BLOOD & MARROW TRANSPLANTION PROGRAM
UNIVERISTY OF MINNESOTA

SINGLE-ARM, OPEN LABEL, INTERVENTIONAL PHASE II CLINICAL TRIAL EVALUATING MGTA-456 IN PATIENTS WITH HIGH-RISK MALIGNANCY

CPRC 2018LS051
MT2018-06
IND # 18372

IND Sponsor:	John E. Wagner, MD
Principal Investigator:	Margaret L. MacMillan, MD
Lead Co-Investigator:	Claudio Brunstein, MD, PhD
Co-Investigators:	Shernan Holtan, MD Christen Ebens, MD
RadiationTherapy:	Kathryn Dusenbery, MD
MCT cGMP Facility:	David H. McKenna, Jr., MD
Biostatistician:	Todd E. DeFor, MS
Development Phase:	2
Version Date:	April 23, 2021

REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Revision
	04/01/2018	Original to CPRC	
1A	05/02/2018	Updated background section 2.2 with pediatric data; changed abbreviations MAC and NMAC to MA and NMA; section 6.4 fixed typos per CPRC stipulations	
1B	06/12/2018	Section 4.2 & eligibility checklist – edits and corrections to bring study to current standard of care; schedules standard and research assessments edited for current standard testing and added research labs; minor administrative edits and corrections.	
1C	08/13/2018	Title page – added IND number; Section 4.2 & Eligibility checklist– added minimum weight requirement (≥11kg); removed Lymphoplasmacytic lymphoma and clarified myelodysplastic syndrome refractory anemia; in Section 4.4 & eligibility checklist – clarified ineligibility after prior autologous or allogeneic transplant and added additional ineligibility criteria added, eg patients with a HLA matched sibling and, patients with a HLA matched unrelated donor who is available at the time the transplant is needed.	No
1D	08/23/2018	Sections 2.2 and Section 2.5 - updated background section of the consent based on IRB stipulations and fixed typographical error in the inclusion criteria and eligibility checklist; changed references of study product from HSC835v1 to MGTA--456	Yes
2	09/28/2018	Updates made by pharmacy to conform to local standard clinical care: -Updated treatment plan and section 6 to reflect Cy to be given on days -7 & -6 -Removed wording that fludarabine had to be given at 10am in section 6.1.1.1 and section 6.1.2.2 -Updated Cytoxan adjusted body weight dosing calculation to local standard in section 6.1.1.2 -Section 6.2.2 clarified peds MMF dosing - Appendix III – added Bartelink dosing nomogram table	
3	12/20/2018	Section 4.1 Updated to note that cord blood selection will be per Magenta's algorithm Section 8 – removed 1 and 2 year chest x-ray; updated research labs Appendix III – Inpatient Pharmacy updated busulfan dosing and monitoring guidelines Consents – updated information on sponsor data use	Yes
3A	01/07/2019	Section 4.1 minor edits for clarity Appendix III – Inpatient Pharmacy updated busulfan dosing and monitoring guidelines Parent consent age 0-3, error correction on study calendar	Yes

4	04/24/2019	Section 1.6 (Exploratory Endpoints), Section 2.6, Section 6.5, Section 7.3, Section 8, Section 12.2, References: Added Immune reconstitution sub-study in order to investigate long term functional immune response Synopsis, Section 4.1, and section 6.3: Clarified selection criteria and parameters of required cell dose Section 8, noted that chimerism will not be drawn if WBC < 500	Yes
5	11/25/2019	Section 6.5, Section 8, - clarified that vaccinations may not be given if active acute or chronic GVHD or other evidence of poor immunologic recovery. Section 6.3, Section 8 – clarified cell dosing; added that there is no lower limit for TNC. Section 8 – Standard of care calendar, added Tcell subsets Removed Appendix I eligibility checklist, per new SOP, this document is kept in Oncore. Throughout document minor edits for consistency and clarity	No
6	04/23/2021	Dr.MacMillan takes over as principal investigator; Dr. Stefanski removed from study	Yes

Table of Contents

Sponsor

John E. Wagner, MD
Director and Professor
Division of Blood and Marrow Transplantation
Department of Pediatrics
660 Cancer Research Building
425 East River Road
Minneapolis, MN 55455
612 626-2961 (phone)
612 626-4074 (fax)
wagne002@umn.edu (email)

Principal Investigator/Contact Information:	**Lead Co- Investigator/Contact Information:**
Margaret L. MacMillan, MD, MSc, FRCPC Professor of Pediatrics Blood and Marrow Transplantation & Cellular Therapy Program University of Minnesota MMC 484 420 Delaware Street SE Minneapolis, MN 55455 612 626-2961 (phone) 612-626-2815 (fax) macmi002@umn.edu (email)	Claudio Brunstein, MD, PhD Associate Professor Division of Hematology, Oncology and Transplantation Department of Medicine Mayo Mail Code 480 420 Delaware Street SE Minneapolis, MN 55455 612 625-3918 (phone) 612 899-6170 (pager) bruns072@umn.edu

LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AE	ADVERSE EVENT
aGVHD	ACUTE GRAFT VERSUS HOST DISEASE
AHR	ARYL HYDROCARBON RECEPTOR
ALL	ACUTE LYMPHOCYTIC LEUKEMIA
ALT	ALANINE AMINOTRANSFERASE
AML	ACUTE MYELOCYTIC LEUKEMIA
ANC	ABSOLUTE NEUTROPHIL COUNT
AST	ASPARTATE AMINOTRANSFERASE
ATG	ANTITHYMOCYTE GLOBULIN
AUC_{cum}	CUMULATIVE AREA UNDER THE PLASMA CONCENTRATION-TIME CURVE
AUC	AREA UNDER THE PLASMA CONCENTRATION-TIME CURVE
AUC_{∞}	AREA UNDER THE PLASMA CONCENTRATION-TIME CURVE FROM TIME ZERO TO INFINITY
AUC_{last}	AREA UNDER THE PLASMA CONCENTRATION-TIME CURVE FROM TIME ZERO TO TIME OF LAST MEASURABLE CONCENTRATION
BMT	BONE MARROW TRANSPLANT
BU	BUSULFAN
CBU	CORD BLOOD UNIT
CFR	CODE OF FEDERAL REGULATIONS
cGVHD	CHRONIC GRAFT VERSUS HOST DISEASE
CLIA	CLINICAL LABORATORY IMPROVEMENT AMENDMENT
C_{max}	MAXIMUM OBSERVED CONCENTRATION
CML	CHRONIC MYELOGENOUS LEUKEMIA
CMV	CYTOMEGALOVIRUS
COBLT	CORD BLOOD TRANSPLANT CONSORTIUM
CRF	CASE REPORT FORM
CsA	CYCLOSPORINE A
CTCAE	COMMON TOXICITY CRITERIA ADVERSE EVENT
CTO	CLINICAL TRIALS OFFICE
CY	CYCLOPHOSPHAMIDE
DNA	DEOXYRIBONUCLEIC ACID
DMC	DATA MONITORING COMMITTEE
DUCBT	DOUBLE UMBILICAL CORD BLOOD TRANSPLANTATION
ECG	ELECTROCARDIOGRAM
EBV	EPSTEIN-BARR VIRUS
EFS	EVENT FREE SURVIVAL
FLU	FLUDARABINE
FDA	FOOD AND DRUG ADMINISTRATION
GCP	GOOD CLINICAL PRACTICE

ABBREVIATION	DEFINITION
G-CSF	GRANULOCYTE-COLONY STIMULATING FACTOR
GVHD	GRAFT VERSUS HOST DISEASE
HBcAb	HEPATITIS B CORE ANTIBODY
HBsAg	HEPATITIS B SURFACE ANTIGEN
hCG	HUMAN CHORIONIC GONADOTROPIN
HCV	HEPATITIS C VIRUS
HIV	HUMAN IMMUNODEFICIENCY VIRUS
HLA	HUMAN LEUKOCYTE ANTIGEN
HSC	HEMATOPOIETIC STEM CELLS
HSCT	HEMATOPOIETIC STEM CELL TRANSPLANTATION
HSV	HERPES SIMPLEX VIRUS
HTLV1/2	HUMAN T CELL LYMPHOTROPIC VIRUS 1/2
IB	INVESTIGATOR'S BROCHURE
ICF	INFORMED CONSENT FORM
ICH	INTERNATIONAL COUNCIL ON HARMONIZATION
IEC	INDEPENDENT ETHICS COMMITTEE
IRB	INSTITUTIONAL REVIEW BOARD
IUD	INTRAUTERINE DEVICE
IUS	INTRAUTERINE SYSTEM
IV	INTRAVENOUS
LDH	LACTATE DEHYDROGENASE
LFT	LIVER FUNCTION TEST
LLOQ	LOWER LIMIT OF QUANTIFICATION
LMW	LOW MOLECULAR WEIGHT
LVEF	LEFT VENTRICULAR EJECTION FRACTION
MA	MYELOABLATIVE CONDITIONING
Magenta	MAGENTA THERAPEUTICS, INC.
MDS	MYELODYSPLASTIC SYNDROME
MFI	MEAN FLUORESCENCE INTENSITY
MedDRA	MEDICAL DICTIONARY FOR REGULATORY ACTIVITIES
MMF	MYCOPHENOLATE MOFETIL
MSD	MATCHED SIBLING DONOR
NC	NUCLEATED CELLS
NMA	NON-MYELOABLATIVE CONDITIONING
NOAEL	NO OBSERVED ADVERSE EFFECT LEVEL
PES	PRE-ENGRAFTMENT SYNDROME

ABBREVIATION	DEFINITION
PFT	PULMONARY FUNCTION TEST
PK	PHARMACOKINETIC
PBMC	PERIPHERAL BLOOD MONONUCLEAR CELLS
SAB	SINGLE ANTIGEN BEAD
SAE	SERIOUS ADVERSE EVENT
SOP	STANDARD OPERATING PROCEDURE
SR-1	STEMREGENIN-1
SUCBT	SINGLE UMBILICAL CORD BLOOD TRANSPLANTATION
TBI	TOTAL BODY IRRADIATION
TCF	TOTAL BODY IRRADIATION, CYCLOPHOSPHAMIDE, FLUDARABINE CONDITIONING
TEAE	TREATMENT-EMERGENT ADVERSE EVENTS
TKI	TYROSINE KINASE INHIBITOR
TRALI	TRANSFUSION-RELATED ACUTE LUNG INJURY
TNC	TOTAL NUCLEATED CELL
TREC	T CELL RECEPTOR EXCISION CIRCLES
TRM	TRANSPLANT-RELATED MORTALITY
UCB	UMBILICAL CORD BLOOD
UCBT	UMBILICAL CORD BLOOD TRANSPLANT
ULN	UPPER LIMIT OF NORMAL
US	UNITED STATES
WHO	WORLD HEALTH ORGANIZATION

Protocol Synopsis

SINGLE-ARM, OPEN LABEL, INTERVENTIONAL PHASE II CLINICAL TRIAL EVALUATING MGTA-456 IN PATIENTS WITH HIGH-RISK MALIGNANCY

Study Design:	This is an single arm, open label, interventional phase II trial evaluating the efficacy of umbilical cord blood (UCB) hematopoietic stem and progenitor cells (HSPC) expanded in culture with stimulatory cytokines (SCF, Flt-3L, IL-6 and thromopoietin) in the presence of an aryl hydrocarbon receptor antagonist, LHD221, on lympho-hematopoietic recovery. Patients will receive a uniform myeloablative conditioning and post-transplant immunoprophylaxis.
Investigational Agent:	MGTA-456 is the cellular product composed of the UCB hematopoietic stem and progenitor cells after expansion culture in the presence of the low molecular compound LHD221 and its companion CD34 depleted fraction.
Primary Objective:	Determine if transplantation of MGTA-456 significantly reduces the expected duration of neutropenia and number of hospital days to day 100 relative to that observed in recipients of an unmanipulated single UCB unit
Primary Endpoint:	Incidence of neutrophil recovery by day 14
Secondary Endpoints:	• Number of days alive without hospitalization between days 0 and 100 after transplantation • Incidence of secondary graft failure • Incidence of platelet recovery at day 42 • Incidence of transplant-related mortality (TRM) at 6 months
Transplant Related Secondary Endpoints:	• Incidence of grades II-IV and III-IV acute graft vs host disease (GVHD) at day 100 • Incidence of chronic GVHD at 1 year • Incidence of relapse at 2 years • Incidence of non-catheter associated bacterial infections by day 100 • Probability of overall survival and event-free survival (EFS) at 2 years
Exploratory Endpoints:	• Description of immunological recovery • Incidence of absolute CD4 T cell ≥0.2 x 10^9/L by day 100 • Incidence of opportunistic infection (viral/fungal) by day 100 • Description of hospital costs by day 100 • Description of number of red cell and platelet transfusions by day 100 • Assess B and T cell immune reconstitution as well as functional responses at day 100 and day 180
Eligible Diseases:	Acute myeloid leukemia (AML) Acute lymphocytic leukemia (ALL) Biphenotypic/undifferentiated leukemia

	Chronic myelogenous leukemia excluding refractory blast crisis Myelodysplasia (MDS) IPSS Int-2 or High risk (i.e. RAEB, RAEBt) or other high risk features
Unit Selection for Expansion Culture:	Cell dose ≥1 x 10^7 nucleated cells per kilogram (kg) recipient body weight and HLA match at a minimum of 5 of 8 HLA alleles at high resolution A, B, C, DRB1 typing- based on the current Magenta cord blood algorithm.
Transplant HSC Product:	Cryopreserved MGTA-456 with a cell dose of a minimum of 1 x 10^6 CD34/kg but ideally will be 10 x 10^6 per kg recipient body weight and the cryopreserved CD34-fraction.
Key Inclusion Criteria:	Aged ≤ 55 years Weight ≥11kg Karnofsky performance status ≥70 (≥16 years) or Lansky Play Score ≥50 Adequate organ function (per section 4.3)
Key Exclusion Criteria:	Available HLA matched related or unrelated donor Pregnancy or breastfeeding History of HIV infection or known HIV positive serology Active serious infection Prior autologous or allogeneic hematopoietic stem cell transplant
Accrual Objective:	40 patients enrolled over 3 years

Treatment Plan

All patients will receive MGTA-456 on the day of transplantation after myeloablative conditioning. All patients aged 3-55 years will be conditioned with cyclophosphamide (CY) 120 mg/kg total dose, fludarabine (FLU) 75 m/m2 total dose and total body irradiation (TBI) 1320 cGy total dose as well as tacrolimus (Tac) and mycophenolate mofetil (MMF) immunoprophylaxis and granulocyte-colony stimulating factor (G-CSF) as detailed below:

Day	Conditioning Regimen	Immunoprophylaxis Hematopoietic Growth Factor
-8	FLU 25 mg/m^2 IV over 1 hour (<10 kg: 0.83 mg/kg IV over 1 hour)	
-7	FLU 25 mg/m^2 IV over 1 hour (<10 kg: 0.83 mg/kg IV over 1 hour) CY 60 mg/kg IV over 2 hours	
-6	FLU 25 mg/m^2 IV over 1 hour (<10 kg: 0.83 mg/kg IV over 1 hour) CY 60 mg/kg IV over 2 hours	
-5	Rest	
-4	TBI 165 cGy twice daily	
-3	TBI 165 cGy twice daily	Begin Tac / MMF (section 6.2)
-2	TBI 165 cGy twice daily	
-1	TBI 165 cGy twice daily	
0	MGTA-456 infusion	
+1		Begin G-CSF 5 ug/kg/d until the absolute neutrophil count (ANC) is $\geq$2500/uL for 2 consecutive days (section 6.4)

All young children $\leq$3 years of age at the time of diagnosis will receive MGTA-456 on the day of transplantation after a non-TBI containing myeloablative conditioning as TBI may have a damaging effect on brain development in the very young child. All patients aged 0-3 years will be conditioned with busulfan (BU), FLU and melphalan (MEL) as well as Tac/MMF immunoprophylaxis and G-CSF, as detailed below:

Day	Conditioning Regimen	Immunoprophylaxis Hematopoietic Growth Factor
-8	BU IV once daily with dose based on Pharmacokinetics (PK) calculator over 3 hours	
-7	Bu IV once daily adjusted based on PK results over 3 hours	
-6	Bu IV once daily adjusted based on PK results	

Day	Conditioning Regimen	Immunoprophylaxis Hematopoietic Growth Factor
-5	Bu IV once daily adjusted based on PK results over 3 hours	
-4	FLU 25 mg/m2/day (0.83 mg/kg/day if < 10 kg*) IV over 60 min MEL 50 mg/m2/day (1.7 mg/kg/day if < 10 kg*) IV over 30 min	
-3	FLU 25 mg/m2/day (0.83 mg/kg/day if < 10 kg*) IV over 60 min MEL 50 mg/m2/day (1.7 mg/kg/day if < 10 kg*) IV over 30 min	Begin Tac / MMF (section 6.2)
-2	FLU 25 mg/m2/day (0.83 mg/kg/day if < 10 kg*) IV over 60 min MEL 50 mg/m2/day (1.7 mg/kg/day if < 10 kg*) IV over 30 min	
-1	Rest	
0	MGTA-456 Infusion	
+1		Begin G-CSF 5 ug/kg/d until the absolute neutrophil count (ANC) is $\geq$2500/uL for 2 consecutive days (section 6.4)

*Patients <11kg are currently ineligible because of the expected volume of DMSO in thawed products. Once a wash step after thawing has been validated and approved by the FDA, patients <11 kg may be eligible.

