate is 35%, on average, the DSMB will be consulted 25.3 months after opening, when 7.0 events have been observed in 21.0 patients.

Monitoring rule for graft failure by 100 days post-HCT

Graft failure is expected to be no higher than 10% by 100 days. Graft failure within 100 days will be monitored using a sequential probability ratio test (SPRT) for binary data that compares a rate of 10% under the null hypothesis to a rate of 30% under the alternative hypothesis. This sequential testing procedure preserves the type I error rate at a prespecified level across all of the monthly examinations. The binary SPRT can be represented graphically by plotting the number of evaluable patients against the cumulative number of events. The continuation region of the SPRTis defined by two parallel lines. Only the upper boundary will be used for monitoring in order to protect against excessive Day 100 graft failure rates. If the cumulative number of graft failures falls above the upper boundary the SPRT rejects the null hypothesis and concludes that more graftfailures occurred than should be expected in the observed number of evaluable patients. Otherwise, the SPRT continues until enrollment reaches the target sample size of 40 patients. The binary SPRT can be displayed in a tabular form, as shown in **Table 17**. This table gives the rejection boundaries for the number of Day 100 graft failure events corresponding to the number of evaluable patients. At least three graft failures must be observed in order to trigger review.

This procedure considers only graft failures occurring by Day 100. A binary SPRT contrasting 10% versus 30% Day 100 graft failure rates results in decision boundaries with a common slope of 0.1862 and an upper intercept of 1.7984 with nominal type I and II error rates of 7.5% and 15%, respectively. Because the SPRT employed here is truncated at a sample size of 40 and only uses the upper decision boundary, the actual type I and II errors will vary from these nominal levels.

Table 17: Sequential Monitoring Plan based on Binary SPRT for Graft failure by day 100	
Number of Evaluable Patients	**Rejection Boundary for # of graft failures by day 100**
3-6	3
7-11	4
12-17	5
18-22	6
23-27	7
28-33	8
34-38	9
39-40	10

The actual operating characteristics of the truncated test are shown in **Table 18**, obtained from a simulation study that assumed uniform accrual of 40 patients over a 48-month period. This simulation study implemented monitoring of the SPRT after each additional patient became evaluable. In practical implementation, however, the SPRT is monitored monthly and also reviewed each time a new event occurs to identify any crossing of a toxicity boundary in between monthly assessments.

Table 18: Operating Characteristics of the Binary SPRT for Day 100 graft failure from a Simulation Study with 10,000 Replicates				
True Day 100 Graft Failure Rate	**10%**	**20%**	**25%**	**30%**
Probability Reject Null	0.052	0.484	0.736	0.898
Mean Month Stopped	49.6	37.8	30.0	23.0
Mean # events by Day 100	3.9	5.8	5.7	5.1
Mean # Patients Enrolled	38.7	29.1	22.7	17.0

The testing procedure rejects the null hypothesis in favor of the alternative 5.2% of the time when the true Day 100 graft failure rate is 10% and 89.8% of the time when the rate is 30%. This corresponds to a type I error rate of 5.2% and a type II error rate of 10.2%. If the true Day 100 graft failure rate is 30%, the DSMB will be consulted 23 months after opening on average, when 5.1 events have been observed in 17 patients.

5.4 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized for all patients. Characteristics to be examined may include:

a. Patient factors:

- Genetic mutation (if known)
- Age at diagnosis
- Age at transplantation
- Race/Ethnicity

- Gender
- Karnofsky/Lansky Performance status
- Indication(s) for transplant
- Baseline ferritin and hepatic/cardiac iron level if known
- Disease specific baseline characteristics:
  - Diamond Blackfan anemia – history of steroids (Yes/No)
  - Shwachman-Diamond syndrome
  - PNH – history of eculizumab or other PNH specific therapy (Yes/No)

b. Transplant factors:

- Hematopoietic cell source (BM and PBSC)
- Donor type (HLA-matched related, HLA-matched unrelated, mismatched unrelated)
- Cell dose infused: Total nucleated cell (TNC) per kg recipient body weight, CD34 count per kg recipient body weight, and/or CD3 count per kg recipient body weight

5.5 Analysis Populations and General Analysis Guidelines

5.5.1 Primary Analysis Population

All patients conditioned with a Treosulfan-containing regimen will be included in the primary analysis population. Time to event analyses will start at initiation of conditioning (day -6 of transplant). Analyses for the primary endpoint, all secondary endpoints, and safety endpoints, will use the primary analysis population. The primary analysis will occur once all patients have been followed for 12 months, Endpoint Review Committee adjudication completed, and data is locked.

5.5.2 Replacement of Patients

Patients who are enrolled but do not proceed to conditioning with a Treosulfan-containing regimen will be described (frequency, reason for not proceeding to conditioning), but they will not count towards the target accrual of n=40 patients conditioned with a Treosulfan-containing regimen.

5.5.3 General Analysis Guidelines

Primary analyses for the primary, secondary, safety, and exploratory endpoints will use the primary analysis population. Analyses of each endpoint will follow the analysis plans as describedbelow in **sections 5.6-5.8**. We expect minimal missing data (<5%) for the primary endpoint and secondary endpoints based on past experience with HCT and cellular therapy trials. If more than 10% of a key primary or secondary outcome is missing, we will apply an appropriate imputation method and conduct corresponding sensitivity analyses.

5.6 Analysis of Primary Endpoint

The probability of GEFS at 1 year will be estimated using the Kaplan-Meier method, along with 95% confidence intervals computed using a complementary log-log transformation.

5.7 Analysis of Secondary Endpoints

Analysis of secondary endpoints will be primarily descriptive, using estimates along with 95% confidence intervals as detailed below.

5.7.1 Overall Survival

The probability of OS at 1 year will be estimated using the Kaplan-Meier method, along with 95% confidence intervals computed using a complementary log-log transformation.

5.7.2 GVHD-Free, Event-Free Survival

GVHD-free, event-free survival will be estimated at 1 year using the Kaplan- Meier method, along with 95% confidence intervals computed using a complementary log-log transformation.

5.7.3 Hematologic Recovery

Probabilities of neutrophil recovery by Day 28 and Day 100 will be described with 95% confidence intervals using the cumulative incidence estimate, treating death as a competing event. Complementary log-log transformations will be used to construct the confidence intervals. Similarly, probabilities of platelet recovery by Day 100 will be described with 95% confidence intervals using the cumulative incidence estimate, treating death as a competing event.

5.7.4 Donor Chimerism (CD3 and Myeloid)

Proportions of patients with full (>95%), mixed (5-95%), or rejection (<5%) myeloid chimerism will be tabulated and described using frequencies and percent at each time point.

5.7.5 Acute GVHD

Cumulative incidence of acute grade II-IV GVHD will be estimated using the cumulative incidence function, treating death prior to acute GVHD as a competing risk. Point estimates and confidence intervals will be provided for the cumulative incidence at day 100 and day 180 post-HCT, using the complementary log-log transformation. Time to GVHD will be calculated from transplant date. Cumulative incidence of acute grade III-IV GVHD will be estimated using the cumulative incidence function, treating death prior to acute GVHD as a competing risk. Point estimates and confidence intervals will be provided for the cumulative incidence at day 100 and day 180 post-HCT.

5.7.6 Chronic GVHD

Cumulative incidence of chronic GVHD requiring immune suppression (IS) will be estimated using the cumulative incidence function, treating death prior to chronic GVHD as a competing risk. A point estimate and confidence interval will be provided for the cumulative incidence at 6 months and one-year post-HCT, using the complementary log-log transformation. Frequencies of NIH global severity scores of mild, moderate, and severe will also be provided.

5.7.7 Grade 3-5 Toxicities

All grade 3-5 toxicities according to CTCAE version 5.0 will be categorized by SOC and preferred term using the MedDRA dictionary, and the number of AEs will be summarized by SOC, preferred term, and peak grade. The number and percentage of patients with at least 1 grade 3 or higher AE will be summarized by SOC and the preferred team. Detailed listings of unexpected SAEs, including severity and relationship to treatment, will be presented.

5.7.8 Systemic Infections

The number of microbiologically documented infections or significant infections requiring antibiotic/antifungal/antiviral therapy and the number of patients experiencing infections will be tabulated by type of infection, severity, and time period after transplant. The cumulative incidence of CMV reactivation or EBV reactivation requiring therapy will be estimated at 180 days with a 95% confidence interval, treating death as a competing risk.

5.8 Analysis of Exploratory Endpoints

5.8.1 PK Studies, Biological Studies, and Mutational Testing

Analyses of lab sample endpoints will be described in a future statistical analysis plan separate from this protocol. This protocol only describes processes for collection of the samples.

5.8.2 Health-Related Quality of Life

PROMIS Scale total score and subdomain scores will be summarized using means and SDs at baseline, 6 months, and 1 year. Changes from baseline to day +180 and day +365 post-HCT will be analyzed using the paired t-test and linear mixed models for longitudinal data.

APPENDIX A
HUMAN SUBJECTS PROTECTION

APPENDIX A HUMAN SUBJECTS

1. Subject Consent

Candidates for the study will be identified as described in **Chapter 4** of the protocol. The Principal Investigator or his/her designee at each transplant center will contact the candidates, provide them with information about the purpose of the study and obtain voluntary consent if the candidates agree to participate. The BMT CTN will provide a template of the assent and the consent forms to each center. Each center will add their NMDP IRB-approved boiler-plate language to these documents and submit it for review by the NMDP Internal Review Board (IRB). The DCC will verify the adequacy of the assent and consent forms prior to submission to the IRB. The NMDP IRB will provide evidence of IRB approval.

2. Confidentiality

Confidentiality will be maintained by individual names being masked and assigned a patient identifier code. The code relaying the patient's identity with the ID code will be kept separately at the center. The ID code will be transmitted to the network.

Participation of Women and Minorities

Women and ethnic minorities and other populations will be included in this study. Accrual of women and minorities at each center will be monitored to determine whether their rates of enrollment are reflective of the distribution of potentially eligible women and minorities expected from data reported to the CIBMTR and from published data on incidence of leukemia and lymphoma in these groups. Centers will be notified if their rates differ significantly from those expected and asked to develop appropriate recruitment reports.

3. GCP

This study will be conducted in accordance with standards of Good Clinical Practice (as defined by the International Council for Harmonization) and all applicable national and local regulations.