1 Hypothesis and Objectives

1.1 Hypothesis

In vitro expansion of the hematopoietic stem and progenitor cells (HSPC) obtained from a single umbilical cord blood (UCB) unit prior to transplantation will significantly reduce the number of days of neutropenia relative to that observed after the transplantation of an unmanipulated single UCB unit (standard of care).

1.2 Primary Objective

Determine if transplantation of MGTA-456 significantly reduces the expected duration of neutropenia and number of hospital days to day 100 relative to that observed in recipients of an unmanipulated single UCB unit.

1.3 Primary Endpoint

Determine the incidence of neutrophil recovery by day 14 after transplantation in recipients of MGTA-456.

1.4 Secondary Endpoints

- Number of days alive without hospitalization between days 0 and 100 after transplantation
- Incidence of secondary graft failure
- Incidence of platelet recovery at day 42
- Incidence of TRM at 6 months

1.5 Transplant Related Secondary Endpoints

- Incidence of grades II-IV and III-IV acute GVHD at day 100
- Incidence of chronic GVHD at 1 year
- Incidence of relapse at 2 years
- Incidence of non-catheter associated bacterial infections by day 100
- Probability of overall survival (OS) and event-free survival (EFS) at 2 years

1.6 Exploratory Endpoints

Additional analyses will be performed that may be important for the design of future trials. These include:

- Description of immunological recovery
- Incidence of absolute CD4 T cell $\geq 0.2 \times 10^9$/L by day 100
- Incidence of opportunistic infection (viral / fungal) by day 100
- Description of hospital costs by day 100
- Description of number of red cell and platelet transfusions by day 100
- Assess B and T cell immune reconstitution as well as functional responses at day 100 and day 180.

2 Background

Hematopoietic stem cell transplantation (HSCT) is a standard treatment option for an increasing number of malignant and non-malignant disorders [1]. To reconstitute hematopoiesis after an intensive myeloablative therapy, the transplantation of hematopoietic stem cells (HSCs) is required. Such HSCs are typically recovered from the bone marrow or apheresed peripheral blood of the patients themselves, or suitably HLA matched related and unrelated volunteer donors [1,2]. Unfortunately, suitable donors are frequently not available either because the patient's own marrow is contaminated with tumor cells or potential allogeneic marrow donors are HLA mismatched or not healthy enough due to preexisting co-morbidities [3]. UCB is an established alternative source of HSCs that is capable of reconstituting hematopoiesis after intensive myeloablative therapy. It is immediately available at the time it is needed (in contrast to unrelated volunteer donors which takes a median of 3-4 months) and poses no risk to the donor [4,5].

2.1 Overview of UCB Transplantation

As a result of the early successes with UCBT from sibling donors [6,7], pilot programs for the banking of unrelated donor UCB were initiated in many countries worldwide [8,9,10]. In addition to rapid availability, absence of donor risk, absence of donor attrition, and very low risk of transmissible infectious diseases, such as CMV and EBV, UCB is associated with a lower risk of acute GVHD despite HLA mismatch. UCB is especially beneficial for patients of ethnic and racial minority descent for whom suitably matched adult marrow and blood donors often cannot be identified [11]. UCBT is also associated with a potent anti-leukemia effect [12, 13, 14], lost in recipients of T cell depleted HSCs. While there is growing interest in the use of HLA mismatched haploidentical related donors with promising early data using post-transplant cyclophosphamide (CY) [15, 16], risks of relapse and clonal hematopoiesis has been observed [17]. Therefore, UCB remains an important alternative particularly for children and young adults.

The University of Minnesota has the largest single center experience worldwide with UCBT, having performed more than 1400 primary transplants with UCB between January 1, 1992 and the present.

2.1.1 Single Unit UCBT

2.1.1.1 University of Minnesota Single Center Experience [18]

Between 1994 and 2001, 102 consecutive patients (median age 7.4 years) received a single, unrelated UCB unit after a myeloablative conditioning for malignant (n = 65; 68% high-risk) and non-malignant diseases (n = 37). The median infused cell dose of UCB was 3.1 x 10^7 nucleated (NC)/kg (range 0.7-57.9), and 2.8 x 10^5 CD34+ cells/kg (range 0.4-39.1). Fourteen percent had an HLA matched unit with the remaining 86% having a mismatch at 1 to 3 HLA-antigens. Neutrophil recovery (defined as an absolute neutrophil count [ANC] ≥ 500/uL) occurred at a median of 23 days (range 9-54) with the cumulative incidence of engraftment of 88% (95% CI: 81-95) by day 42. Speed of neutrophil recovery and engraftment were strongly associated with cell dose, with markedly inferior engraftment (72% at a median of 34 days) in patients receiving a CD34+ cell dose <1.7 x 10^5 cells/kg.

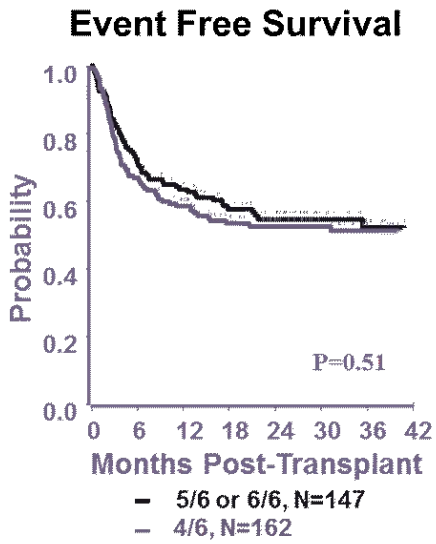
The incidences of grade II-IV and III-IV acute GVHD were 39% (95% CI: 29-49) and 11% (95% CI: 5-17), respectively, at day 100, with 10% (95% CI: 4-14) of patients having chronic GVHD at 1 year. One year TRM was 30% (95% CI: 21-39) which was strongly associated with CD34+ cell dose. The probabilities of 1 and 2-year survival were 58% (95% CI: 49-70) and 47% (95% CI: 36-57), respectively. Importantly, with a graft cell dose $\geq$1.7 x 10^5 CD34+ cells/kg, survival was 70% (95% CI: 49-90) at 1 year.

The principal conclusions of this study were: 1) a CD34 dose threshold is $\geq$1.7 x 10^5/kg and nucleated cell dose threshold is $\geq$2.5 x 10^7/kg; 2) neutrophil recovery is slow at a median of 23 days with more rapid recovery in recipients of higher cell doses, 3) risks of acute and chronic GVHD are low despite HLA mismatch; 4) cell dose significantly limits the applicability of UCB, particularly in adult size recipients.

2.1.1.2 National COrd BLood Transplant (COBLT) Study [19]

On the basis of promising single center results and registry reports, the National Heart, Lung and Blood Institute (NHLBI) proposed COBLT study, a phase 2 trial to determine if unrelated UCB could serve as an adequate HSC source. The primary endpoint of the study was survival at 180 days after transplantation. The secondary endpoints included engraftment (neutrophil and platelet), acute and chronic GVHD, disease-free survival, long-term survival, relapse, and regimen-related toxicities. For children with malignancy over the age of 2 years, the conditioning regimen and GVHD prophylaxis consisted of TBI 1350 cGy, CY 120 mg/kg and anti-thymocyte globulin (ATG, equine) 90 mgkg and CsA and methylprednisolone (MP) for GVHD prophylaxis.

Figure 1



One hundred and ninety-three children with hematologic malignancy were enrolled. The median age was 7.7 years (range, 0.9-17.9 years) and median weight was 25.9 kg (range, 7.5-118.4 kg) with 40% being nonwhite, 61% being male, and 51% being seropositive for cytomegalovirus (CMV). The majority of patients had either ALL (n = 109 [57% of total patients] with 17 in first complete remission [CR]; 65 in second CR; 20 in third CR; 6 in relapse; and, 1 with primary induction failure) or AML (n = 51 [27%] with 13 in first CR; 21 in second CR; 6 with primary induction failure; and 11 in relapse). The UCB grafts were 6/6 HLA matched (8.9%), 5/6 matched (30.4%) or 4/5 matched (58.1%). The median TNC, $CD34^+$, and CD3 doses of the UCB grafts were 5.1 × 10^7 cells/kg (range, 1.5-23.7), 1.9 × 10^5/kg (range, 0.0-25.3), and 7.9 × 10^6/kg (range, 0.1-35.6), respectively.

The incidence of neutrophil recovery by day 42 was 79.9% (95% confidence interval [CI] 75.1%-85.2%) with 14 patients recovering neutrophils between days 43 and 90. At 6 months, 86.9% of patients had sustained neutrophil engraftment. The median time to neutrophil recovery was 27 days (range, 11-90 days). Primary and secondary graft failure occurred in 21 and 2 patients, respectively. The incidence of platelet engraftment (defined as platelet count ≥ 50 000/uL without transfusion for 1 week) was 50.0% (95% CI, 42.1%-56.5%) at day 180. Notably, the probability of EFS was 49.5% at 2 years with no impact of HLA match based on HLA A and B at antigen level and DRB1 at allele level (Figure 1) and the incidences of grade II-IV acute GVHD at day 100, chronic GVHD at 2 years, and relapse at 2 years were 19.5%, 21% and 20%, respectively.

In univariate analysis, lower recipient weight (p=.02), better allele level HLA match (p=.03), higher TNC (p=.03), and higher $CD34^+$ dose (p=.01) positively impacted neutrophil recovery. In a multivariate Cox model, only better allele level HLA match (p=.04) and higher TNC dose were independently significant (p=.04).

The principal conclusions of this study were: 1) slow neutrophil recovery and poor engraftment are significant limitations of UCBT, 2) risks of GVHD are low relative to other HSC sources, and 3) HLA mismatch is tolerable with improved survival in recipients of better HLA matched grafts with higher TNC.

2.1.2 Double Unit UCBT

2.1.2.1 Minnesota Pilot Experience [20, 21]

Since cell dose was identified as a major limitation of UCBT, often preventing the consideration of UCB for adult recipients, we explored the possibility of infusing two partially HLA matched units to augment cell dose. After 2000, two UCB units were increasingly utilized, particularly in adults and adolescents for whom an adequately dosed single UCB unit could not be identified. The underlying hypothesis was that the addition of the second unit would enhance the engraftment and the speed of hematopoietic recovery.

In the first pilot study [20] of double UCBT (DUCBT), 31 adult and adolescent patients [median age 24 years (range: 13-53); median weight 73 kg (range: 48-120)] with high-risk hematologic malignancy were transplanted with two partially HLA-matched UCB units after a myeloablative conditioning. The median total infused dose 3.7 x 10^7 nucleated cells (NC) per kilogram (range 1.1-6.3) and 4.9 x 10^5 CD34 per kilogram (range, 0.9-14.5).

Of 29 patients surviving 21 days after transplant, 100% engrafted at a median of 23 days (range 14-41), comparing favorably with 65% engraftment in the prior 23 adults treated with a single UCBT. The incidence of platelet recovery (>50,000/uL) was 73% (95% CI, 51-95) at day 180. Incidence of grades II-IV and III-IV acute GVHD was 65% (95% CI, 42-88) and 17% (95% CI, 2-32) at day 100. EFS was 72% at 1 year for patients transplanted in CR.

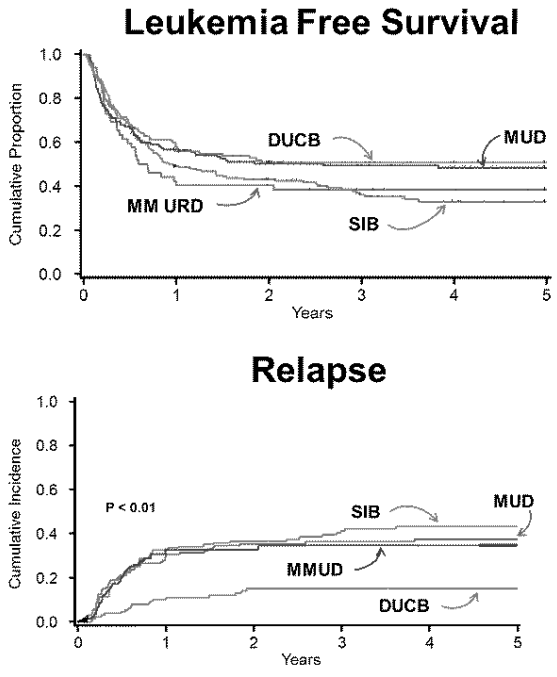
The principal conclusions of this study were: 1) DUCBT is safe with engraftment observed in all patients, 2) one unit predominates within the first 100 days, 3) >90% of adults will be able to identify two units that are partially HLA matched with the patient and each other, 4) incidence grade III-IV acute GVHD is similar to that observed after single UCBT; and 5) survival exceeds that of historical data in adult recipients of a single UCB unit.

2.1.3 Comparisons with Other HSC Sources [12]

In order to determine the relative effectiveness of DUCBT compared to other HSC sources (marrow or peripheral blood from matched and mismatched sibling and unrelated donors), consecutive patients aged ≥ 10 years and undergoing first allogeneic HSCT for hematologic malignancy were evaluated. All 536 patients received myeloablative conditioning with CY 120 mg/kg and TBI 1200 to 1320 cGy with the addition of FLU 75 mg/m^2 in recipients of DUCBT; GVHD immunoprophylaxis included a calcineurin inhibitor. Allografts were from a matched unrelated donor (MRD, n=204), matched unrelated donor (MUD, n=152), mismatched unrelated donor (MMUD, n=52), or DUCB (n=128). Differences between donor groups included, higher median age in MRD recipients, more frequent diagnosis of CML in recipients of MUD or MMUD grafts, lower incidence of CMV seropositivity in recipients of MUD grafts, and greater HLA mismatch and shorter follow-up in recipients of DUCB.

The primary endpoint of this analysis was leukemia free survival (LFS). LFS at 5 years was similar for all 4 groups. After adjusting for disease risk and time from diagnosis to transplantation, multivariable analysis did not reveal any difference in the relative risk of EFS by donor type.

Figure 2



The incidence of relapse at 5 years was comparable in recipients of MRD, MUD, and MMUD grafts but lower in recipients of DUCBT (Figure 2) with similar outcomes in the subset of patients ≤ 45 years. After adjusting for disease risk, interval from diagnosis to transplantation, age at transplantation and diagnosis, multivariable analysis showed lower relative risk of relapse was associated with DUCBT. Similarly, the relative risk of relapse remained lower among DUCBT recipients regardless of risk group and additional analyses revealed similar outcomes when restricted to patients aged ≥18 years. Leukemia relapse was the most frequent cause of mortality in recipients of MRD, MUD, and MMUD grafts.

The incidence of TRM at 2 years, however, was higher in recipients of UCB even after adjusting for age at transplantation and diagnosis. Analysis of risk factors for TRM among DUCB recipients demonstrated a higher risk in patients with delayed neutrophil recovery (41% [95% CI, 26%-56%] if neutrophil recovery took ≥26 days (which was the median time to neutrophil recovery) compared 16% [95% CI, 7%-25%] if recovery was <26 days). If restricted to DUCB recipients with earlier (<median 26 days) neutrophil recovery after UCBT, the incidence of TRM was similar in all groups, emphasizing that delayed engraftment is the single greatest barrier to successful UCBT and the most important contributor to early TRM. Infection was the most common cause of death among recipients of UCB.

The principal conclusions of this study were: 1) Higher TRM after DUCBT is still driven by prolonged neutropenia, and 2) relapse is low after DUCBT relative to other HSC sources.