APPENDIX B
PROTOCOL ELIGIBILITY REVIEW COMMITTEE

APPENDIX B

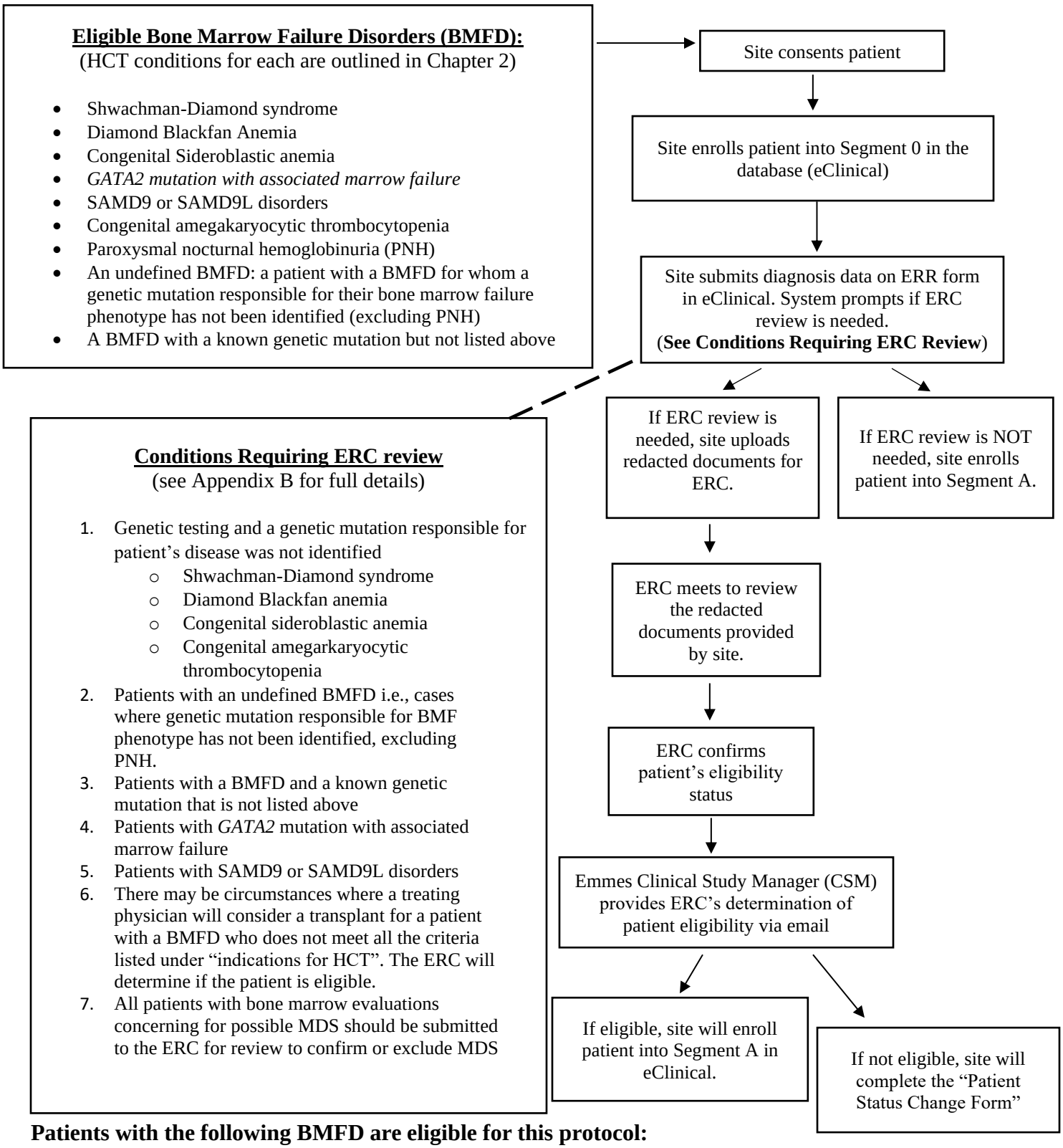
ELIGIBILITY REVIEW COMMITTEE

Eligible Bone Marrow Failure Disorders (BMFD):
(HCT conditions for each are outlined in Chapter 2)

- Shwachman-Diamond syndrome
- Diamond Blackfan Anemia
- Congenital Sideroblastic anemia
- *GATA2 mutation with associated marrow failure*
- SAMD9 or SAMD9L disorders
- Congenital amegakaryocytic thrombocytopenia
- Paroxysmal nocturnal hemoglobinuria (PNH)
- An undefined BMFD: a patient with a BMFD for whom a genetic mutation responsible for their bone marrow failure phenotype has not been identified (excluding PNH)
- A BMFD with a known genetic mutation but not listed above

Conditions Requiring ERC review
(see Appendix B for full details)

1. Genetic testing and a genetic mutation responsible for patient's disease was not identified
   - Shwachman-Diamond syndrome
   - Diamond Blackfan anemia
   - Congenital sideroblastic anemia
   - Congenital amegarkaryocytic thrombocytopenia
2. Patients with an undefined BMFD i.e., cases where genetic mutation responsible for BMF phenotype has not been identified, excluding PNH.
3. Patients with a BMFD and a known genetic mutation that is not listed above
4. Patients with *GATA2* mutation with associated marrow failure
5. Patients with SAMD9 or SAMD9L disorders
6. There may be circumstances where a treating physician will consider a transplant for a patient with a BMFD who does not meet all the criteria listed under "indications for HCT". The ERC will determine if the patient is eligible.
7. All patients with bone marrow evaluations concerning for possible MDS should be submitted to the ERC for review to confirm or exclude MDS



Patients with the following BMFD are eligible for this protocol:

1. Shwachman-Diamond syndrome
2. Diamond Blackfan Anemia
3. Congenital Sideroblastic anemia
4. *GATA2* mutation with associated marrow failure
5. SAMD9 or SAMD9L disorders
6. Congenital amegakaryocytic thrombocytopenia
7. Paroxysmal nocturnal hemoglobinuria
8. An undefined BMFD: a patient with a BMFD for whom a genetic mutation responsible fortheir bone marrow failure phenotype has not been identified (excluding PNH) will be eligible for this clinical trial following approval by BMT CTN 1904 ERC.
9. A BMFD with a known genetic mutation but not listed above will be eligible for this clinical trial following approval by BMT CTN 1904 ERC.

The following patients MUST be reviewed by the BMT CTN 1904 protocol eligibility review committee (ERC) in order to determine if they are eligible for this trial:

1. All patients with Shwachman-Diamond syndrome, Diamond Blackfan anemia, congenital sideroblastic anemia, or congenital amegakaryocytic thrombocytopenia who have had genetic testing and a genetic mutation responsible for their disease was not identified.
2. All patients with an undefined BMFD: a patient with a BMFD for whom a genetic mutation responsible for their bone marrow failure phenotype has not been identified, excluding PNH.
3. All patients with a BMFD and a known genetic mutation that is not listed above
4. All patients with *GATA2* mutation with associated marrow failure
5. All patients with SAMD9 or SAMD9L disorders
6. There may be circumstances where a treating physician will consider a transplant for a patient with a BMFD who does not meet all the criteria listed under “indications for HCT”. In these situations, treating physicians may submit their patient to the BMT CTN1904 ERC for review in order to determine if the patient is eligible for this clinical trial based on additional clinical or laboratory information.
7. Many patients with BMFD can have bone marrow evaluations that raise concern for possible MDS including but not limited to dysplastic bone marrow evaluations or cytogenetic abnormalities. However, in patients with BMFD these findings are not necessarily diagnostic or consistent with MDS. Therefore, given the complexities of diagnosing MDS in patients with BMFD, all patients with bone marrow evaluations concerning for possibleMDS should be submitted to the ERC for review to confirm or exclude MDS. This is particularly important as we do not want to exclude potentially eligible patients due to an incorrect diagnosis of MDS.

The protocol ERC will consist of a minimum of 3 members of the BMT CTN protocol 1904 team. All potential patients will be identified by their local transplant physician who will determine if the patient meets the eligibilitycriteria for BMT CTN 1904.

Once the patient has signed the consent for this study, certain screening information outlined below will need to be submitted to the protocol ERC who will review and confirm whether the patient is eligible for BMT CTN 1904 protocol. As outlined above, there are specific situations that require

ERC review. For example, patients who lack a genetic mutation following genetic testing. In addition, we also recognize there **may be circumstances where the transplant physician will consider a transplant for a patient with a BMFD who does not meet all the criteria listed under "indications for HCT". In these situations, the transplant physician may submit their patient to the BMT CTN 1904 ERC for review in order to determine if the patient is eligible for this clinical trial based on additional clinical or laboratory information.**

The patient does NOT have to complete the pre-transplant evaluations specified in BMT CTN 1904 prior to ERC review. However, the ERC may ask for additional patient data to determine protocol eligibility which may include some of the clinical assessments recommended/required by the protocol. If the ERC deems the patient eligible for BMT CTN 1904, the patient will need to complete the recommended/required baseline pre-transplant clinical assessments in the time period outlined in the protocol. The patient must also continue to meet protocol eligibility criteria as outlined in BMT CTN 1904 at time of study enrollment and at the time of starting conditioning.

The following screening information is recommended and should be uploaded in Advantage eClinical prior to ERC review:

1. Disease Assessments:
    a. All genetic testing available including genetic testing that was negative for a mutation (this excludes patients with PNH)
    b. Patients who lack a genetic mutation (excluding patients with PNH) must have negative testing for Fanconi anemia via Diepoxybutane (DEB) or Mitomycin C (MMC) testing on peripheral blood or skin fibroblasts (at any time prior to screening assessment for disease eligibility). Centers should upload testing results even if normal.
    c. Patients who lack a genetic mutation (excluding patient with PNH) must have telomere testing by flow-FISH. Centers should upload testingresults even if normal.
    d. For patients with PNH: flow cytometry for PNH per institutional practice.
    e. Additional disease specific evaluations should also be included (<u>if performed</u>) if a genetic mutation responsiblefor the patient's bone marrow failure phenotype has not been identified including the following:

1. Shwachman-Diamond syndrome

- Pancreatic isoamylase, trypsinogen, or fecal elastase
- Abdominal ultrasound demonstrating fatty pancreas
- Imaging demonstrating skeletal abnormalities

2. Diamond Blackfan anemia

- eADA if available at diagnosis prior to transfusions
- Hemoglobin F
- Reticulocyte count
- Bone marrow demonstrating a deficiency of erythroid precursors in anotherwise cellular bone marrow

3. Congenital sideroblastic anemia

- Bone marrow demonstrating the presence of ringed sideroblasts
- Lead and zinc level testing to exclude an acquired cause of ringedsideroblasts

4. Congenital amegakaryocytic thrombocytopenia

- Bone marrow demonstrating megakaryocyte hypoplasia

2. History, physical exam, height, and weight.
3. Additional labs (if performed):
    a. CBC with differential
    b. Liver function studies: ALT, AST, alkaline phosphatase, and total bilirubin/direct bilirubin
    c. BUN/creatinine
    d. Serum ferritin and % iron saturation
    e. Additional labs deemed clinical important for review by the treating physician for review by the ERC
4. Bone Marrow Evaluation (if performed):
    a. Bone marrow aspirate/biopsy for pathology, flow cytometry, cytogenetics and MDS FISH panel. The MDS FISH panel should evaluate for deletion 5q/monosomy 5, monosomy 7/deletion 7q, trisomy 8, and deletion 20q. Given thecomplexities of diagnosing MDS in patients with BMFD, the ERC will review all pre-transplant bone marrow evaluations in patients who lack a genetic mutation aswell as review all patients with bone marrow evaluations concerning for possible MDS in order to aid with the diagnosis of MDS. This is particularly important as we do not want to exclude potentially eligible patients due to an incorrect diagnosisof MDS.
5. Procedures/Imaging (if performed):
    a. Assessment of hepatic and potentially cardiac (age ≥18 years old) iron status is required for all patients with a history of significant transfusions defined as ≥8 packed red blood cell transfusions per year for ≥1 year or have received ≥20 packed red blood cell transfusions (lifetime cumulative). In addition, patients with prior history of elevated hepatic or cardiac iron will require formal assessment prior to study enrollment. Formal assessment will include a liver MRI using a validated methodology such as T2* MRI or ferriscan or liver biopsy as well as formal assessment of cardiac iron contentusing T2* MRI.
    b. Additional imaging deemed clinical important by the treating physician for review by the ERC.

All uploaded source documentation must be de-identified, including redaction of all patient identifiers and/or PHI (name, patient initials, medical record number, date of birth) as well as redaction of institutional name and physician/advanced practice provider name(s).

Following completion of Segment 0 enrollment form and upload of source documentation, the DCC protocol coordinator will be notified via email. The protocol coordinator will review the data and source documentation in Advantage eClinical. If all case information is present, the protocol coordinator will alert the protocol ERC. The ERC will review the case at the next scheduled ERC meeting which usually occurs weekly. The protocol coordinator will notify the enrolling center in writing once the panel has completed their review.

APPENDIX C
KARNOFSKY/LANSKY PERFORMANCE STATUS SCALE

Karnofsky Scale (recipient age ≥ 16 years)	Lansky Scale (recipient age ≥ 1 year and <16 years)
Able to carry on normal activity; no special care is needed	**Able to carry on normal activity; no special care is needed**
100 - Normal, no complaints, no evidence of disease	**100** - Fully active
90 - Able to carry on normal activity	**90** - Minor restriction in physically strenuous play
80 - Normal activity with effort	**80** - Restricted in strenuous play, tires more easily, otherwise active
Unable to work, able to live at home, cares for most personal needs, a varying amount of assistance is needed	**Mild to moderate restriction**
70 - Cares for self, unable to carry on normal activity or to do active work	**70** - Both greater restrictions of, and less time spent in active play
60 - Requires occasional assistance but is able to care for most needs	**60** - Ambulatory up to 50% of time, limited active play with assistance/supervision
50 - Requires considerable assistance and frequent medical care	**50** - Considerable assistance required for any active play, fully able to engage in quiet play
Unable to care for self, requires equivalent of institutional or hospital care, disease may be progressing rapidly	**Moderate to severe restriction**
40 - Disabled, requires special care and assistance	**40** - Able to initiate quite activities
30 - Severely disabled, hospitalization indicated, although death not imminent	**30** - Needs considerable assistance for quiet activity
20 - Very sick, hospitalization necessary	**20** - Limited to very passive activity initiated by others (e.g., TV)
10 - Moribund, fatal process progressing rapidly	**10** - Completely disabled, not even passive play

APPENDIX D
ANTHROPOMETRY (Height, Weight and BSA calculations)

APPENDIX D:
Anthropometry (Height, Weight, and BSA calculations)

Drug Dosing by Body Size

Drug dosing will be based on either body surface area (BSA) or body weight.