2.1.4 National BMT-CTN 0501 Study - Single versus Double UCBT [22].

As a result of these promising single institutional data, a national trial was developed to compare single versus DUCBT to determine if two UCB units would confer a survival advantage. Between December 1, 2006, and February 24, 2012, a total of 224 patients 1 to 21 years of age with hematologic cancer were randomly assigned to undergo DUCBT (111 patients) or single UCBT (113 patients) after a uniform myeloablative conditioning regimen regimen of FLU 75 mg/m^2, TBI 1320 cGy and CY 120 mg/kg. The primary end point was 1-year overall survival. Treatment groups were balanced with respect to all baseline covariates, including sex, age, results of serologic testing for cytomegalovirus, disease type and status at transplantation, performance score, degree of donor–recipient HLA matching and ABO matching, and self-reported Hispanic or Latino background and race or ethnic group. The median age at transplantation was 9.9 years (range, 1.1 to 21.2) for DUCBT recipients and 10.4 years (range, 1.4 to 21.4) for single UCBT recipients, and the median body weight was 37.0 kg (range, 9.7 to 81.7) and 35.7 kg (range, 10.0 to 93.0), respectively. All received a graft containing $\geq$2.5 x 10^7 NC/kg. The median number of infused NC/kg was 7.2 x 10^7 for recipients of DUCBT and 3.9 x 10^7 for recipients of single UCBT grafts, and the number of infused CD34+ cells/kg was 3.7 x 10^5 and 1.9 x 10^5, respectively.

The overall survival rate was 65% (95% CI, 56 to 74) among DUCBT recipients and 73% (95% CI, 63 to 80) among single UCBT recipients (p=0.17). In a multivariate analysis, the risk of death did not differ significantly between recipients of DUCBT and recipients of single UCBT (hazard ratio, 1.34; 95% CI, 0.86 to 2.09; p=0.20, with the single-unit group used as the referent), even after adjustment for disease type (AML vs. other), which was the only factor associated with an increased risk of death.

The 1-year EFS rate was 64% (95% CI, 54 to 72) among DUCBT recipients and 70% (95% CI, 60 to 77) among single UCBT recipients (p=0.11). In a multivariate analysis, the risk of relapse or death (i.e., the risk of treatment failure, the inverse of the EFS rate) did not differ significantly between groups, even after adjustment for leukemia type, self-

reported race (white vs. nonwhite), and HLA match score (better HLA match had worse outcome), which were the factors associated with EFS.

Notably, the incidence of neutrophil recovery was 88% (95% CI, 82 to 94) among DUCBT recipients and 89% (95% CI, 83 to 95) among single UCBT recipients (p=0.29), at a median of 23 days (range, 11 to 133) and 21 days (range, 11 to 62) after transplantation, respectively. No patients in either group had secondary graft failure. The incidence of platelet recovery, however, was significantly higher and recovery occurred more quickly among recipients of a single UCBT than among recipients of a DUCBT (incidence, 76% [95% CI, 68 to 85] vs. 65% [95% CI, 56 to 74]; P=0.04). The median time to platelet recovery was 58 days (range, 28 to 295) in the single unit group and 84 days (range, 22 to 716) in the double-unit group.

For the other endpoints, the incidence of grade II–IV acute GVHD was similar in the two treatment groups (p=0.78); however, recipients of DUCBT had a higher incidence of grade III and IV acute GVHD (23% [95% CI, 15 to 31] vs. 13% [95% CI, 7 to 20], p=0.02). The incidence of any chronic GVHD at 1 year was 32% (95% CI, 23 to 40) and 30% (95% CI, 22 to 39), respectively (p=0.51), with a higher incidence of extensive disease (15% [95% CI, 8 to 22] vs. 9% [95% CI, 4 to 14], respectively p=0.05). Among the 220 patients (91%) with at least one infection after transplant, 120 (55%) had a severe infection, and 32 (15%) had a life-threatening or fatal infection. No significant differences were observed between the treatment groups. The incidences of relapse and TRM were also similar in the two treatment groups. Notably, the incidence of relapse at 1 year was low in both groups: 14% (95% CI, 7 to 21) in recipients of a DUCBT and 12% (95% CI, 6 to 18) in recipients of a single UCBT (p=0.12). The incidence of TRM at 1 year was 22% (95% CI, 14 to 30) among DUCBT recipients and 19% (95% CI, 11 to 26) among single UCBT recipients (p=0.43).

The principal conclusions of this study were: 1) rates of engraftment, relapse and survival were similar between recipients of DUCBT and an adequately dosed single UCBT, and 2) patients in both treatment groups had better engraftment and survival outcomes than those previously reported, supporting the use of FLU rather than ATG in the conditioning, and MMF rather than methylprednisolone in the post-transplant immunoprophylaxis in future trials.

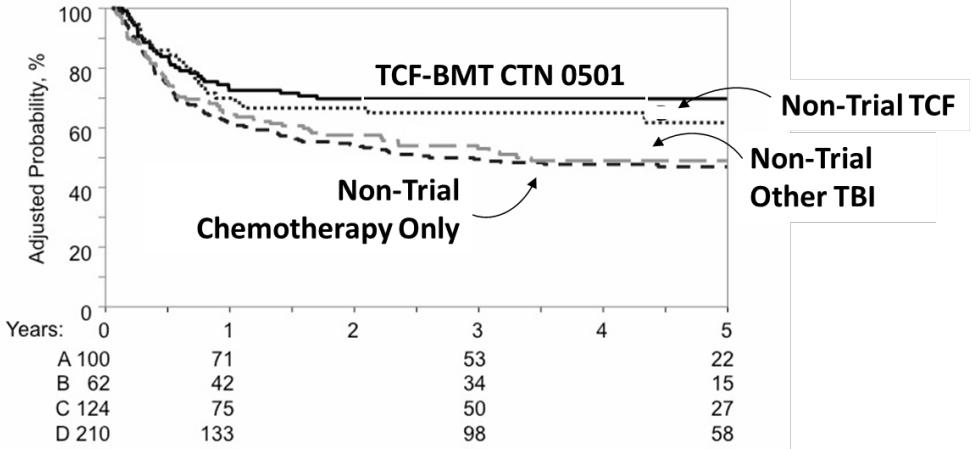
2.1.5 Impact of Conditioning on Engraftment and Survival after UCBT [23]

The BMT CTN 0501 trial not only demonstrated similar survival rates in recipients of double vs single UCBT in children with hematologic malignancies but suggested that the treatment package, ie conditioning and post-transplant immunoprophylaxis, might be superior to other regimens. This prompted a comparison of survival of 0501 trial versus nontrial patients to determine the generalizability of trial results and whether survival was better because of the trial treatment regimen. During the trial period, 396 recipients of a single UCB unit met trial eligibility but were not enrolled. Trial patients (n = 100) received TBI 1320 cGy, CY120 mg/kg, and FLU 75 mg/m2 (TCF). Non-trial patients either received the same TCF regimen (n = 62; nontrial TCF) or alternative regimens

(n = 334; nontrial regimens). Five-year survival between trial and nontrial patients conditioned with TCF was similar (70% versus 62%) (Figure 3). However, 5-year survival was significantly lower with nontrial TBI-containing (47%; hazard ratio [HR], 1.97; P = .001) and chemotherapy-only regimens (49%; HR, 1.87; P = .007). The survival difference between the trial-specified regimen and other regimens indicate the importance of the treatment package for UCBT.

The principal conclusions of this study were: 1) engraftment was better in recipients of TCF as compared to non-TCF regimens, and 2) extended survival outcomes to 5 years for patients treated on BMT CTN 0501 was stable at 70%. Together, these data support the use of TCF as employed in BMT CTN 0501 developed at the University of Minnesota and used in all UCBT after 2000.

Figure 3 Overall Survival



2.2 MGTA-456 – Aryl Hydrocarbon Receptor (AHR)-Antagonist Mediated HSPC Expansion

StemRegenin-1 (SR-1) was first identified in an unbiased screen for compounds that promoted expansion of CD34+ hematopoietic progenitors [25]. SR-1 is an AHR antagonist which impedes HSC differentiation during cytokine-driven expansion culture. Thus far, 36 patients with hematological malignancy have been treated with UCB CD34+ cells after a 15-day expansion culture using this strategy (cross-reference IND# 14822 Novartis Sponsor). The cellular product that results from this expansion culture as well as its companion CD34 depleted fraction (cryopreserved on the day the CD34+ cells are placed in expansion culture), was previously referred to as HSC835 and is now referred to as 'MGTA-456'. MGTA-456 has been tested in 2 human studies in patients with hematologic malignancies Study CHSC835X2201 and Study CHSC835X2202.

Study CHSC835X2201 was a first-in-human, single-arm, single-center, open-label, proof-of-concept study to evaluate the safety, tolerability, and preliminary efficacy of infusing MGTA-456 (expanded UCB HSC) in patients with hematological malignancies who have undergone myeloablative conditioning. The conditioning regimen comprised fludarabine administered at

25 mg/m2/day i.v. for three days (Day -8 to -6), cyclophosphamide administered at 60 mg/kg/day for two days (Day -7 to -6) and total body irradiation (TBI) at 165 cGy given twice daily for four days (Day -4 to -1). The study initially enrolled adult patients who were administered a DUCBT - one unit consisting of unmanipulated cells and the other of the expanded MGTA-456 product. After the first planned interim analysis at which five evaluable patients demonstrated that administration of MGTA-456 showed no unexpected infusional toxicity and resulted in hematopoietic reconstitution within the expected time frame, the protocol was amended to include pediatric patients (≥ 10 years) into the study. A subsequent interim review of the data for the first nine adult patients showed that the safety findings were consistent with the published findings for DUCBT [12]. The expanded cord blood product led to sustained, long-term hematopoietic engraftment in an accelerated fashion that correlated with the CD34+ cell dose. Furthermore, patients in whom MGTA-456 predominated recovered their neutrophils faster than patients in whom the unmanipulated CBU predominated. Following this, the protocol was amended such that adult patients were transplanted with a SUCBT with MGTA-456. Once safety data of SUCBT in adults and of DUCBT in the pediatric population were available, the protocol was amended such that pediatric patients were transplanted with SUCBT. A total of 31 patients were enrolled in this study; 27 of whom were transplanted during the study and 16 of whom completed the study.

In the initial analysis of the trial identified by CHSC835X2201, 18 patients were treated with MGTA-456 along with a second unmanipulated UCB unit, in case the MGTA-456 did not engraft. All 18 patients engrafted at a median of 14.5 days (range, 7-23 days), substantially better than identically treated historical controls (n=151) in whom the incidence of engraftment was 89% (95% CI 83-93) at a median of 23 days (range, 19-31 days) ($p<0.01$).

In two Phase 2 clinical trials using MGTA-456 as a stand-alone graft, 18 patients with high-risk hematologic malignancy and a partially HLA matched UCB unit were treated. Patients received the CD34-positive fraction along with the CD34-negative fraction of the expanded UCB unit which was separated prior to initiating the expansion culture. It is known that CD34-negative cells contain CD3+ T lymphocytes and have been shown to be important in unit predominance long term and therefore may have a protective effect. Nine were treated with myeloablative (MA) conditioning consisting of CY 120 mg/kg, FLU 75 mg/m2 and TBI 1320 cGy, which was an extension of CHSC835X2201 and 9 were treated with non-myeloablative (NMA) conditioning consisting of CY 50 mg/kg, FLU 200 mg/m2 and TBI 200 cGy, identified by CHSC835X2202 with all receiving CsA and MMF immunoprophylaxis.

The AHR antagonist expansion culture yielded a median of 1,227 x 10^6 CD34+ cells (range, 201-8969) as compared to the input number of 4.2 x 10^6 (range, 1.4-16.3) after CD34 selection – a 324-fold (range, 42-1643) expansion of CD34+ cells. As transplant results vary by intensity of the conditioning regimen, patient outcomes were compared to similarly treated historical cohorts (n=151 MA conditioning; 32 NMA conditioning). For both groups, demographics were similar except for more recent year of transplant for recipients receiving MGTA-456. For recipients of MA, MGTA-456 engrafted in 100% of patients at a median of 14 days (range, 7-32 days) as compared to 89% engraftment at a median of 23 days in historical cohort (range, 19-31 days) ($p<0.01$). Similar to the historical cohort, complete chimerism was

rapid for both myeloid and T cells with no late graft failures in recipients of MGTA-456. For recipients of NMA conditioning, MGTA-456 also engrafted in 100% of patients but at a median of 7 days (range, 6-14 days) as compared to 95% of patients at a median of 15 days (range, 7-22 days) in the historical cohort. In contrast to MA conditioning, chimerism was often mixed for the first month in both myeloid and T cells after NMA conditioning. Compared to the historical cohort, recipients of MGTA-456 had more rapid chimerism after NMA conditioning. CD34 cell dose correlated with speed of recovery but only in recipients with MA conditioning; in recipients of non-MA conditioning, recovery was uniformly rapid regardless of CD34 cell dose. With regard to other transplant outcomes, results are encouraging. For recipients of MGTA-456 compared to the historical cohort after MA conditioning, incidence of AGVHD 3-4 was 22% vs 24%; CGVHD, 11% vs 21%; TRM, 11% vs 34%; and OS, 67% vs 55%). After NMA conditioning, results are similar between cohorts, specifically, incidences of acute GVHD 3-4 were 56% vs 15%; CGVHD, 0% vs 19%; TRM, 22% vs 20%; and OS, 44% vs 49%).

2.3 Human Pharmacokinetic Data

Patients enrolled onto Novartis study CHSC835X2201 were evaluated for the presence of the AHR antagonists, LFU835 or LHD221, infused with the washed cells of MGTA-456. Pharmacokinetic (PK) data from patients receiving MGTA-456 expanded with LFU835, showed an exposure to LFU835 slightly lower than predicted from animal data. Safety margins were very large (based on one-fifth of NOAEL in female monkeys) and maximum observed concentration (C_{max}) and area under the plasma concentration-time curve (AUC) ranged from 9.9 to >10,000 ng/mL and from 68 to 2734 h × ng/mL in individual patients who received total cell doses between 4 and 114 million cells/kg body weight.

For LHD221, which will be used in the present study, Table 1 shows the available results. The amount of co-infused LHD221 remaining on the washed cells ranged from 55 to 693 µg (predicted based on in vitro measurements of 0.044 pg/cell). The highest measured concentration in a plasma sample was 1.36 ng/mL and in many samples no LHD221 could be detected (lower limit of quantification [LLOQ]: 0.1 ng/mL). No pharmacokinetic parameters (AUC, C_{max}) were derived, but compared to the exposure in female monkeys at the no observed adverse effect level (NOAEL) (0.5 mg/kg of LHD221; AUC=637 h × ng/mL, C_{max}=1112 ng/mL) it can be assumed that the safety margins are very large.

Table 1: LHD221 Plasma Concentrations in Patients Receiving MGTA-456

Study ID	Patient No.	Calculated LHD221 Dose (µg)	Scheduled Time (hours)		
			1.0	5.0	12.0
			Concentration (ng/mL)		
CHSC835X2201	0001-05123	226	NA	0.00	NA
	0001-05124	125	1.09	0.17	0.00
	0001-05126	55	0.00	0.00	0.00
	0001-05127	203	0.34	0.00	0.00
CHSC835X2202	1001-25101	76	0.00	0.00	0.00

Study ID	Patient No.	Calculated LHD221 Dose (µg)	Scheduled Time (hours)		
			1.0	5.0	12.0
			Concentration (ng/mL)		
	1001-25102	693	0.14	0.00	0.00
	1001-25103	169	1.36	0.34	0.00
	1001-25104	95	0.00	0.00	0.00
	1001-25105	592	0.28	0.00	0.00

For LFU835 the calculated safety margins are very large. As the LHD221 concentrations were very low and no PK parameters could be derived, it can be assumed that the safety margins are also very large for LHD221 as well. For additional information, please refer to the IND Master File.

2.4 Cryopreserved MGTA-456

A manufacturing process was developed to allow the formulation of the expanded CD34+ fraction as a cryopreserved product. The cryopreserved product improves on the quality of key manufacturing reagents and results in a final product with a pre-defined volume and DMSO content. DMSO content in the cryopreserved product is lower in concentration than the amount present in the FDA-approved, cryopreserved unmanipulated UCB units. The total amount of administered DMSO is expected to be generally well tolerated with some risk of infusional toxicities. Such infusional toxicities may include but are not limited to fever, nausea, vomiting, excessive sweating (diaphoresis), labored breathing (dyspnea), chest discomfort, desaturation (reduced oxygen saturation), hypotension, hypertension, headache, and bradycardia (slow heart rate). If thawed product units are washed prior to infusion, the amount of DMSO will be significantly reduced and expected to be very well tolerated.

2.5 Summary and Rationale for the Proposed Study

MGTA-456 is an allogeneic UCB cell therapy product that consists of 2 cell fractions derived from the same UCB unit. The manufacturing process consists of expanding the CD34-positive (CD34+) cells derived from the UCB in vitro in the presence of cytokines and a low molecular weight (LMW) compound (AHR antagonist) and the resulting product is provided to the patient directly after the manufacturing process. The other cell fraction consists of CD34-depleted cells from the same UCB unit. Results with MGTA-456 in 38 patients with hematological malignancies support additional evaluation.