1. BSA is calculated in m^2. The formula by definition adjusts for both under and overweight individuals. The formula for this calculation is:

$$\text{BSA } (m^2) = \frac{\sqrt{actual\ weight\ (kg)\ x\ height(cm)}}{60}$$

2. Body weight is measured in kg. The ideal body weight (IBW) will be calculated in the following ways:

Age ≥ 18 years

Gender (height)	
Male (>5ft)	50 kg + (2.3 kg/inch over 5 feet)
Female (>5ft)	45.5 kg + (2.3 kg/inch over 5 feet)
Subject <5ft	subtract 2.3 kg/inch
Rounding	Height in inches will be rounded to the nearest whole number
Height with *inches < ½ inch*	*Round down* to the nearest whole inch (e.g., 5ft 5 ¼ in will be rounded to 5ft 5 in)
Height with *inches ≥ ½ inch*	*Round up* to the nearest whole inch (e.g., 5ft 6 ½ in will be rounded to 5ft 7 in)

Age <18 years

Pre-pubertal males (<14 years) females (<12 years)	Ideal body weight (IBW) will be calculated by matching body weight at the 50th percentile for length-for-age or height-for-age at the 50th percentile CDC growth chart
Post-pubertal adolescents males (≥14 years) females (≥12 years)	Ideal body weight will be assessed using the body mass index (BMI). If the child's normal BMI [body weight in kg/ height in meters^2] is between the 25-75th percentile on the CDC growth chart, the child may be considered at IBW
Adolescents whose BMI is > 75th percentile	Ideal body weight will be the 75th percentile BMI body weight
Adolescents whose BMI is < 25th percentile	Ideal body weight will be the 25th percentile BMI body weight

Note: When deviating from these age ranges (based on early or late maturity), the dietitian will document the rationale in the nutrition assessment.

Body Weight/Adjusted Body Weight for Drug Dosing

Adult and Pediatric Subjects:

Adjusted Body Weight will be calculated as follows:

Ideal Body Weight + 0.25 (actual body weight – ideal body weight)

Body Weight Shifts After Initial Evaluation:

Individuals with significant body weight shifts after the initial evaluation will have the adjusted body weight reassessed by the study site's Clinical Nutrition Staff as appropriate.

APPENDIX E
LABORATORY ASSESSMENTS

APPENDIX E:
Protocol-Based Laboratory Procedures

There are three research laboratory studies associated with the BMT CTN 1904 clinical trial that are optional.

5. Treosulfan Pharmacokinetics (PK; Optional; Table 19)

Studies have demonstrated highly predictable PK in adult patients; however, limited data are available for pediatric patients. In addition, very little data is available on the relationship between Treosulfan exposure and HCT outcomes including early and late toxicities and engraftment. We plan on collecting PK samples on patients enrolled on this clinical trial in order to gain a better understanding of the relationship between the exposure to Treosulfan AUC to engraftment, transplant related mortality, overall and event-free survival for patients with BMFD. This information will help guide future studies using Treosulfan-based conditioning in patients with non-malignant diseases. This is an optional research study and Treosulfan PK data will not be used to adjust the patient's Treosulfan dose.

Treosulfan pharmacokinetic sampling and analysis on peripheral blood samples will be done on days -6 and -5. The same peripheral blood sample will be used for both Treosulfan and Treosulfan monoepoxide quantitation (samples will be split in PK Lab during analysis process). Peripheral blood samples (1 mL per sample) will be collected at 6 timepoints associated with both the day -6 and day -5 Treosulfan infusions. PK samples must be drawn from a different lumen/venous access than the drug infusion. If this is not possible and blood sampling needs to occur from the same lumen for Treosulfan infusion, document on the Treosulfan PK worksheet.

We will perform Treosulfan PK studies in approximately 5 patients for each of the following age groups: 1) 1 year to < 2 years, 2) 2 years to <4 years, 3) 4 years to <12 years and 4) ≥ 12 years (total patients: approximately 20).
For patients who enroll in the Treosulfan PK, the following will be required:

1) Clinical sites will need to accurately record infusion dates, infusion start and end times, as well as the timing of the PK blood sample collections.
2) Patient height, weight, BSA (within 3 days of starting Treosulfan)
3) Serum hematocrit and creatinine will need to be drawn with the patient's morning labs (prior to the start of Treosulfan) on days -6 and -5.
4) Due to Treosulfan interpatient variability which may be dependent on the patient's fluid status, sites will be required to document the total fluids given to patients (IV plus po) from the time point of admission to the hospital for initiation of conditioning through 24 hours after the Treosulfan infusion has completed.
5) Treosulfan and the monoepoxide degrade rapidly. Collected blood must be immediately placed on ice, centrifuged within 30 minutes of collection, and placed at -20C or below within 1 hour of blood draw. Within 5 days of blood collection, frozen plasma should be stored at -70C or below until shipment.

PK sampling kits will be provided by the BMT CTN, and locally processed frozen plasma samples will be periodically batch-shipped directly to the Seattle Cancer Care Alliance (SCCA) Pharmacokinetics Lab for temporary storage and batched PK testing and analysis. Comprehensive instructions detailing Treosulfan infusion, PK blood sample collection, sample stabilization, blood

sample processing and batched frozen plasma sample shipping will be provided in the BMT CTN 1904 Research Sample Information Guide.

Table 19: PK Sample Collection Schedule for Day -6 and Day -5 Treosulfan Infusions

Time Point	Collection Window	Blood Volume
Pre-infusion	Within 5 minutes before starting Treosulfan infusion	1 mL
End-of-infusion (EOI)	EOI + 5 minutes	1 mL
EOI + 20 minutes	+/- 5 minutes	1 mL
3.5 hours after start of infusion	+/- 10 minutes	1 mL
6 hours after start of infusion	+/- 10 minutes	1 mL
9 hours after start of infusion	+/- 10 minutes	1 mL

6. Biological Studies and Mutational Testing (Optional; Table 20)

Biological Studies

Recent attention has focused on genomic analysis of somatic mutations for risk stratification to inform treatments tailored to the individual patient. A subset of patients with BMFD are at high risk for regimen-related toxicities. In addition, a subset of patients with BMFD develop malignancies, predominantly solid tumors, post-HCT. Identification of patients at risk for these complications would allow appropriate adjustment of HCT regimens and institution of surveillance strategies for early tumor detection. Such genomic studies also stand to advance our understanding of the molecular pathogenesis of bone marrow failure to develop novel treatments.

The objective of the second laboratory correlate to this clinical trial will be to collect blood and/or marrow samples (if performed for clinical reasons) from consenting patients prior to the initiation of conditioning as well as around 1-year post-HCT. The goal of this research is to assess somatic genomic analysis of patients with severe bone marrow failure before and 1-year post-HCT, T-cell phenotypes, and comparison of cytokine levels before and after HCT. The objective is to identify risk factors associated with HCT complications. If samples have been donated and subject does not continue to transplant, samples will be stored for future research.