Therefore, we propose a phase II trial evaluating the effectiveness of MGTA-456 in children and adults with selected hematological malignancies eligible to receive MA conditioning. Eligible patients will undergo single UCBT consisting of the investigational therapy of MGTA-456. In addition to the conventional endpoints of neutrophil and platelet engraftment, acute and chronic GVHD, TRM, relapse and survival, other functional endpoints, such as non-catheter associated bacterial infections by day 100, risk of opportunistic infections, number of red cell and platelet transfusions will be evaluated. Surrogate endpoints, such as number of hospital days and treatment costs over the first 100 days will also be evaluated. With regard

to the primary endpoint, this trial is powered to verify the effectiveness of MGTA-456 as measured by the proportion of patients engrafting by day 14 after transplantation.

This trial is supported by an NCI Program Project Grant and other local support. Magenta Therapeutics will provide LHD221 (StemRegenin-1) for the proposed trial and permit use of the manufacturing SOPs previously developed at the University of Minnesota under Novartis/Magenta sponsorship as well as provide support for the manufacture of MGTA-456.

2.6 Immune Reconstitution Sub-study

Immunity is impaired after hematopoietic stem cell transplant (HSCT), and the recovery of cell counts can take years. The innate immune system is comprised of neutrophils, monocytes, macrophages, dendritic cells, and natural killer (NK) cells. These cells are able to recognize and eradicate pathogens without the need for antigen presentation. In contrast, the adaptive immune system is comprised of T and B cells. These cells require priming by the antigen, leading to long-term specific responses using unique receptor sequences. The desired outcome of HSCT is the reestablishment of an effective immune system that accurately delineates self from non-self. If adequate antigen-specific immune function is present, functional immune reconstitution has the potential to decrease leukemic relapse and TRM. Clear understanding of the functional immune recovery is needed for successful HSCT. Also, understanding the different time courses of functional recovery of each immune cell subset can help to predict the course of disease in individual patients after HSCT.

Recent work has been done to understand immune reconstitution. In a recent study by Park et al (2015) 95 patients with hematologic malignancies who underwent allogeneic HSCT were evaluated [45]. Patients received different conditioning regimens depending on donor type which included sibling matched, unrelated matched or haploidentical HSCT. Lymphocyte subsets showed different sequential patterns according to HSCT type but no differences were seen according to primary diagnosis or conditioning regimen. Specifically they found that the number of white blood cells (WBCs) recovered within 1 month of HSCT and T and B lymphocyte numbers recovered at 2 months after HSCT. Interestingly, the CD4/CD8 ratio was reversed during all periods after HSCT. Among T cell subpopulations, NK-T cells, and effector memory T cells recovered within 1–2 months after HSCT, but central memory T cells were not detected in peripheral blood throughout the study period. There were low levels of naïve thymic T cells after HSCT, while the number of naive central T cells peaked immediately after HSCT. The percentage of Tregs started to increase at 3 months after HSCT, but maintained at a relatively low level during the entire study period.

B cell reconstitution is less studied and understood after HSCT. Recent work form Abdel-Azim et al (2017) evaluated 71 pediatric patients undergoing HSCT from 2004-2011 [46]. They found that tetanus toxoid antibody levels were normal at 1 year after HSCT whereas antipolysaccharide carbohydrate antibodies remained persistently low for up to 5 years. While naive B cell counts normalized by 6 months, IgM memory B cell deficiency persisted for up to 2 years. Moreover, switched memory B cell deficiency normalized by 1 year after HSCT. CD4+ T cell play a large part in humoral immunity; data showed the CD4 cell immune reconstitution correlated with that of switched memory B cells as early as 6 months after

HSCT but did not correlate with IgM memory B cells at any time point after HSCT. Taken together, this data suggests that antibody immune reconstitution is impaired mainly due to IgM memory B cell maturation block. They also found that the use of naïve UCB was associated with better naive and IgM [47] memory B cell immune reconstitution at 6 months after HSCT, compared with the use of bone marrow and peripheral blood stem cells. Moreover, the use of UCB was also associated with better switched memory B cell immune reconstitution at 6 months after HSCT, compared with the use of PBSC. As there is currently an unmet need in regards to understanding both B and T cell immune reconstitution as well as functional responses post expanded UCB HSCT this sub-study aims to prospectively assess both subsets of immune cells and determine functional immune responses at certain time points.

3 Study Design

This is a prospective single-arm, open label, interventional phase II clinical trial evaluating MGTA-456 in children and adults with selected hematopoietic malignancies to elucidate its effect on reducing the duration of neutropenia and thrombocytopenia, and consequently reducing risks of bacterial infections and numbers of platelet transfusions, and risks of infections. Success will be measured by 33% of patients achieving neutrophil recovery by day 14.

4 Patient Selection

Study entry is open to persons aged ≤55 years regardless of gender or ethnic background. While there will be every effort to seek out and include females and minority patients, the patient population is expected to reflect those with high risk leukemia and MDS referred to the institution.

4.1 Age, Weight, Unit Cell Dose and HLA Match Criteria

- Subjects must be ≤55 years of age.
- Subjects must weigh >11 kg
  - Subjects must have a partially HLA matched UCB unit with a pre-cryopreserved TNC dose ≥1.0×10^7 per kilogram recipient weight. HLA matching is initially based on a minimum of 5 of 8 HLA alleles at high resolution A, B, C, DRB1 typing; searches will be performed according to the current Magenta Cord Blood Search Algorithm.

4.2 Eligible Diseases

- Acute myelogenous leukemia (AML) in morphological complete remission with:
  - Minimal residual disease (MRD) by flow cytometry, or
  - Intermediate to high risk leukemia in first (CR1) based on institutional criteria, eg. not favorable risk AML which is defined as having one of the following:
    - t(8,21) without *cKIT* mutation
    - inv(16) or t(16;16) without *cKIT* mutation
    - Normal karyotype with mutated *NPM1* but *FLT3-ITD* wild type
    - Normal karyotype with double mutated *CEBPA*

  - Acute promyelocytic leukemia (APL) in first molecular remission at the end of consolidation
- Any second or subsequent CR, or
- Secondary AML with prior malignancy that has been in remission for at least 12 months.

- Acute lymphocytic leukemia (ALL) at the following stages:
  - High risk first morphological, cytogenetic and molecular CR with:
    - MRD by flow cytometry, or
    - Diagnosis of Philadelphia chromosome (Ph)+ ALL, or
    - MLL rearrangement at diagnosis with slow early response at Day 14, or
    - Hypodiploidy (< 44 chromosomes or DNA index < 0.81) at diagnosis, or
    - End of induction M3 bone marrow, or
    - End of induction M2 with M2-3 at Day 42.
  - High risk second CR based on institutional criteria (eg, for children, bone marrow relapse <36 months from induction or T-lineage bone marrow relapse or very early isolated central nervous system (CNS) relapse <6 months from diagnosis, or slow re-induction (stage M2-3 at day 28 after induction) regardless of length remission. All patients with MRD by flow cytometry.
  - Any third or subsequent CR.
- Secondary ALL
- Biphenotypic/undifferentiated leukemia in morphological, cytogenetic and molecular CR .
- Chronic Myelogenous Leukemia (CML) in high risk first chronic phase (failure of two tyrosine kinase inhibitors (TKI) or TKI intolerance), accelerated phase or second chronic phase.
- Myelodysplasia (MDS) IPSS Int-2 or High risk (i.e. RAEB, RAEBt <5% blasts) or other high risk features, including multiple cytopenias, high risk cytogenetics or lack of response to standard therapy.
- Relapsed large-cell lymphoma, mantle-cell lymphoma and Hodgkin lymphoma that is chemotherapy sensitive and ineligible for an autologous transplant.
- Burkitt's lymphoma in CR2 or subsequent CR.
- Relapsed T-cell lymphoma that is chemotherapy sensitive in CR/PR that is ineligible for an autologous transplant.

4.3 Organ Specific Inclusion Criteria

- Karnofsky score ≥70 (16 years and older), Lansky play score ≥50 (children 2-16 years, or 'adequate' score for children <2 years, as detailed in Appendix I.
- Adequate organ function defined as:
  - Renal: Serum creatinine within normal range for age, or if serum creatinine outside normal range for age, then creatinine clearance >40 ml/min or GFR normal for age
  - Hepatic: Bilirubin <3x upper limit of normal (ULN) and AST, ALT and alkaline phosphatase <5x ULN.

- Pulmonary function: DLCO, FEV1, FEC (diffusion capacity) >50% of predicted (corrected for hemoglobin); if unable to perform pulmonary function tests, then O2 saturation >95% on room air.
- Cardiac: No uncontrolled arrhythmia and left ventricular ejection fraction at rest must be >45%.

- Available 'back-up' HSPC graft (e.g, second UCB unit, haploidentical related donor).
- Females of child bearing potential and sexually active males must agree to use adequate birth control during study treatment.
- Voluntary written consent signed (adult or parental) before performance of any study-related procedure not part of normal medical care.

4.4 Exclusion Criteria

- Patients with a HLA matched sibling donor or a HLA matched unrelated donor who is available for marrow or peripheral blood stem cell collection at the desired time of transplant.
- Pregnant or breast feeding. The agents used in this study may be teratogenic to a fetus and there is no information on the excretion of agents into breast milk. Females of childbearing potential must have a blood test or urine study within 14 days prior to study enrollment to rule out pregnancy.
- Evidence of human immunodeficiency virus (HIV) infection or known HIV positive serology.
- Active bacterial, viral or fungal infection (currently taking medication and persistence of clinical signs and symptoms) with a minimum of 4 weeks of anti-fungal treatment
- Prior autologous or allogeneic transplant.
- Other active malignancy.
- Subjects >3 years of age unable to receive TBI 1320 cGy due to extensive prior therapy including >12 months alkylator therapy or >6 months alkylator therapy with extensive radiation, or prior Y-90 ibritumomab (Zevalin) or I-131 tostumomab (Bexxar), as part of their salvage therapy.

5 Patient Registration

Registration will occur after the patient or patient's guardian has signed the subject consent but before any treatment has been administered. To be eligible for registration to this study, the patient must meet each criterion listed on the eligibility checklist based on the eligibility assessment documented in the patient's medical record. A copy of the eligibility checklist is under attachments within the study in OnCore.

5.1 Registration with the Masonic Cancer Center Clinical Trials Office

Any patient who has been consented is to be registered in OnCore by the Primary Clinical Research Coordinator (PCRC) or designee. If a patient is consented, but not enrolled, the patient's record is updated in OnCore as a screen failure and reason for exclusion recorded

5.2 Study Enrollment with the Masonic Cancer Center Clinical Trials Office

To be eligible for study enrollment, the patient must sign the treatment consent and meet each of the inclusion criteria and none of the exclusion on the eligibility checklist based on the eligibility assessment documented in the patient's medical record.

The Primary Clinical Research Coordinator (PCRC) or designee will assign the study treatment arm and add the on-treatment date to complete enrollment.

5.3 Patients Who Are Enrolled But Do Not Receive Study Treatment

If a patient is enrolled onto the study and is later found not able to begin the planned study treatment for whatever reason, the patient will be removed from study and treated at the physician's discretion. The study coordinator or designee will update OnCore of the patient's non-treatment status and notify the Principal Investigator. The reason for removal from study prior to starting study treatment will be clearly indicated in OnCore. The patient may be replaced.

6 Treatment Plan

In order to provide optimal patient care and to account for individual medical conditions, investigator discretion may be used in the prescribing of all supportive care drug therapy (i.e. acetaminophen, diphenhydramine, antimicrobials, etc.).

All patients aged 3-55 years inclusive will receive the same conditioning and immunoprophylaxis for the prevention of acute and chronic GVHD, previously demonstrated to offer the best outcomes in recipients of partially HLA matched UCB. Patients 0-3 years will receive a non-TBI containing conditioning regimen which is standard of care.

TBI-Containing Regimen

All patients aged 3-55 years will receive CY 120 mg/kg total dose, FLU 75 m/m2 total dose and TBI 1320 cGy total dose. Standard supportive care, including the use of G-CSF and prophylactic anti-bacterial, protozoal, viral and fungal agents, will also be prescribed. Supportive care will be modified throughout the transplant course at the treating physician's judgement.

Day	Conditioning Regimen	Immunoprophylaxis Hematopoietic Growth Factor
-8	FLU 25 mg/m^2 IV over 1 hour (<10 kg: 0.83 mg/kg IV over 1 hour)	
-7	FLU 25 mg/m^2 IV over 1 hour (<10 kg: 0.83 mg/kg IV over 1 hour) CY 60 mg/kg IV over 2 hours	
-6	FLU 25 mg/m^2 IV over 1 hour (<10 kg: 0.83 mg/kg IV over 1 hour) CY 60 mg/kg IV over 2 hours	
-5	Rest	

Day	Conditioning Regimen	Immunoprophylaxis Hematopoietic Growth Factor
-4	TBI 165 cGy twice daily	
-3	TBI 165 cGy twice daily	Begin Tac / MMF (section 6.2)
-2	TBI 165 cGy twice daily	
-1	TBI 165 cGy twice daily	
0	MGTA-456 infusion	
+1		Begin G-CSF 5 ug/kg/d until the absolute neutrophil count (ANC) is ≥2500/uL for 2 consecutive days (section 6.4)

Non-TBI-Containing Regimen
For children ≤3 years of age will be conditioned with BU, FLU and MEL, as detailed below:

Day	Conditioning Regimen	Immunoprophylaxis Hematopoietic Growth Factor
-8	BU IV once daily with dose based on PK calculator over 3 hours	
-7	Bu IV once daily adjusted based on PK results over 3 hours	
-6	Bu IV once daily adjusted based on PK results over 3 hours	
-5	Bu IV once daily adjusted based on PK results over 3 hours	
-4	FLU 25 mg/m2/day (0.83 mg/kg/day if < 10 kg*) IV over 60 min MEL 50 mg/m2/day (1.7 mg/kg/day if < 10 kg*) IV over 30 min	
-3	FLU 25 mg/m2/day (0.83 mg/kg/day if < 10 kg*) IV over 60 min MEL 50 mg/m2/day (1.7 mg/kg/day if < 10 kg*) IV over 30 min	Begin Tac / MMF (section 6.2)
-2	FLU 25 mg/m2/day (0.83 mg/kg/day if < 10 kg*) IV over 60 min MEL 50 mg/m2/day (1.7 mg/kg/day if < 10 kg*) IV over 30 min	
-1	Rest	
0	MGTA-456 Infusion	
+1		Begin G-CSF 5 ug/kg/d until the absolute neutrophil count (ANC) is ≥2500/uL for 2 consecutive days (section 6.4)

*Patients <11kg are currently ineligible because of the expected volume of DMSO in thawed products. Once the washed product has been validated and approved by the FDA, patients <11 kg may be eligible.

6.1 Conditioning Regimen (day – 8 through day -1)

Conditioning will be administered according to institutional guidelines. Potential toxicities for each agent are detailed in section 7.1.

6.1.1 TBI-Containing Regimen

6.1.1.1 Fludarabine

FLU will be administered at dose of 25 mg/m^2 once a day as a 1 hour IV infusion on days -8, -7 and -6; total dose: 75 mg/m^2. The dose is calculated based on actual body weight (ABW); however, if < 10 kg dosing will be 0.83 mg/kg/dose once a day as a 1 hour IV infusion). The dose will be adjusted if the GFR is ≤ 70. There are no special precautions.

6.1.1.2 Cyclophosphamide

CY will be administered at a dose of 60mg/kg once a day as a 2 hour IV infusion on days -7 and -6; total dose: 120 mg/kg. The dose will be administered after a high volume fluid flush and mesna administration per institutional guidelines.

Cyclophosphamide dosing is calculated based on actual weight (ABW) unless ABW >150% above Ideal Body Weight (IBW). Then the dose should be computed using adjusted body weight. The adjusted body weight = IBW + 0.5(ABW - IBW). For patients 1-18 years of age, the ideal body weight is calculated using the Traub and Johnson formulas: i.e, <60 inches = ((height in cm)^2 x 1.65)/1000; >/= 60 inches = 39 +[2.27 x (height in inches - 60)] for males and 42.2 + [2.27 x (height in inches – 60)] for females. For patients >18 years of age, the ideal body weight is calculated using Devine's formula: i.e., 50 kg + (2.3 kg x (height in inches - 60)) for men; 45.5 kg + (2.3 kg x (height in inches - 60)) for women.

6.1.1.3 Total Body Irradiation

TBI at a dose of 165 cGy will be administered twice a day on days -4, -3, -2 and -1; total dose 1320 cGy. TBI administration procedure is described in Appendix II.

6.1.2 Non-TBI-Containing Regimen

6.1.2.1 Busulfan

BU compounding, administration and monitoring should be performed per institutional guidelines and infused over 3 hours. The initial BU dose will be determined based on the weight of the patient upon admission and per our institutional BU standard of practice dose calculator. The first BU dose will be determined by a weight-based dosing nomogram. Refer to institutional guidelines for dosing of BU (Appendix III).