Mutational Testing

Over the past 5-10 years, there have been significant strides made in identifying new genetic mutations in patients with inherited BMFD. However, there remain a fair number of patients who are diagnosed with an underlying BMFD but lack a genetic mutation. One common reason is that commercial genetic panels are very different and do not analyze for all the known genetic mutations for these diseases. As has been mentioned above, a definite diagnosis is important for future management and surveillance of these patients. Dr. Adrianna Vlachos of Cohen Children's Medical Center, in collaboration with Dr. David Bodine at the National Human Genome Research Institute, will analyze peripheral blood on patients who lack a genetic mutation, and perform whole exome or whole genome sequencing with the goal of identifying new mutations for patients with BMFD.

Table 20: Optional Research Study Samples

Study Purpose	Sample Type	Sample Collection Summary	Sample Collection Time Points	Shipping Summary	Shipping Location
Biologic Studies	**Bone Marrow Aspirate** 8-10 mL (if a clinical marrow occurs)	Collect bone marrow aspirate sample and place: • 5 mL into a green top plastic BD Vacutainer® tube containing sodium heparin anticoagulant. • 3-5 mL into a lavender top plastic BD Vacutainer® tube containing EDTA anticoagulant.	**Pre-transplant** Within 60 days prior to enrollment **Post-transplant** Day 365 post-HCT	Blood and marrow samples will be shipped at ambient temperature on the day of collection to the TransLab project laboratory by priority overnight FedEx delivery for processing and research testing. Detailed instructions for specimen collection, handling, and shipment to TransLab will be provided in the BMT CTN 1904 Research Sample Information Guide.	**TransLab** Boston Children's Hospital
	Peripheral Blood 8-10 mL	Collect peripheral blood sample and place: • 5 mL into a green top plastic BD Vacutainer® tube containing sodium heparin anticoagulant. • 3-5 mL into a lavender top plastic BD Vacutainer® tube containing EDTA anticoagulant.			
Mutational Testing	**Peripheral Blood** 10 mL For Patients <30 kg 20 mL For Patients ≥30 kg	Collect peripheral blood sample and place into one or more lavender top plastic BD Vacutainer® tubes containing EDTA anticoagulant.	**Pre-transplant** Prior to the initiation of conditioning	Blood samples will be shipped at ambient temperature on the day of collection to the Bodine/NHGRI laboratory by priority overnight FedEx delivery for processing and research testing. Detailed instructions for specimen collection, handling, and shipment to the Bodine/NHGRI lab will be provided in the BMT CTN 1904 Research Sample Information Guide.	**Bodine Laboratory** National Human Genome Research Institute

APPENDIX F
DIAGNOSIS AND SEVERITY SCORING FOR ACUTE GVHD

Harris (MAGIC) Criteria

GVHD Target Organ Staging[67]

Stage	Skin (active erythema only)	Liver (total bilirubin)	Upper GI	Lower GI (stool output/day)
0	No active (erythematous) GVHD rash	< 2 mg/dl	No or intermittent nausea, vomiting or anorexia	Adult: < 500 ml/day or <3 episodes/day Child: < 10 ml/kg/day or <4 episodes/day
1	Maculopapular rash <25% BSA	2–3 mg/dl	Persistent nausea, vomiting or anorexia	Adult: 500–999 ml/day or 3–4 episodes/day Child: 10–19.9 ml/kg/day or 4–6 episodes/day
2	Maculopapular rash 25 – 50% BSA	3.1–6 mg/dl	-	Adult: 1000–1500 ml/day or 5–7 episodes/day Child: 20 – 30 ml/kg/day or 7–10 episodes/day
3	Maculopapular rash > 50% BSA	6.1–15 mg/dl	-	Adult: >1500 ml/day or >7 episodes/day Child: > 30 ml/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) 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).

Overall clinical grade (based upon most severe target organ involvement):

- Grade 0: No stage 1–4 of any organ
- Grade I: Stage 1–2 skin without liver, upper GI or lower GI involvement
- Grade II: Stage 3 rash and/or stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI
- Grade III: Stage 2–3 liver and/or stage 2–3 lower GI, with stage 0–3 skin and/or stage 0–1 upper GI
- Grade IV: Stage 4 skin, liver, or lower GI involvement, with stage 0–1 upper GI

Categories of Acute and Chronic GVHD

Categories of Acute and Chronic GVHD			
Category	**Time of Symptoms after HCT**	**Presence of Acute GVHD Features**	**Presence of Chronic GVHD Features**
Acute GVHD			
Classic acute GVHD	≤ 100 d	Yes	No
Late-onset acute GVHD	> 100 d	Yes	No
Chronic GVHD			
Classic chronic GVHD	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes

APPENDIX G
DIAGNOSIS AND SEVERITY SCORING FOR CHRONIC GVHD

(2014 NIH Consensus Criteria[62])

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capabl of self-care, >50% of waking hours ou of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN† **SCORE % BSA** ☐ *GVHD features to be scored by BSA:* **Check all that apply:** ☐ Maculopapular rash/erythema ☐ Lichen planus-like features ☐ Sclerotic features ☐ Papulosquamous lesions or ichthyosis ☐ Keratosis pilaris-like GVHD	☐ No BSA involved	☐ 1-18% BSA	☐ 19-50% BSA	☐ >50% BSA
SKIN FEATURES SCORE:	☐ No sclerotic features		☐ Superficial sclerotic features "not hidebound" (able to pinch)	**Check all that apply:** ☐ Deep sclerotic features ☐ "Hidebound" (unable to pinch) ☐ Impaired mobility ☐ Ulceration
Other skin GVHD features (NOT scored by BSA) **Check all that apply:** ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Poikiloderma ☐ Severe or generalized pruritus ☐ Hair involvement ☐ Nail involvement ☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
MOUTH *Lichen planus-like features present:* ☐ **Yes** ☐ **No**	☐ No symptoms	☐ Mild symptoms **with** disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				

Organ scoring of chronic GVHD. ECOG indicates Eastern Cooperative Oncology Group; KPS, Karnofsky Performance Status, Lansky Performance Status; BSA, body surface area; ADL, activities of daily living; LFTs, liver function tests; AP, alkaline phosphatase; ALT, alanine aminotransferase; ULN, normal upper limit. *Weight loss within 3 months. Skin scoring should use both percentage of BSA involved by disease signs and the cutaneous features scales. When a discrepancy exists between the percentage of total body surface (BSA) score and the skin feature score, OR if superficial sclerotic features are present (Score 2), but there is impaired mobility or ulceration (Score 3), the higher level should be used for the final skin scoring. To be completed by specialist or trained medical providers. **Lung scoring should be performed using both the symptoms and FEV1 scores whenever possible. FEV1 should be used in the final lung scoring where there is discrepancy between symptoms and FEV1 scores.

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist:* ☐ **Yes** ☐ **No** ☐ **Not examined**	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	☐ Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
GI Tract ***Check all that apply:*** ☐ Esophageal web/ proximal stricture or ring ☐ Dysphagia ☐ Anorexia ☐ Nausea ☐ Vomiting ☐ Diarrhea ☐ Weight loss ≥5%* ☐ Failure to thrive	☐ No symptoms	☐ Symptoms without significant weight loss* (<5%)	☐ Symptoms associated with mild to moderate weight loss* (5-15%) **OR** moderate diarrhea without significant interference with daily living	☐ Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
LIVER	☐ Normal total bilirubin and ALT or AP < 3 x ULN	☐ Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥ 3 x ULN	☐ Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	☐ Elevated total bilirubin > 3 mg/dL
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
LUNGS** **Symptom score**:	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring 0_2)
Lung score: **% FEV1** ☐	☐ FEV1≥80%	☐ FEV1 60-79%	☐ FEV1 40-59%	☐ FEV1 ≤39%
Pulmonary function tests ☐ Not performed				
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GENITAL TRACT (*See Supplemental figure*‡) ☐ Not examined *Currently sexually active* ☐ Yes ☐ No	☐ No signs	☐ Mild signs‡ and females with or without discomfort on exam	☐ Moderate signs‡ and may have symptoms with discomfort on exam	☐ Severe signs‡ with or without symptoms
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

Other indicators, clinical features or complications related to chronic GVHD (check all that apply and assign a score to severity (0-3) based on functional impact where applicable none – 0,mild -1, moderate -2, severe – 3)

☐ Ascites (serositis)___	☐ Myasthenia Gravis___	
☐ Pericardial Effusion___	☐ Peripheral Neuropathy___	☐ Eosinophilia > 500/µl___
☐ Pleural Effusion(s)___	☐ Polymyositis___	☐ Platelets <100,000/µl ___
☐ Nephrotic syndrome___	☐ Weight loss>5%* without GI symptoms ___	☐ Others (specify):_____

Overall GVHD Severity *(Opinion of the evaluator)*	☐ **No GVHD**	☐ **Mild**	☐ **Moderate**	☐ **Severe**

APPENDIX H
QUALITY OF LIFE QUESTIONAIRE USING THE PATIENTREPORTED OUTCOMES MEASUREMENT INFORMATIONSYSTEM (PROMIS)

QOL Study Design

The following instruments will be used to assess QOL within one month before transplant (Baseline), at 180 days and 1-year post-transplant. Only English- or Spanish-speaking trial participants aged 5 years and older will be included in the QOL collection. Patients aged 8-17 at the time of assessment will complete pediatric instruments. Proxy reports (e.g., parents,guardians) will be collected for patients aged 5 to 8.

At the Baseline time point, center or CIBMTR staff will administer QOL instruments electronically or on paper. At 180 day and 1 year time points, the CIBMTR will administer the QOL surveys electronically or on paper, per respondent need or preference. Each time point will take about 2-3 minutes to complete.

PROMIS Global

The PROMIS Global measure contains 10 items for adults and 9 items for pediatric patients (self- or proxy-report), with two summary scores for physical and mental functioning. The median score is 50 with a standard deviation of 10. Higher scores indicate better QOL[68].

Subject/Patient Reported Outcomes (PRO)

This includes all items/questions included on each patient- or proxy-reported QoL survey.

PROMIS Scale – Global Health (adult self-report)

Please respond to each question or statement by marking one box per row

	Excellent	Very good	Good	Fair	Poor
In general, would you say your health is:	☐	☐	☐	☐	☐
In general, would you say your quality of life is:.	☐	☐	☐	☐	☐
In general, how would you rate your physical health?	☐	☐	☐	☐	☐
In general, how would you rate your mental health, including your mood and your ability to think?	☐	☐	☐	☐	☐
In general, how would you rate your satisfaction with your social activities and relationships?	☐	☐	☐	☐	☐
In general, please rate how well you carry out your usual social activities and roles. (This includes activities at home, at work and in your community, and responsibilities as a parent, child, spouse, employee, friend, etc.)	☐	☐	☐	☐	☐

	Completely	Mostly	Moderately	A little	Not at all
To what extent are you able to carry out your everyday physical activities such as walking, climbing stairs, carrying groceries, or moving a chair?	☐	☐	☐	☐	☐

In the past 7 days…	Never	Rarely	Sometimes	Often	Always
To what extent are you able to carry out your everyday physical activities such as walking, climbing stairs, carrying groceries, or moving a chair?	☐	☐	☐	☐	☐

In the past 7 days…	None	Mild	Moderate	Severe	Very severe
How would you rate your fatigue on average?	☐	☐	☐	☐	☐

In the past 7 days…											
How would you rate your pain on average?	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	0 No pain	1	2	3	4	5	6	7	8	9	10 Worst pain imaginable

PROMIS Scale – Global Health (pediatric self-report)

Please respond to each question or statement by marking one box per row

	Excellent	Very good	Good	Fair	Poor
In general, would you say your health is:	☐	☐	☐	☐	☐
In general, would you say your quality of life is:	☐	☐	☐	☐	☐

	Excellent	Very good	Good	Fair	Poor
In general, how would you rate your physical health?	☐	☐	☐	☐	☐
In general, how would you rate your mental health, including your mood and your ability to think?	☐	☐	☐	☐	☐

	Never	Rarely	Sometimes	Often	Always
How often do you feel really sad?	☐	☐	☐	☐	☐

	Always	Often	Sometimes	Rarely	Never
How often do you have fun with friends?	☐	☐	☐	☐	☐
How often do your parents listen to your ideas?					