Seizure and VOD prophylaxis are prescribed according to institutional guidelines. Concomitant administration of azole anti-fungal agents (except fluconazole) and acetaminophen should be avoided within 72 hours before and after BU administration.

6.1.2.2 Fludarabine

FLU will be administered at dose of 25 mg/m^2 once a day (<10 kg, dosing will be weight based: 0.83 mg/kg/day IV) as a 1 hour IV infusion on days -4, -3 and -2; total dose: 75 mg/m^2. The dose is calculated based on ABW. The dose will be adjusted if the GFR is ≤ 70. There are no special precautions.

6.1.2.3 Melphalan

MEL 50 mg/m2 IV will be administered over 30 minutes, 12 hours after FLU on days -4 to -2 (total of 150 mg/m^2). For children <10 kg dosing will be weight based, with MEL 1.7 mg/kg (total of 5.1 mg/kg). Maintenance hydration of >1500 ml/m^2/day will be administered with MEL and for 24 hours following infusion.

6.2 Immunoprophylaxis

GVHD immunoprophylaxis will consist of Tacrolimus and MMF and will be administered according to institutional guidelines. Potential toxicities for each agent are detailed in section 7.2.

6.2.1 Tacrolimus

Tacrolimus will start day -3 and will be administered as a continuous IV infusion at a starting dose of 0.03 mg/kg/day. Goal trough levels will be 10-15 ng/mL for the first 14 days post-transplant and then decreased to a goal of 5-10 ng/ml thereafter. Tacrolimus dosing will be monitored and altered as clinically appropriate per institutional pharmacy guidelines. Dose adjustments will be made on the basis of toxicity and/or tacrolimus levels outside of goal range. Conversion from IV to oral tacrolimus will be done per institutional pharmacy guidelines as the patient tolerates and prior to discharge. Potential toxicities are detailed in section 7.2.

Patients will receive tacrolimus until day +100. If no GVHD, the dose will be tapered 10% per week beginning on day 101, to discontinue at approximately day +180.

In the presence of severe tacrolimus toxicities, other alternative agents may be used after review and approval by the co-PIs.

6.2.2 Mycophenolate Mofetil

MMF 3 gram/day IV/PO for adult patients divided in 2 or 3 doses. Pediatric patients will receive MMF at the dose of 15 mg/kg/dose (max 1 gram per dose) every 8 hours beginning day -3. MMF dosing will be monitored and altered as clinically appropriate based on institutional guidelines. Patients will be eligible for MMF dosing and pharmacokinetics studies.

Stop MMF at day +30 or 7 days after the ANC is >0.5 x 10^9 /L, whichever day is later. However, if there is acute GVHD, MMF should be continued for an additional 7 days after initiation of systemic therapy for acute GVHD. Afterward, use of MMF is at the discretion of the attending physician.

6.3 MGTA-456 Infusion

All cell products will be handled by the Cell Therapy Laboratory in the Molecular and Cellular Therapeutics (MCT) facility using validated standard operating procedures (SOPs). The cell product will be released for human use to the inpatient unit after assessing the identity, sterility, purity and viability of the product.

The target cell dose is >10 x 10^6 CD34/kg. However, if the cell dose is < the target of 10 x 10^6 CD34/kg, it may be infused as long as it exceeds 1 x 10^6 CD34/kg. With regard to TNC, the maximum cell dose is 2.7 x 10^8/kg for children (<18 years) and 8.1 × 10^8 cells/kg for adults [expanded product only] based on the highest cell dose windows evaluated in prior studies. There is no lower limit for TNC dose, only a lower limit for CD34 dose.

The infusion will be performed according to FACT accredited institutional policies. The pre-medication and hydration regimen will be given following institutional guidelines (refer to section 6.4). No steroids will be given prior to infusion.

Under no circumstances will MGTA-456 (both the expanded product and its CD34 negative fraction) be irradiated. There must be no in-line leukocyte filter used with product infusion nor any medications or fluids infused in the same line with MGTA-456.

Vital signs will be checked before and every 15 minutes during the infusion, at the completion of the infusion, and one hour post infusion per institutional guidelines. More frequent vital signs may be required depending on reactions to the product infusion. Potential toxicities are detailed in section 7.3.

The infusion will be around 30 minutes for all patients at a rate per institutional guidelines.

Emergency drugs for hypersensitivity reactions should be available and administered according to institutional guidelines. Oxygen with nasal prongs for standby use should be present in the room.

Recipients of MGTA-456 will receive the associated CD34 negative fraction 1 hour after the infusion of the expanded product. However, if the expanded product results in a reaction that necessitates intervention, the CD34 negative fraction infusion may be delayed but should be given within 24 hours after the infusion of the expanded product.

6.4 Hematopoietic Growth Factor and Other Supportive Care

All patients will receive G-CSF 5 mcg/kg/day IV (maximum dose 480 mcg) based on the actual body weight IV beginning on day +1 after MGTA-456 infusion. G-CSF will be administered daily until the ANC is ≥2.5 x 10^9/L for two consecutive days and then

discontinued. If the ANC decreases to <1.0 x 10^9/L, G-CSF should be reinstituted as needed to maintain an ANC >1.0 x 10^9/L. Potential toxicities are detailed in section 7.2.

Patients will receive transfusions, infection prophylaxis and nutritional support according to institutional guidelines. Infection prophylaxis should include, but is not limited to, agents or strategies (e.g., PCR screening and preemptive therapy) to prevent herpes simplex, cytomegalovirus (CMV), Pneumocystis carinii, and fungal infections. Transfusion thresholds for blood product are based on standard institutional guidelines. All blood products other than MGTA-456 will be irradiated.

IVIG infusions (500 mg/kg/dose) is recommended for immunoprophylaxis for total serum IgG levels <400. IgG levels will be monitored monthly for first 3 months and then as directed by the care team.

6.5 Immunizations (Immune Reconstitution Sub-study)

To evaluate the pace of 'functional' immune recovery, Pediarix and Pneumovax will be administered at day 60, day 120, day 180 unless there is active acute or chronic GVHD. We will test for serologies for Hepatitis B, polio, pertussis, diphtheria, tetanus and pneumococcus pre-transplant, day 60, day 100, day 180 and one year post transplant. Other standard immunizations will be given at 12 months and two years unless there is active acute or chronic GVHD or other evidence of poor immunologic recovery.

6.6 Duration of Study Participation

Patients will be followed for 2 years after transplant.

Follow-up after 2 years will be institutional standard of care follow up for all patients treated by HSCT at the institution.

7 Expected Treatment Related Toxicities

7.1 Potential Toxicities of the Conditioning Regimen

Cyclophosphamide		
Common	**Less Common**	**Rare**
• low white blood cell count with increased risk of infection • hair loss or thinning, including face and body hair (usually grows back after treatment) • nausea • vomiting • loss of appetite • sores in mouth or on lips • bleeding from bladder, with blood in urine • diarrhea • long-term or short-term infertility in women and men	• low platelet count (mild) with increased risk of bleeding • darkening of nail beds • acne • tiredness • infection • fetal changes if pregnancy occurs while taking cyclophosphamide	• heart problems with high doses, with chest pain, shortness of breath, or swollen feet • severe allergic reactions • skin rash • scarring of bladder • kidney damage (renal tubular necrosis) which can lead to kidney failure • heart damage, with trouble getting your breath, swelling of feet, rapid weight gain • scarring of lung tissue, with cough and shortness of breath • second cancer, which can happen years after taking this drug • death from infection, bleeding, heart failure, allergic reaction, or other causes

Busulfan		
Common	**Less Common**	**Rare**
• Nausea/vomiting • Mucositis • Rash • Severe suppression of blood counts • Diarrhea • Fluid weight gain/edema • Alopecia • Hyperpigmentation	• Veno-occlusive disease • Alveolar hemorrhage • Pulmonary fibrosis • Interstitial pneumonitis	• Seizures (low frequency with anti-seizure prophylaxis) • Hepatic fibrosis/liver failure

Fludarabine		
Common	**Less Common**	**Rare**
• low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • low red blood cell count (anemia) with tiredness and weakness • tiredness (fatigue) • nausea • vomiting • fever and chills • infection	• pneumonia • diarrhea • loss of appetite • weakness • pain	• numbness and tingling in hands and/or feet related to irritation of nerves • changes in vision • agitation • confusion • clumsiness • seizures • coma • cough • trouble breathing • intestinal bleeding • weakness • death due to effects on the brain, infection, bleeding, severe anemia, skin blistering, or other causes

Melphalan		
Common	**Less Common**	**Rare**
• Hypotension • Nausea and vomiting • Diarrhea • Hives/allergic reaction	• Veno-occlusive disease	• Dyspnea • Lung scarring

Total Body Irradiation (TBI)		
Common	**Less Common**	**Rare**
• nausea and vomiting • diarrhea • cataracts • sterility • endocrinopathies • growth failure • intestinal cramps • mucositis	• parotitis • interstitial pneumonitis • generalized mild erythema • veno-occlusive disease	• dysphagia • vertebral deformities • nephropathy • risk of 2nd malignancy years later (when given along with chemotherapy)

7.2 Potential Toxicities of GVHD Immunoprophylaxis and G-CSF

GVHD Immunoprophylaxis	
Tacrolimus (Tac)	**Mycophenolate mofetil (MMF)**
• high blood pressure • abnormalities in blood chemicals • seizures • headaches • renal dysfunction to renal failure requiring dialysis	• nausea and vomiting • diarrhea • constipation • lowering of blood counts • leg cramps • skin rash • difficulty sleeping • chemical imbalances including high blood sugar • headaches • dizziness • high blood pressure

G-CSF Hematopoietic Growth Factor
• bone pain • headaches • body aches • fatigue • nausea/vomiting • insomnia • dyspnea • rash • edema

7.3 Potential Side effects of Immunizations

Pediarix
• injection site reactions • fever • joint pain • body aches, • loss of appetite • nausea • vomiting • diarrhea

Pneumovax
• injection site reactions • muscle or joint aches or pain • fever • chills • headache • nausea • vomiting

- stiffness of the arm or the leg where the vaccine was injected
- weakness
- fatigue
- skin rash

7.4 Potential Adverse Events (AE) associated with the Infusion of Cryopreserved HSPC Products (including MGTA-456)

Cryopreserved HSPC Products / MGTA-456
Infusional Toxicities within 48 Hours
• nausea and vomiting • possible allergic reaction (including itching, hives, flushing [red face], shortness of breath, wheezing, chest tightness, skin rash, fever, chills, stiff muscles, or trouble breathing) • acute hemolytic reactions in setting of ABO incompatibility • febrile nonhemolytic reactions • anaphylactoid or anaphylactic reactions • transfusion-related acute lung injury (TRALI) • DMSO toxicities (eg headache, hypertension, cardiac toxicity, nausea and vomiting) • transmission of occult bacterial, viral or protozoal infections • transfusion-associated circulatory overload (TACO) • hypothermia • non-immunologic hemolysis
Later AEs after the Initial 48 Hours
• Failure to engraft • GVHD – acute and chronic • Slow immune recovery and high risks of opportunistic infection • Diminished graft-versus-leukemia effect and greater risk of relapse

7.5 Recommended Treatment of Adverse Events

The clinical management of the complications discussed below will be per institutional guidelines. The following text is considered general guidance only.

7.5.1 Slow Engraftment/Graft Failure

The clinical management of slow engraftment/graft failure will be per institutional guidelines. Management of slow engraftment is triggered by the assessment of peripheral blood and bone marrow on Day 21. If at Day 21, the ANC is ≤0.5 × 109/L and bone marrow cellularity is ≤5%, G-CSF dose may be doubled or granulocyte macrophage-colony stimulating factor (GM-CSF) may be added at 250 mcg/m2/d, the availability of the back-up UCB unit(s) that was put on hold at the time of patient enrolment is confirmed and a Day 28 bone marrow biopsy is scheduled.

If on Day 28 ANC remains ≤0.5 × 10^9/L and bone marrow cellularity is ≤5%, consideration will be given to performing a second HSC infusion using the previously identified back up source after some form of preconditioning per institutional standard of care.

7.5.2 Anemia

Transfusions of packed red blood cells are indicated for symptomatic management of anemia. An attempt should be made to maintain the hematocrit >24% and hemoglobin >8 g/dL. Irradiated (1500 to 3000 cGy) blood products will be used in accordance with the institutional standard of care.

7.5.3 Thrombocytopenia

Prophylactic platelet transfusions should be given to maintain the platelet count >10 × 10^9/L or above the level at which signs of bleeding are known to occur, whichever is greater. All aspirin containing drugs are to be avoided. The patients should receive no intramuscular injections while thrombocytopenic. Irradiated (1500 to 3000 cGy) blood products will be used in accordance with the institutional standard of care.

7.5.4 Nutrition

All patients will be candidates for total parenteral nutrition; length of use is per institutional guidelines.

7.5.5 Acute GVHD

Patients will be considered evaluable for acute GVHD unless they are known to have autologous recovery or die before Day 21 without GVHD. Organ involvement will be staged using the criteria outlined in the Appendix IV. Biopsy of each organ site at diagnosis or major change in disease activity will be performed unless clinical circumstances make it impossible.

7.5.6 Infusional Reaction

MGTA-456 expanded cell product, like other blood products, may cause infusional reactions, including hemodynamic effects or acute hypersensitivity. Furthermore, the co-infusion of residual components of the expansion culture carries theoretical attendant risks. These time-limited events are expected to occur within the first 24 hours [38, 39], and may range from mild to moderate in severity and may include gastrointestinal (nausea, vomiting, diarrhea and abdominal cramps), respiratory (cough, dyspnea), cardiovascular (hypotension, hypertension, bradycardia), neurological, or dermatological (skin flushing, rash) events, infection, and anaphylaxis [40].

Emergency drugs should be available and administered per institutional guidelines. Oxygen with nasal prongs for standby use should be present in the room.

7.5.7 Engraftment Syndrome/Transfusion Related Acute Lung Injury (TRALI)

Engraftment syndrome is a syndrome characterized by fever, fluid retention, rash and pulmonary infiltrates proximal to the time of neutrophil recovery after high dose conditioning and HSCT, likely mediated by activated leukocytes and proinflammatory cytokines. Treatment is supportive, including antipyretics, oxygen and diuretics, and systemic corticosteroids or therapies directed to specific proinflammatory cytokines (eg, IL6).

TRALI is a rare but serious syndrome characterized by sudden acute respiratory distress following transfusion. It is defined as new, acute lung injury during or within six hours after blood product administration in the absence of other risk factors for acute lung injury. TRALI is thought to be caused by activation of recipient neutrophils by donor-derived antibodies targeting HLA or human neutrophil antigen, in most cases. Non-antibody-mediated cases occur and may be mediated by biologic response modifiers present in the transfused blood product, along with recipient factors. Treatment is supportive and consists of oxygen, ventilator support and management of fluid balance.

8 Schedule of Patient Activities

Scheduled evaluations after screening and until engraftment may be performed +/-3 days from the targeted date; assessments performed after engraftment and through day 100 may be done +/-7 days of the targeted date. After day 100 assessments may be done +/- 30 days of the targeted date. In addition, targeted days may be altered as clinically appropriate (e.g., intercurrent illness, maximum blood volume for clinical care preventing research lab testing, patient refusal).