In the past 7 days…	Never	Almost Never	Sometimes	Often	Always
I got tired easily	☐	☐	☐	☐	☐
I had trouble sleeping when I had pain	☐	☐	☐	☐	☐

PROMIS Scale – Global Health (pediatric parent proxy report)

Please respond to each question or statement by marking one box per row

	Excellent	Very good	Good	Fair	Poor
In general, would you say your child's health is:	☐	☐	☐	☐	☐
In general, would you say your child's quality of life is:.	☐	☐	☐	☐	☐
In general, how would you rate your child's physical health?	☐	☐	☐	☐	☐
In general, how would you rate your child's mental health, including your mood and your ability to think?	☐	☐	☐	☐	☐

	Never	Rarely	Sometimes	Often	Always
How often does your child feel really sad?	☐	☐	☐	☐	☐

	Always	Often	Sometimes	Rarely	Never
How often does your child have fun with friends?	☐	☐	☐	☐	☐
How often does your child feel that you listen to his or her ideas?	☐	☐	☐	☐	☐

In the past 7 days…	Never	Almost Never	Sometimes	Often	Always
My child got tired easily	☐	☐	☐	☐	☐
My child had trouble sleeping when he/she had pain	☐	☐	☐	☐	☐

APPENDIX I
DERIVATION OF A SQUENTIAL TEST STATISTIC FORCENSORED EXPONENTIAL DATA

APPENDIX I

DERIVATION OF A SQUENTIAL TEST STATISTIC FOR CENSORED EXPOENTIAL DATA

Background – The Sequential Probability Ratio Test

Let $f(.,\theta)$ be the density function for random variable X. According to Neyman and Pearson, the most powerful test of $H_0:\theta=\theta_o$ versus $H_1:\theta=\theta_1$ decides in favor of H_1 or H_0 if $L_n > c_\alpha$ or $L_n < c_\alpha$ or $L_n < c_\alpha$, respectively, where $L_n = \prod_i^n f(x_i;\theta_1)/f(x_i;\theta_0)$ is the likelihood ratio, and c_α is determined to have the size α. When the sample size is not fixed in advance, further improvement is possible by using Wald's Sequential Probability Ratio Test (SPRT). The SPRT continues to sample as long as $B < L_n < A$ for some constant $B<1<A$, stops sampling and decides in favor of H_1 as soon as $L_n > A$, and stops sampling and decides in favor of H_0 as soon as $L_n < B$.

The usual measures of performance of such a procedure are the error probabilities α and β of rejecting H_0 when $\theta=\theta_0$, and of accepting H_0 when $\theta=\theta_1$, respectively, and the expected sample size $E(N\mid\theta_j)\equiv E_j(N)$. Wald and Wolfowitz showed that among all tests, sequential or not, for which $\Pr_0(\text{reject } H_0)\le\alpha$ and $\Pr_1(\text{reject } H_0)\le\beta$, and for which $E_j(N)$ are finite, j=0,1, the SPRT with error probabilities α and β minimizes $E_0(N)$ and $E_1(N)$. If, in addition, the $x1, x2,\ldots$ are independent and identically distributed (i.i.d.) with density function $f(x,\theta)$, with monotone likelihood ratio in $\tau(x)$, then any SPRT for testing θ_0 against $\theta_1(>\theta_0)$ has non-decreasing power function.

For the SPRT with error probabilities α and β, the SPRT boundaries are given approximately by $A=(1-\beta)/\alpha$ and $B=\beta/(1-\alpha)$. The operating characteristics of the SPRT are given by $O(\theta,\alpha,\beta,\theta_0,\theta_1)=(A^{h(\theta)}-1)/(A^{h(\theta)}-B^{h(\theta)})$ where $h(\theta)$ is the non-trivial solution to the equation $\int(f(x;\theta_1)/f(x,\theta_2))^{h(\theta)}f(x;\theta)dx=1$.

The formula $E(N;\theta)=[[(1-O(\theta)]\log A+O(\theta)\log B]/E(z;\theta)$ provides the average sample number for an arbitrary θ. The sample size distribution is very highly skewed, $Var(N)\approx[E(N)]^2$. Thus, we will consider a truncated test with maximum sample size of N_0 and simulate to obtain the operating characteristics of the test.

Derivation of the SPRT for Censored Exponential Survival Times

Suppose that we wish to construct a sequential test for the composite null hypothesis that the rate of overall mortality at an early time point t is less than or equal to p_0 versus the alternative hypothesis that it is greater than or equal to p_0. Let us assume that the survival times, $T_1, T_2, ..., T_n$, are i.i.d. with exponential density function $f(T,\theta) = \theta e^{-\theta T}$. Although an exponential model may not fit well for overall mortality, it usually provides a reasonable model over a short time frame for modeling toxicity, so in all discussion below we assume that exponential survival times are censored at time point t. In the exponential parameterization, a t-day survival rate of p_0 translates into a mean survival of μ_0=-t/ln(1-p_0) (rate parameter θ_0 = -ln(1-p_0)/t).

The SPRT is derived with reference to a simple null and alternative hypothesis for the rate parameter, in this case, $H_0 : \theta = \theta_o$ versus $H_1 : \theta = \theta_1$. The log-likelihood ratio for the exponential in the presence of censoring is $\log\prod_i^n f(x_i;\theta_1) - \log\prod_i^n f(x_i,\theta_0) = d(\log(\theta_1) - \log(\theta_0)) - (\theta_1 - \theta_0)\sum_i^n T_i$, where d is the number of events. The SPRT can be represented graphically when plotting the number of deaths (d) on the y axis against the total time on study $\sum_i^n T_i$ on the x axis. The continuation region in terms of d is bounded by two parallel lines given by

$$\left[\frac{\log(B)}{(\log\theta_1 - \log\theta_0)}\right] + \left[\frac{(\theta_1 - \theta_0)}{(\log\theta_1 - \log\theta_0)}\right]\sum_i^n T_i < d < \left[\frac{\log(A)}{(\log\theta_1 - \log\theta_0)}\right] + \left[\frac{(\theta_1 - \theta_0)}{(\log\theta_1 - \log\theta_0)}\right]\sum_i^n T_i$$

with common slope $(\theta_1 - \theta_0)/(\log\theta_1 - \log\theta_0)$, and intercepts $\log A/(\ln\theta_1 - \ln\theta_0)$ and $\log B/(\ln\theta_1 - \ln\theta_0)$, for the upper and lower bounds, respectively. For monitoring purposes, at an interim analysis calendar time point s, suppose that d(s) events have occurred and that the total time on study is $\sum_i^n T_i(s)$ The cumulative number of events d(s) is plotted on the y axis against the total time on study, $\sum_i^n T_i(s)$ When this graph crosses the upper boundary, the null hypothesis is rejected.

In practice, monitoring will be scheduled monthly after the start of enrollment to the study.

A truncated version of the SPRT can be obtained by specifying a maximum sample size. We truncate the SPRT by declaring that if the test has failed to terminate after the maximum sample size, that the null hypothesis will be accepted. Since the probability that the untruncated SPRT would reject the null at the maximum sample size is negligible, it makes little difference how the final boundary value is selected, and this rule is chosen for simplicity. The operating characteristics of this proposed truncated SPRT for censored exponential data can be estimated by simulation.

APPENDIX J
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