Schedule of Assessments – Standard of Care

Study Phase	**Screening**	**Conditioning** [24]	**MGTA-456**	**Follow up**									
Study Day(s)	**-60 to -10**	**-8 to -1**	**0**	**7 ±3**	**14 ±3**	**21 ±3**	**28 ±3**	**35 ±3**	**42 ±3**	**60 ±3**	**100 ±10**	**180 ±30**	**360±30 and 720±30**
Informed Consent	X SCRF												
Pregnancy test[1]	X SCRF												X (at 360 only)
Medical history/current medical conditions	OCRF At every visit with a medical provider												
Concomitant therapies	Reviewed at every visit with a medical provider												
Body height	X SCRF												
Vital signs[2]	X SCRF		X ICRF										
Physical examination	X OCRF At every visit with a medical provider												
Karnofsky / Lansky Performance status	X SCRF									X OCRF	X OCRF	X OCRF	X OCRF
Echocardiogram ECHO or MUGA	X SCRF												X OCRF
Adults: chest x-ray/ chest CT Peds: chest CT	X SCRF												
PFT (if capable) otherwise S02	X SCRF												X OCRF
GFR or creatinine clearance	X SCRF												
Hematology[3]	X SCRF	X Daily	X	X	X	X	X		X	X	X	X	X
Blood chemistries	X SCRF		X										

Study Phase	**Screening**	**Conditioning** [24]	**MGTA-456**	**Follow up**									
Study Day(s)	**-60 to -10**	**-8 to -1**	**0**	**7 ±3**	**14 ±3**	**21 ±3**	**28 ±3**	**35 ±3**	**42 ±3**	**60 ±3**	**100 ±10**	**180 ±30**	**360±30 and 720±30**
Immunoglobulin levels	X OCRF						X OCRF			X OCRF	X OCRF	X OCRF	X OCRF
Viral screen[4]	X SCRF												
CMV and EBV surveillance	X SCRF			X OCRF	X OCRF	X OCRF	X OCRF	X OCRF	X OCRF	X OCRF	X OCRF		
HHV6 surveillance (pcr)	X SCRF			X OCRF	X OCRF	X OCRF	X OCRF	X OCRF	X OCRF				
Anti-HLA antibody	X SCRF												
GVHD score[5]				X	X	X	X	X	X	X	X OCRF	X OCRF	X OCRF
Adverse events[6]		SAEs only	X										
Disease Status Assessments	X SCRF										X OCRF	X OCRF	X OCRF
Chimerism[7] (CD3, CD33/66)						X OCRF				X OCRF	X OCRF	X OCRF	X OCRF
T-cell subsets							X OCRF			X OCRF	X OCRF	X OCRF	X OCRF

Abbreviations: LTFU=long term follow-up; ECHO=echocardiogram; MUGA=multigated acquisition; PFT=pulmonary function tests; GFR=glomerular filtration rate; CMV=cytomegalovirus;HHV6=human herpes virus 6; GVHD=graft-versus-host disease; SAE=severe adverse event; SCRF=result recorded in a screening case report form; OCRF=result recorded in outcomes case report form; ICRF=result recorded in infusion case report form; x=result retained in electronic medical record with specific results eg days to ANC and platelet recovery, retained in the BMT database. Dates of transfusions, resource utilization and costs will be obtained from the electronic medical record and Fairview Health System after day 100 with summary files maintained in the BMT database.

1 For females of child bearing potential (post-menarche), serum pregnancy testing is required at screening and 1 year visits. If not done within 7 days of Day -9, pregnancy test must be repeated prior to initiating conditioning.

2 Vital signs include body weight, body temperature, blood pressure, pulse, and pulse oximetry. Vital signs will be checked before and every 15 minutes during the infusion, at the completion of the infusion, and one hour post infusion per institutional guidelines. More frequent vital signs may be required depending on reactions to the product infusion. Weight will be collected at pre-infusion only. The highest temperature recorded within 24 hr period will be reported.

3 Hematology will need to be repeated daily during inpatient hospitalization, then at every scheduled visit. The patient will remain hospitalized until neutrophil engraftment is achieved.

4 All patients will be screened for hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb), hepatitis C virus (HCV), herpes simplex virus (HSV), cytomegalovirus, (CMV), Epstein-Barr virus (EBV), and human T cell lymphotropic virus 1/2 (HTLV1/2). After Screening, infection surveillance will include human herpesvirus 6 (HHV-6) (to be tested weekly through Day 42), and CMV and EBV (to be tested weekly through Day 100).

5 Weekly assessments for GVHD is collected starting at Day 7 until hospital discharge, and then at every scheduled visit. aGVHD will be collected until Day 100 and cGVHD will be collected starting at Day 100 and then at Day 180 and 360/EOS

6 AEs must be reported starting from the day of transplant Day 0 and per the timeline specified in Section 9. SAEs will be collected from the time conditioning is initiated at Day -9 and per the timeframe specified in Section 9.

7 Chimerism will be assessed by sorting for 4 subsets - CD3 T cells, CD33/66 myeloid cells, CD19 B cells and CD56 NK cells. Evaluation of CD3 and CD33/66 subsets is considered SOC assessments on days 21, 28, 35, 42, 60, 100, 180, 360 and 720. Blood volume limits will be monitored for all patients; if the blood volume for SOC blood work exceeds the maximum allowable, research blood samples will either be cancelled or delayed until a date and time it is considered appropriate based on institutional criteria.

Schedule of Assessments – Research Only

Study Phase	**Screening**	**Conditioning**	**MGTA-456**	**Follow up**										
Study Day(s)	**-60 to -10**	**-8 to -1**	**0**	**7 ±3**	**14 ±3**	**21 ±3**	**28 ±3**	**35 ±3**	**42 ±3**	**60 ±3**	**100 ±10**	**120[6] ±10**	**180 ±30**	**360±30 and 720±30**
MGTA-456 Infusion[1,2]			X OCRF											
Chimerism[3, 8] (CD19/20) (CD16/56)				X OCRF	X OCRF	X OCRF	X OCRF			X OCRF	X OCRF		X OCRF	X OCRF
Chimerism[3, 8] (CD3, CD33/66)				X OCRF	X OCRF		X OCRF	X OCRF	X OCRF					
Cellular immune reconstitution[4]	X OCRF						X OCRF		X OCRF	X OCRF	X OCRF		X OCRF	X OCRF
T cell repertoire[4]	X OCRF[3]						X OCRF		X OCRF	X OCRF	X OCRF		X OCRF	X OCRF
TREC[5]							X OCRF		X OCRF	X OCRF	X OCRF		X OCRF	X OCRF
Serologies: Hep B, polio, pertussis, diphtheria, tetanus, pneumococcal	X OCRF									X OCRF	X OCRF		X OCRF	X
Pediarix and Pneumovax vaccines[9]										X OCRF		X OCRF	X OCRF	

Abbreviations: OCRF=result recorded in outcomes case report form

1 Two therapeutic cell preparations will be infused sequentially in the following order. First= a target cell dose is >10 x 10^6 CD34/kg. However, if the cell dose is < the target of 10 x 106 CD34/kg, it may be infused as long as it exceeds 1x10^6 CD34+/kg BW cells will be administered. With regard to TNC, the maximum cell dose is 2.7 x 10^8/kg for children (<18 years) and 8.1 × 10^8 cells/kg for adults [expanded product only] based on the highest cell dose windows evaluated in prior studies. There is no lower limit for TNC dose, only a lower limit for CD34 dose.. The exact dose level achieved after stem cell expansion should be recorded. Second = CD34- negative fraction from the same UCB unit. All available CD34-negative cells will be administered.

2.Graft Evaluation. The total number of CD3+ and CD34+ cells and methylcellulose colony-forming cells will be enumerated for all UCB grafts beinfused into the patient regardless prior processing (standard of care). Proportions of CD34+CD133+CD90+ cells and T cell subsets will also be determined (research).

3.Chimerism will be assessed by sorting for 4 subsets - CD3 T cells, CD33/66 myeloid cells, CD19 B cells and CD56 NK cells. Evaluation of CD19 and CD56 is research at all time points; evaluation of CD3 and CD33/66 subsets is research on days 7 and 14 but not for other time points, as this would be considered SOC assessments on days 21, 28, 35, 42, 60, 100, 180, 360 and 720. Blood volume limits will be monitored for all patients; if the blood volume for SOC blood work exceeds the maximum allowable, research blood samples will either be cancelled or delayed until a date and time it is considered appropriate based on institutional criteria.

4 Immune Reconstitution. Patients will also have an extensive evaluation of immune reconstitution, including NK and B cell subset but particular emphasis on T cell subsets, ie TCR rearrangement excision circles (TREC) and naïve (CD45RA+/CD27+), central memory (CD45RA+CD27-), effector memory (CD45RA-CD27-) and stem/memory (Tsm, CD8/45RA/62L/95/CCR7+) populations, as well as TCR repertoire diversity. In addition, Quantitative recovery of immunoglobulin synthesis and functional T and B cell generation and antigen driven responses in response to immunizations will be evaluated. For adult patients, six 10 mL green heparin tubes and one 10 mL red top tube at each time point. For pediatric patients, two 10 mL green heparin tubes and one 5 mL red top tube (not to exceed 2 mL/kg in patients ≤ 40kg) per time point

5 Frozen cells (no extra blood)

6 At subject's home clinic/provider

7 Diphtheria and tetanus ab panel(lab 792) 0.8 ml-2 ml red top; polio antibody (lab 653) 0.6 ml-2.0 ml red top; hepatitis B surface antibody (lab 6383) 1.2 ml-2 ml red top; B. pertussis lab 6759 0.8-2 ml red top; pneumo 7 (lab 5896) 0.7-3 ml red top.

8 If at any time point WBC < 500, do not draw sample for chimerism

9 Unless there is active acute or chronic GVHD. Missed immunizations will not be considered study deviations.

9 Adverse Event Monitoring, Recording and Reporting

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 5.0 (CTCAE) and reported on the schedule below. A copy of the CTCAE events can be downloaded from http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50

9.1 Definitions

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21CFR312.32(a)).

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

Treatment-Emergent Adverse Event: Any event not present prior to the initiation of the treatment or any event already present that worsens in either intensity or frequency following exposure to the treatment. A treatment emergent AE refers to an event temporally related to the study treatment regardless of the causality assessment by the investigator.

Life-Threatening Adverse Event Or Life-Threatening Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. Note: a life-threatening event does not necessarily equate to a CTCAE grade 4.

Serious Adverse Event Or Serious Suspected Adverse Reaction: An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.

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

Unexpected adverse event or unexpected suspected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as

amended. Thus, as defined by the FDA adverse events that occur as part of the disease process or underlying medical conditions are considered *unexpected;* however, for the purposes of this study they will not be documented or reported.

9.2 Adverse Event Monitoring, Recording and Reporting

9.2.1 Event Monitoring

Monitoring for adverse events will begin with MGTA infusion and continue through 2 year post-transplant anniversary visit.

9.2.2 Event Recording/Documentation

Due to the intentional use of chemotherapy and radiation to eliminate residual leukemia and provide adequate immune suppression to reduce the risk of graft rejection, numerous toxicities are anticipated in this patient population as detailed in section 7. Therefore, adverse event documentation in OnCore for the purposes of this study will focus on:

- All grade 4 or 5 non-hematologic adverse events through neutrophil engraftment or Day 42, whichever is shorter
- All serious unexpected suspected adverse reactions after Day 42 (or engraftment) through 2 years post-transplant upon knowledge
- Targeted toxicities (appendix V) for all patients at the following time points:
  - Prior to the MGTA infusion
  - 4 hours (+/- 1 hour) post UCB infusion
  - 24 hours (+/- 1 hour) post UCB infusion
  - 48 hours (+/- 1 hour) post UCB infusion

Transplant related outcomes will be collected as routine by the BMT Database.

9.2.3 Event Reporting to the IRB, FDA and Cancer Center

Certain events will require prompt reporting according to the table below:

Agency	Criteria for reporting	Timeframe	Form to Use	Submission information	Copy to:
IRB	Events requiring prompt reporting including, but not limited to unanticipated death of a locally enrolled subject(s); new or increased risk; any adverse event that require a change to the protocol or consent form or any protocol deviation that resulting in harm refer to http://www.research.umn.edu/irb/forms.html	within 5 business days of discovery	Report Form	irb@umn.edu	SAE Coordinator mcc-saes@umn.edu
Masonic Cancer Center SAE Coordinator	Events that meet the definition of dose limiting toxicity or an early study stopping rule	At time of reporting	Event Form	mccsaes@umn.edu	

FDA	Unexpected fatal or unexpected life threatening suspected adverse reaction	As soon as possible but no later than 7 calendar-days	Institutional SAE form	Submit as an amendment to IND	
	1) Serious and unexpected suspected adverse reaction or 2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure or 3) findings from other sources (other studies, animal or in vitro testing)	As soon as possible but no later than 15 calendar-days			
	Note: Events due to the disease under treatment or an preexisting medical condition will not require expedited reporting to the FDA for the purposes of this study				
Magenta	1) Product manufacturing failures 2) Failure of hematopoietic engraftment				

At the time of continuing review/IND annual report, relevant events recorded in OnCore will be reported in summary format by those persons responsible for such reporting.

9.3 Monitoring for Stopping Rule Events

In addition to the event monitoring and reporting described in the previous section, the following events will be considered excess toxicity and be reported using the Event Form found in OnCore:

- Excess graft failure at day 42
- Excess TRM at day 100
- Excess grade III-IV acute GVHD

A copy of the completed Event Form must be sent to the SAE Coordinator (mcc-saes@umn.edu) at the time it is submitted to the study statistician.

As these events are outcomes of the treatment, not toxicity as found in CTCAE, they should only be recorded/reported as an AE, if they meet the criteria found in section 9.2

10 Study Data Collection and Monitoring

10.1 Data Management

This study will collect regulatory and clinical data using University of Minnesota CTSI's instance of OnCore® (Online Enterprise Research Management Environment).

The Oncore database resides on dedicated secure and PHI compliant hardware consisting of 3 physical servers: dev, DR, and production. The dev server is located in the University of Minnesota (UMN) datacenter (WBOB) and houses six database

instances (test, train, sandbox, mcc reports, oncdm, and vendor) that are backed up locally because the data is refreshed from Oncore production data. The production server is located in the UMN datacenter (WBOB). All the data servers are managed by the Academic Health Center – Information Systems (AHC-IS) virtual servers which utilize clustered infrastructure to provide real-time failover of virtual servers. This real-time clustering is physically limited to the UMN data center. All relevant AHC IS procedures related for PHI compliant servers (as required by the Center of Excellence for HIPAA Data) apply to Oncore databases.

The integrated data will be stored in PHI compliant servers managed by AHC IS with access given to those authorized users in the Clinical and Translation Science Institute Informatics team (CTSI BPIC and MCC CISS). The data will be integrated and extracted to researchers through the CTSI Informatics team and will be delivered through secure and compliant mechanisms (e.g. AHC IE data shelter, BOX, sftp, etc). If data de-identification is needed, then compliant AHC IE data de-identification tools will be used. The informatics team will grant the IRB approved study team members access to data.

Additional data about correlative laboratory samples generated by the Masonic Cancer Center Translational Therapy Laboratory (TTL) from the protocol-directed correlative research samples is stored in their Laboratory Information Management System (LIMS).The LIMS database application is also stored on a production server located in the UMN datacenter (WBOB) and is managed by the Academic Health Center

Key study personnel are trained on the use of OnCore and will comply with protocol specific instructions embedded within the OnCore.

10.2 Case Report Forms

Participant data will be collected using protocol specific CRFs developed within OnCore, based on its library of standardized forms. The CRFs will be approved by the study's Principal Investigator and the Biostatistician prior to release for use. There is a screening case report form (SCRF) which the Study Coordinator or designee will use to register the patient into OnCore at time of study entry. An infusion case report form (ICRF) will be used to document adverse events during the infusion of MGTA-456 (expanded product and CD34 depleted product) and outcomes case report form (OCRF) will record specific outcomes data for submission to the BMT Database. As medications, blood product administration, provider examinations and laboratory/radiographic data are accessible to the BMT Database through a Data Sharing Agreement, CRFs will only be needed for specific data collection.

10.3 Data and Safety Monitoring Plan

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://z.umn.edu/dmsp

For the purposes of data and safety monitoring, this study is classified as high risk (under a locally held IND). Therefore the following requirements will be fulfilled:

- The Masonic Cancer Center Data and Safety Monitoring Council (DSMC) will review the study's progress at least quarterly with the understanding the Cancer Protocol Review Committee (CPRC) may require more frequent reporting.
- The PI will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The PI will oversee the submission of all reportable adverse events per the definition of reportable in section 9.2.3 to the Masonic Cancer Center's SAE Coordinator, the University of Minnesota IRB, and the FDA.

In addition, at the time of the continuing review with the University of Minnesota IRB, a copy of the report with any attachments will be submitted to the CPRC.

10.4 IND Annual Reports

In accordance with regulation 21 CFR § 312.33, the sponsor-investigator with assistance from the MCC Clinical Trials Office (CTO) will submit a progress report annually. The report will be submitted within 60 days of the anniversary date that the IND went into effect.

10.5 Data Sharing with Magenta

Only data related to MGTA-456 product manufacturing data and product failures (lot release, failure to engraft) will be shared with Magenta.

10.6 Monitoring

The investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, and University of Minnesota compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

10.7 Study Record Retention

The investigator will retain study records including source data, copies of case report form, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at 6 years after the study file is closed with the IRB and FDA.

In addition, the CTO will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient.

Please contact the CTO before destroying any study related records.

11 Definition of Study Endpoints

11.1 Neutrophil Recovery

All patients will have daily complete blood counts beginning on day +1 with white blood cell differential assessments beginning when the total WBC ≥0.5 x 10^9/L. The day of neutrophil recovery will be the first of three consecutive days when the ANC is ≥0.5 x 10^9/L.

11.2 Platelet Recovery

The frequency of platelet count assessments will vary over the course of the transplant period. During the firsts 100 days after transplant, all patients will have at least weekly platelet counts. The day of platelet recovery will be the first of three consecutive measurements when the platelet count is ≥20,000 x 10^9/L without a platelet transfusion in the prior 7 days.

11.3 Engraftment

Engraftment is determined by neutrophil recovery with hematopoietic cells of donor origin (chimerism). Complete chimerism is defined as 90-100% of marrow cells or CD33/66+ peripheral blood cells of donor origin. Partial chimerism is defined as 10-90% donor cells and autologous recovery is defined as 0-10% donor cells.

11.4 Graft Failure

Graft failure is the absence of neutrophil recovery or lack of chimerism from any etiology.

- **Primary Graft Failure** is defined by the absence of neutrophil recovery by day 42 after UCBT or absence of chimerism before day 42. Note: Patients surviving ≥14 days will be considered evaluable and patients receiving a 'back-up' unmanipulated graft within 5 days of the transplant, as a result of bioassays outside the specification limits for MGTA-456, will not be considered evaluable
- **Secondary Graft Failure** is defined by a recurrence of neutropenia (ANC <0.5 x 10^9/L lasting >7 days) or autologous recovery after prior engraftment.

11.5 Graft-versus-Host Disease

GVHD is the immune response of donor cells directed against host tissues. There are two distinct forms – acute and chronic.

- **Acute GVHD** is syndrome that typically occurs in the first 100 days after transplant and is characterized by abdominal pain or cramps, nausea, vomiting, and diarrhea, jaundice or other liver function abnormalities, and skin rash. The diagnosis is based on the presence of one or more of these symptoms without other etiology and often supported by tissue biopsy. Patients will be assigned an overall GVHD score based on extent of skin rash, volume of diarrhea and maximum bilirubin level. At day 100, the maximum severity will be determined using the NIH Consensus Criteria.
- **Chronic GVHD** is a syndrome that typically occurs 3 months or more after transplant and is characterized by dry eyes or vision changes, dry mouth with increased sensitivity to foods, fatigue, muscle weakness, arthralgias, arthritis, contractures, skin

rash, hyper and hypopigmentation, scleroderma, obliterative bronchiolitis, steatorrhea, weight loss. The diagnosis is based on the presence of one or more of these symptoms without other etiology and often supported by tissue biopsy.

11.6 Transplant-Related Mortality

TRM is a death in continuous complete remission.

11.7 Relapse

Relapse is the recurrence of the underlying disease for which the transplant was performed. The diagnosis of relapse is based on morphological evidence supported by cytogenetic and/or molecular testing consistent with disease characteristics noted at diagnosis or a relapse prior to transplant.

11.8 Non-Catheter Associated Bacterial Infection

All blood or invasive bacterial infections (i.e. excluding surveillance cultures of the nose, throat, stool and urine; catheter infections) which are not considered to be a central line-associated infection (CLABSI) based on published criteria from the U.S. Centers for Disease Control, will be considered an event. A second positive bacterial culture with the same organism from the same site will not be considered a new event unless symptoms resolved after treatment and negative cultures were documented for at least one week.

11.9 Event Free Survival (EFS)

EFS is the patient's status of being alive with sustained engraftment and in continuous complete remission.

12 Statistical Considerations

12.1 Study Design, Objectives and Endpoints

The principal objective of this phase II trial is to determine if transplantation of MGTA-456 significantly reduces the expected duration of neutropenia relative to that estimated in recipients of an unmanipulated single UCB unit transplantation receiving the same conditioning and GVHD immunoprophylaxis for hematological malignancy. The primary endpoint of engraftment will be evaluated using a two stage enrollment procedure, while TRM, graft failure, and excessive acute GVHD will be monitored using continual stopping boundaries. Stage 1 will enroll 18 patients. If 4 or more demonstrate engraftment by day 14, an additional 22 patients will be enrolled in stage 2 for a maximum total of 40 enrolled patients. If 9 or more demonstrate engraftment in both stages combined, the treatment will be considered a success.

The aim is to evaluate the impact of MGTA-456 containing $\geq 10 \times 10^6$ CD34 cells/kg. Patients may still receive MGTA-456 at lower cell doses ($<10 \times 10^6$ CD34 cells/kg but $\geq 2 \times 10^6$ CD34 cells/kg) but endpoints and stopping rules will be evaluated separately by study design.

12.1.1 Primary Endpoint

- Incidence of neutrophil recovery by day 14 after transplantation

12.1.2 Secondary and Transplant Related Secondary Endpoints

- Number of days alive without hospitalization between days 0 and 100 after transplantation
- Incidence of secondary graft failure
- Incidence of platelet recovery at day 60
- Incidence of TRM at 6 months
- Incidence of grades II-IV and III-IV acute GVHD at day 100
- Incidence of chronic GVHD at 1 year
- Incidence of relapse at 2 years
- Incidence of non-catheter associated bacterial infections by day 100
- Probability of overall survival and event-free survival at 2 years

12.1.3 Exploratory Endpoints

- Description of immunological recovery
- Incidence of absolute CD4 T cell ≥0.2 x 10^9/L by day 100
- Incidence of opportunistic infection (viral / fungal) by day 100
- Description of hospital costs by day 100
- Description of number of red cell and platelet transfusions by day 100.
- Assess B and T cell immune reconstitution as well as functional responses at day 100 and day 180.

12.2 Statistical Analysis

Our primary hypothesis is that subjects transplanted with MGTA-456 will demonstrate neutrophil recovery by day 14 at a substantially higher frequency relative to the historical estimate for identically treated patients transplanted with unmanipulated UCB. Neutrophil engraftment by day 14 will be estimated with simple proportions. Given our experience from historical data as well as the pilot data for MGTA-456, it is unlikely that there will be any deaths without engraftment prior to day 14. If such a scenario arises, however, neutrophil engraftment will be estimated by cumulative incidence, treating non-event death as a competing risk [41]. Cumulative incidence will also be used to estimate platelet recovery, TRM, GVHD, relapse and infection treating non-event deaths or relapse in the case of TRM as a competing risk. Simple proportions will be used to estimate secondary graft failure among engrafted patients only. Overall survival and EFS will be estimated with Kaplan-Meier curves [43]. If assumptions are satisfied, a sample mean along with a 95% confidence interval will be used to assess the number of surviving discharged days from the hospital prior to day 100 post-transplant. In addition, a one-sample t-test will be run to compare the study group average to a null hypothesis estimate of 60 days. If assumptions are not satisfied, descriptive statistics such as medians, ranges and interquartile ranges will be used. These will similarly be used for the endpoints of immune reconstitution and hospital costs. Descriptive endpoints may also be summarized with boxplots and dot plots. No specific hypothesis tests will be

used to investigate B and T cell immune reconstitution or functional response. Descriptive statistics such as medians and ranges at days 100 and 180 along with descriptive plots will be used to assess these parameters.

12.3 Rationale for Sample Size

Although the primary endpoint is the incidence of patients reaching neutrophil recovery by day 14, the sample size is based on the more restrictive test for the number of surviving hospital-free days prior to day 100. We want to detect a minimum 10 day improvement in the average number of days surviving outside of the hospital. Based on the historical estimate of 60 days and using a standard deviation of 25.0 from pilot data, a sample size of 40 patients will provide 80% power to detect a minimum improvement of 10 days over the null hypothesis of 60 days. This power estimate uses a one-sided one-sample t-test. In the assessment of our primary endpoint, historical data show that the standard control populations have an estimated neutrophil engraftment of 13% by day 14 post-HSCT. Pilot data among patients receiving the expanded MGTA-456 unit potentially show engraftment of 60% by day 14. We want to detect a minimum improvement of 20% (from 13% to 33%). Based on a Simon's optimal two stage design with a type I error of 5%, 40 patients will provide power of 87% [48].

12.4 Monitoring Guidelines

Early stopping rules for excess graft failure, TRM and grade III-IV acute GVHD will be in place to monitor safety. In the event that a monitoring boundary is triggered, study enrollment will be suspended and the PI, IRB and the Cancer Center's DSMC will be notified.

The stopping rules were developed using an adaptation of Pocock stopping boundaries [44].

- Graft Failure by day 42: We will construct a boundary such that the probability of early stopping is at most 5% if the graft failure rate is equal to 5%. The monitoring boundary for the MGTA-456 patients will be 2/4, 3/12, 4/21, 5/31 or 6 graft failures out of 40 patients. The probability of stopping early if the actual probability of graft failure is 20% is 88%.
- TRM by 6 months: We will construct a boundary such that the probability of early stopping is at most 5% if the TRM rate is equal to 20%. The monitoring boundary for the MGTA-456 patients will be 3/3, 4/5, 5/8, 6/11, 7/14, 8/17, 9/21, 10/24, 11/28, 12/31, 13/35, 14/38 or 15 deaths out of 40 patients. The probability of stopping early if the actual probability of TRM is 40% is 80%.
- Grade III-IV Acute GVHD day 100: We will construct a boundary such that the probability of early stopping is at most 5% if the GVHD rate is equal to 15%. The monitoring boundary for the MGTA-456 patients will be 3/4, 4/7, 5/10, 6/14, 7/18, 8/22, 9/27, 10/32, 11/36 or 12 events out 40 patients. The probability of stopping early if the actual probability of TRM is 30% is 66%. Operating characteristics are calculated using software available at www.bios.unc.edu/~qaqish/software.

- We understand that inclusion of the stopping rules may slightly reduce the overall power of our study.

12.5 Gender and Ethnicities Statement

This study is open to both males and females and to all racial/ethnic groups. The patient enrollment pattern is expected to be similar to that of other hematological malignancy studies. It is not anticipated that the outcome will be affected by either race or gender. The study will not have separate accrual targets for different subgroups.

13 Conduct of the Study

13.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files will be established at the beginning of the study, maintained for the duration of the study and retained in a centralized file within the Clinical Trials Office of the Masonic Cancer Center according to the appropriate regulations.

13.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

13.3 Informed Consent

All potential study participants will be given a copy of the IRB-approved consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

14 References

1) Cheuk DKL. Optimal stem cell source for allogeneic stem cell transplantation for hematological malignancies. World. J. Transplant. 2013; 3 (4): 99-112.
2) Eapen M, Rocha V. Principles and analysis of hematopoietic stem cell transplantation outcomes: the physician's perspective. Lifetime Data Anal 2008; 14: 379-88.
3) Gragert L, Eapen M, Williams E, Freeman J, Spellman S, Baitty R, Hartzman R, Rizzo JD, Horowitz M, Confer D, Maiers M. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. Registry. New Engl J Med 2014;371: 339-348.
4) Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. Blood. 2013; 122 (4): 491-498.
5) Smith AR, Wagner JE. Alternative hematopoietic stem cell sources for transplantation: place of umbilical cord blood. Br. J. Haematol. 2009; 147 (2): 246-61.
6) Wagner JE, Kernan NA, Steinbuch M, Broxmeyer HE, Gluckman E. Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease. Lancet. 1995;346:214-219.
7) Gluckman E, Rocha V, Boyer-Chammard A, et al. Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. N Engl J Med. 1997;337:373-381.
8) Rubinstein P, Adamson JW, Stevens C. The placental/umbilcal cord blood program of the New York BloodCenter: a progress report. Ann NY Acad Sci. 2006;872:328–35.
9) Hackenberg P, Kögler G, Wernet P. NETCORD: a cord blood allocation network. Bone Marrow Transplant. 1998;22:S17– 8.
10) Lazzari L, Corsini C, Curioni C, Lecchi L, Scalamogna M, Rebulla P, et al. The Milan Cord Blood Bank the Italian Cord Blood Network. J Hematother. 1996;5:117–22.
11) Barker JN. Umbilical cord blood (UCB) transplantation: an alternative to the use of unrelated volunteer donors? Hematology (Am Soc Hematol Educ Program) 2007; 2007:55-61
12) Brunstein CG, Gutman JA, Weisdorf DJ, Woolfrey AE, Defor TE, Gooley TA, et al. Allogeneic hematopoietic cell transplantation for hematological malignancy: Relative risks and benefits of double umbilical cord blood. Blood. 2010;116:4693-9
13) Verneris MR, Brunstein CG, Barker J, et al. Relapse risk after umbilical cord blood transplantation: enhanced graft-versus-leukemia effect in recipients of 2 units. Blood. 2009;114:4293-4299.
14) Rocha V, Labopin M, Mohty M, et al. Outcomes after double unit unrelated cord blood transplantation compared to single UCBT in adults with acute leukemia in remission: An Eurocord ELWP Collaboration Study. Blood. 2010;116:401
15) Showel M, Fuchs EJ. Recent developments in HLA-haploidentical transplantations. Best Pract Res Clin Haematol 2015; 28: 141-6.
16) Kasamon YL, Bolaños-Meade J, Prince GT, et al. Outcomes of nonmyeloablative HLA-haploidentical blood or marrow transplantation with high-dose post-transplantation cyclophosphamide in older adults. J Clin Oncol 2015; 33: 3152-61.
17) Shaw BE. Related haploidentical donors are a better choice than matched unrelated donors: Counterpoint. Blood Advances 2017; 1: 401-406.

18) Wagner JE, Barker JN, DeFor TE, Baker KS, Blazar BR, Eide C, Goldman A, Kersey J, Krivit W, MacMillan ML, Orchard PJ, Peters C, Weisdorf DJ, Ramsay NK, Davies SM. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood 2002;100: 1611-8.
19) Kurtzberg J, Prasad VK, Carter SL, Wagner JE, Baxter-Lowe LA, Wall D, et al. Results of the Cord Blood Transplantation Study (COBLT): clinical outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with hematologic malignancies. Blood. 2008;112:4318-27.
20) Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, Miller JS, Wagner JE. Transplantation of two partially HLA matched umbilical cord blood units to enhance engraftment in adults with hematological malignancy. *Blood*. 2005; 105 (3): 1343-7.
21) Brunstein CG, Barker JN, Weisdorf DJ, DeFor TE, Miller JS, Blazar BR, McGlave PB, Wagner JE. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematological disease. *Blood*. 2007; 110 (8): 3064-70.
22) Wagner JE, Jr., Eapen M, Carter S, Wang Y, Schultz KR, Wall DA, et al. One-unit versus two-unit cord-blood transplantation for hematologic cancers. N Engl J Med. 2014; 371: 1685-94.
23) Eapen M, Kurtzberg J, Zhang M-J, Hattersely G, Fei M, Mendizabal A, Chan KW, De Oliveira S, Schultz KR, Wall D, Horowitz MM, Wagner JE. Umbilical Cord Blood Transplantation in Children with Acute Leukemia: Impact of Conditioning on Transplantation Outcomes. Biol Blood Marrow Transplant 2017; 23: 1714–1721.
24) Wagner JE, Brunstein CG, Boitano AE, DeFor TE, McKenna D, Sumstad D, Blazar BR, Tolar J, Le C, Jones J, Cooke CP, Bleul CC. Safety and efficacy of StemRegenin-1 expanded umbilical cord blood hematopoietic cells after myeloablative conditioning. Cell Stem Cell 2016;18:144-55.
25) Boitano, A. E.,Wang, J.,Romeo, R.,Bouchez, L. C.,Parker, A. E.,Sutton, S. E.,Walker, J. R.,Flaveny, C. A.,Perdew, G. H.,Denison, M. S.,Schultz, P. G.,Cooke, M. P. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. Science 2010, 329 (5997), 1345-8.
26) de Lima M, McMannis J, Gee A, Komanduri K, Couriel D, Andersson BS, Hosing C, Khouri I, Jones R, Champlin R, et. al. Transplantation of ex vivo expanded cord blood cells using the copper chelator tetraethylenepentamine: a phase I/II clinical trial. Bone Marrow Transplant. 2008; 41 (9): 771-8.
27) Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RI, Bernstein ID. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. Nat. Med. 2010; 16 (2): 232-6.
28) de Lima M, McNiece I, Robinson SN, Munsell M, Eapen M, Horowitz M, Alousi A, Saliba R, McMannis JD, Kaur I, et.al. Cord-blood engraftment with ex vivo mesenchymal-cell culture. New Engl. J. Med. 2012; 367 (24): 2305-15.
29) Horwitz ME, Chao NJ, Rizzieri DA, Long GD, Sullivan KM, Gasparetto C, Chute JP, Morris A, McDonald C, Waters-Pick B, et. al. Umbilical cord blood expansion with nicotinamide provides long-term multi-lineage engraftment. J. Clin. Invest. 2014; 124 (4): 3121-8.

30) Radtke S, Gorgens A, Kordelas L, Schmidt M, Kimmig KR, Koninger A, Horn PA, Giebel B. CD133 allows elaborated discrimination and quantification of haematopoietic progenitor subsets in human haematopoietic stem cell transplants. Brit J Haematol 2015; 169: 868-878.
31) Wong WM, Sigvardsson M, Astrand-Grundstom I, Hogge D, Larsson J, Qian H, Ekblom M. Expression of integrin α2 receptor in human cord blood CD34+CD38-CD90+ stem cells engrafting long-term in NOD/SCID-IL2R□c null mice. Stem Cells 2013; 31:360-371.
32) Gutman JA, Turtle CJ, Manley TJ, Heimfeld S, Riddell SR, Delaney C. Single-unit dominance after double-unit umbilical cord blood transplantation coincides with a specific CD8+ T-cell response against the nonengrafted unit. Blood. 2010; 115 (4): 757-785.
33) Ramirez P, Wagner JE, DeFor TE, Blazar BR, Verneris MR, Miller JS, McKenna DH, Weisdorf DJ, McGlave PB, Brunstein CG. Factors predicting single-unit predominance after double umbilical cord blood transplantation. Bone Marrow Transplant. 2012; 47 (6): 799-803.
34) Lund TC, Boitano AE, Delaney CS, Shpall EJ, Wagner JE. Advances in umbilical cord blood manipulation-from niche to bedside. Nat Rev Clin Oncol. 2015;12:163-74.
35) Yahata T, Ando K, Miyatake H, Uno T, Sato T, Ito M, Kato S, Hotta T. Competitive repopulation assay of two gene-marked cord blood units in NOD/SCID/gammac(null) mice. Mol Ther 2004; 10: 882-91.
36) Shu Z, Heimfeld S, Gao D. Hematopoietic SCT with cryopreserved grafts: adverse reactions after transplantion and cryoprotectant removal before infusion, Bone Marrow Transplant 2014; 49: 469-476.
37) Konuma T, Ooi J, Takahashi S, Tomonari A, Tsukada N, Kobayashi T, Sata A, Kato S, Kasahara S, Ebihara Y, Nagamura-Inoue T, Tsuji K, Tojo A, Asano S. Cardiovascular toxicity of cryopreserved cord blood cell infusion, Bone Marrow Transplant 2008; 41: 861-865.
38) Regan DM, Wofford JD, Wall DA. Comparison of cord blood thawing methods on cell recovery, potency, and infusion. Transfusion. 2010; 50:2670-75.
39) Kharbanda S, Smith AR, Hutchinson SK, McKenna DH, Ball JB, Lamb LS, Agarwal R, Weinberg KI, Wagner JE. Unrelated donor allogeneic hematopoietic stem cell transplantation for patients with hemoglobinopathies using a reduced- intensity conditioning regimen and third-party mesenchymal stromal cells. Biol Blood Marrow Transplant.2014; 20:581-586.
40) Martín-Henao GA, Resano PM, Villegas JM, Manero PP, Sánchez JM, Bosch MP, Codins AE, Bruguera MS, Infante LR, Oyarzabal AP, Soldevila RN, Caiz DC, Bosch LM, Barbeta EC, Ronda JR. Adverse reactions during transfusion of thawed haematopoietic progenitor cells from apheresis are closely related to the number of granulocyte cells in the leukapheresis product. Vox Sang. 2010; 99:267-73.
41) Lin, DY. Non-parametric inference for cumulative incidence functions in competing risks studies. Statistics in Medicine 1997, 16 (8), 901-10.
42) Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. Ann Stat. 1988;16:1141-54.
43) Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53:457-81.

44) Ivanova A, Qaqish B, Schell M. Continuous toxicity monitoring in phase II trials in oncology. Biometrics. 2005 61:540-, 2005.
45) Park BG, Park CJ, Jang S, et al. Reconstitution of lymphocyte subpopulations after hematopoietic stem cell transplantation: comparison of hematologic malignancies and donor types in event-free patients. Leuk Res. 2015;39(12):1334-1341.
46) Abdel-Azim H, Elshoury A, Mahadeo KM, Parkman R, Kapoor N. Humoral Immune Reconstitution Kinetics after Allogeneic Hematopoietic Stem Cell Transplantation in Children: A Maturation Block of IgM Memory B Cells May Lead to Impaired Antibody Immune Reconstitution. Biol Blood Marrow Transplant. 2017;23(9):1437-1446.
47) Wagner JE, Brunstein C, McKenna D, et al. StemRegenin-1 (SR1) Expansion Culture Abrogates the Engraftment Barrier Associated with Umbilical Cord Blood Transplantation (UCBT). Vol. 124; 2014.
48) Simon R. Optimal two-stage designs for Phase II Clinical Trials. Controlled Clinical Trials 1989;10:1-10.

Appendix I – Karnofsky and Lansky Scales

Karnofsky Scale (recipient age ≥ 16 years)	Lansky Scale (recipient age <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

https://www.cibmtr.org/manuals/fim/1/en/topic/appendix-l-karnofsky-lansky-performance-status

Lansky scale above will only be applied to children aged 2-15 years, inclusive. For children <2 years, scores will be severe, mild to moderate or no restrictions:

Levels of Restriction:

Severe	All activities require more than any age appropriate assistance
Mild to moderate	Some activities require more than age appropriate assistance
None	All activities require assistance that is age appropriate

Appendix II - Recommended TBI Guidelines

Fractionated Total Body Irradiation (In Lateral Position):

1320 cGy administered in an 8 fractions of 165 cGy each with 2 fractions being given each day.

Total body irradiation is given at a dose rate of 10-19 cGy/minute prescribed to the midplane of the patient at the level of the umbilicus.

The total body irradiation will be delivered with right and left lateral fields, with the patient supine on a specially designed couch.

Based on measurements of transverse thicknesses, aluminum compensators will be used to ensure that the dose homogeneity across the field is within 10 % of the prescribed dose. Usually head/neck, leg and lung compensators are used (although based on calculated mid-mediastinal doses, lung compensators are often not needed).

Total body irradiation will be delivered with a linear accelerator using 6, 10, and 18 MeV X-rays. The energy used will be based on the calculated dose to the midline at points up and down the patient's torso. The lowest energy that gives 90-100% of the prescription point dose will be used.

A beam "spoiler" will be used to ensure a full skin dose.

Half value layer lung and kidney blocks will not be utilized.

Testicular boosts should be used for all males with ALL (and according to institutional practice for other diseases). The testicular boost is given in a single 400 cGy fraction with either electrons prescribed to Dmax or photons prescribed to the midplane of the scrotum. If electrons are used, the energy for the testicular boost depends on the thickness of the testicles and is chosen so that the D90 corresponds > to the posterior surface of the scrotum.

Appendix III – Busulfan Dose Selection, AUC Monitoring and Algorithm for Dose Modification Using Once Daily IV Dosing

This protocol will use once daily (every 24 hour) intravenous BU dosing. Four (4) total doses of BU will be given over 4 days in the preparative regimen. AUC determination after each of the first 3 doses will be performed (AUC1, AUC2, and AUC3 following Doses #1, #2 and #3, respectively). For all patients, each busulfan dose will be administered over 3 hours by central venous line per University of Minnesota BMT Program standard guidelines..

THE TOTAL regimen busulfan AUC (AUC_{cum}) targeted will be = **14,080-16,400 (μM·Min)/L [57.7-67.2 (mg · h)/L] with ideal target AUC_{cum} of 15,200 (μM·Min)/L [62 (mg · h)/L]** in order to best optimize engraftment while minimizing toxicity. If at any time upon interim analysis and review of patients transplanted on this protocol with the principal investigators and pharmacy team, the subsequent busulfan levels (following doses #2 and #3) are deemed reasonable and consistent, the decision may be made to only do kinetics with the first dose for all remaining patients treated on this protocol. In that event, kinetics for a subsequent dose (i.e. AUC monitoring) may be performed whenever a dose change is required but will not be mandatory. These protocol changes (if enacted) will not require a treatment deviation or protocol amendment.

Steady State Concentration (Css) equivalent to 14,080-16,400 (μM·Min)/L = **600-700 ng/ml**

DETERMINATION OF FIRST BUSULFAN DOSE (DOSE #1)

The initial empiric busulfan dosing (Dose #1) for ALL patients will be determined as follows:

a. For patients weighing <12 kg: initial dose will be based on the formula according to the population PK model developed by Long-Boyle.
b. For patients weighing ≥12kg and <66 kg: initial dose will be determined by the nomogram adapted from Bartelink.
c. For patients weighing ≥66kg: initial busulfan dose = 3 mg/kg IV Once Daily

1. **For patients <12 kg: the first busulfan dose will be based on the population PK formula developed by Long-Boyle which calculates the first dose as a function of weight and age:**
    - Use the Dosing Calculator (Long-Boyle Busulfan 1st Dose) on the BMT website to determine the precise dose for the patient;
    - Age: enter the age in years (to the hundredths place) by dividing the patient's age in days by 365
    - Weight: enter the weight in kg (to the tenths place)
    - The Calculator is constructed using the following function:
        - Dose (mg) = $AUC_{(target)}$ x CL_i
        - $AUC_{(target)}$ = 15.6 mg·hr·L-1 (equivalent to 3800 μM·min·L-1)
        - CL_i is a function of weight and age
            - CL_i = 2.3 L/h x (Mat_{mag} + (1- Mat_{mag}) x [1-$e^{(-age \times Kmat)}$]) x (weight/8 kg)$^{0.75}$
            - Maturation magnitude (Mat_{mag}) = 0.46; Maturation rate constant (k_{mat}) = 1.4

- See Table A-1 below for EXAMPLE first Busulfan doses for hypothetical children under 12 kg; this table can be used to ensure your patient's calculator output does not carry significant error. However, the patient's first dose should be determined directly from the calculator.

Table A-1: Sample First Busulfan Doses for Various Ages and Weights Using the Long-Boyle Method for Determination of First Busulfan Dose for patients < 12 kg & for ideal target AUC_{cum} of 15,200 (μM·Min)/L [62 (mg · h)/L. Use this table to check against error from the calculator.

Sample Age	Sample Weight (kg)	1st Busulfan Dose Per Long-Boyle Method (mg)
1 month (0.08 years)	4	11
	4.4	11.9
	5	13
3 months (0.25 years)	5.8	17.4
	6.4	18.7
	6.8	19.6
6 months (0.50 years)	7.2	24.1
	7.8	25.5
	8.4	27
9 months (0.75 years)	8.2	29.4
	9	31.6
	9.6	33.1
12 months (1 years)	9.6	35.4
	10.2	37.1
	11	39.2
15 months (1.25 years)	10.2	38.8
	11	41
	11.8	43.3

2. **For patients >/= 12 kg to <66 kg:** the first busulfan dose will be according to weight-based dosing nomogram developed by Bartelink, which determines the first dose as a function of **weight** only.
    - Determine the first dose directly from the nomogram chart (no calculator is used)
    - Round the patient's weight to the nearest kg. For weights in kg ending in "x.5", round UP to the nearest kg. For instance, for a weight of 43.5 kg, use 44 kg to determine the first busulfan dose from the nomogram.
    - See Table A-2 for nomogram

Table A-2: Nomogram for the Determination of First Busulfan Dose for Patients ≥ 12 kg and < 66kg for ideal target AUC_{cum} of 15,200 (μM·Min)/L [62 (mg · h)/L. (Adapted from Bartelink).
Round the patient's weight to the nearest kg in order to determine the first busulfan dose from this nomogram

Weight (kg)	Dose (mg)	**Weight (kg)**	Dose (mg)	**Weight (kg)**	Dose (mg)	**Weight (kg)**	Dose (mg)	**Weight (kg)**	Dose (mg)	**Weight (kg)**	Dose (mg)
		20	64.7	30	86.3	40	103.5	50	117.8	60	130.5
		21	67.1	31	88.2	41	105	51	119.1	61	131.6
12	41.8	22	69.5	32	90.2	42	106.5	52	120.5	62	132.6
13	45	23	71.9	33	92.1	43	108	53	121.8	63	133.7
14	48.2	24	74.3	34	94.1	44	109.5	54	123.2	64	134.7
15	51.2	25	76.5	35	96	45	111	55	124.5	65	135.8
16	54	26	78.5	36	97.5	46	112.4	56	125.7		
17	56.8	27	80.4	37	99	47	113.7	57	126.9		
18	59.5	28	82.4	38	100.5	48	115.1	58	128.1		
19	62.2	29	84.3	39	102	49	116.4	59	129.3		

3. **For patients weighing ≥ 66kg:** first busulfan dose = 3 mg/kg IV.

BUSULFAN THERAPEUTIC DRUG MONITORING (TDM)

Area under the curve (AUC) analyses will be calculated in-house per University of Minnesota BMT standard guidelines for all patients. The AUC will be calculated for each dose using serum busulfan concentrations (in ng/ml) performed by the University of Minnesota Medical Center Drug Analysis Lab and obtained at the following time-points:

- Immediately prior to busulfan infusion (*ONLY to be drawn if obtaining levels with doses 2, 3 or 4)
- Immediately after the end of the busulfan infusion
- 15 minutes after the end of the busulfan infusion
- 1 hour after the end of the busulfan infusion
- 3 hours after the end of the busulfan infusion
- 5 hours after the end of the busulfan infusion
- 7 hours after the end of the busulfan infusion

* “trough” or pre-dose serum busulfan level will only be obtained when performing AUC on 2nd or higher doses, as serum busulfan level prior to dose #1 is assumed to be zero.

After determination of the patient's clearance and AUC, subsequent doses will be calculated linearly to achieve a goal cumulative AUC exposure.

For patients receiving 4 total busulfan doses the goal AUC_{cum} = 14,080-16,400 (μM·Min)/L [57.7-67.7 (mg · h)/L] with ideal target AUC_{cum} of 15,200 (μM·Min)/L [62 (mg · h)/L]

GUIDELINES FOR CHANGING SUBSEQUENT BUSULFAN DOSES BASED ON A PATIENT'S TDM RESULTS

Subsequent busulfan dose changes will be made according to the following guidelines:

1. The **goal cumulative AUC range** (AUC_{cum} for the entire 4-dose course) **will be 14,080-16,400 (µM·Min)/L [57.7-67.7 (mg · h)/L] with ideal target AUC_{cum} of 15,200 (µM·Min)/L [62 (mg · h)/L]**
2. Cumulative AUC is defined as the sum of each individual dose AUC:
   AUC_{cum} = AUC1 + AUC2 + AUC3 + AUC4
3. Patients will undergo Dose #1 AUC analysis (AUC1), Dose #2 AUC analysis (AUC2) and Dose #3 AUC analysis (AUC3).
4. Changes will be made to subsequent doses only if the **projected AUC_{cum}** falls outside of the goal range
   a. After the first busulfan dose, **projected AUC_{cum}** = AUC1 x 4
   b. After the second busulfan dose, **projected AUC_{cum}** = AUC1 + [AUC2 x 3]
   c. After the third busulfan dose, **projected AUC_{cum}** = AUC1 + AUC2 + [AUC3 x 2]

5. If dose adjustment is necessary, new doses will be calculated based on **an ideal target AUC_{cum} of exactly 15,200 µM·min·L-1 [62 (mg · h)/L].**

REFERENCES

1) Bartelink IH. Body weight-dependent pharmacokinetics of busulfan in paediatric haematopoietic stem cell transplantation patients: towards individualized dosing. Clin Pharmacokinet. 2012;51:331-45.
2) Bartelink IH, van Kesteren C, Boelens JJ, Egberts TC, Bierings MB, Cuvelier GD, Wynn RF, Slatter MA, Chiesa R, Danhof M, Knibbe CA. Predictive performance of a busulfan pharmacokinetic model in children and young adults. Ther Drug Monit. 2012;34:574-83.
3) Savic RM, Cowan MJ, Dvorak CC, Pai SY, Pereira L, Bartelink IH, Boelens JJ, Bredius RGM, Wynn RF, Cuvelier GDE, Shaw PJ, Slatter MA, Long-Boyle J. Effect of weight and maturation on busulfan clearance in infants and small children undergoing hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2013;19:1608-1614.

Appendix IV - GVHD Scoring

Organ involvement will be staged using the criteria outlined in the table below. Biopsy of each organ site at diagnosis or major change in disease activity will be performed unless clinical circumstances make it impossible.

Consensus Clinical Stage and Grade of Acute GVHD (Przepiorka *et al*, 1995)

Stage	Skin	Liver	Lower Gastrointestinal Tract	Upper Gastrointestinal Tract
1	Maculopapular rash <25% of body surface	Bilirubin 2.0 – 3.0 mg/dl	Diarrhea 500 – 1000 mL/day or 280 – 555 mL/m^2	No protracted nausea and vomiting
2	Maculopapular rash 25-50% body surface	Bilirubin 3.1 – 6.0 mg/dl	Diarrhea 1001 – 1500 mL/day or 556 – 833 mL/m^2	Persistent nausea, vomiting or anorexia
3	Generalized erythroderma	Bilirubin 6.1 – 15.0 mg/dl	Diarrhea >1500 mL/day or >833 mL/m^2	
4	Generalized erythroderma with bullous formation and desquamation	Bilirubin > 15 mg/dl	Severe abdominal pain, with or without ileus, or stool with frank blood or melena	

Grading for Treatment Criteria:

Mild GVHD Skin stage I-II only (Equivalent to Seattle Grade I).

Moderate GVHD Skin stage I-III and/or liver I-IV and/or Gastrointestinal tract (GI) I-III and/or Upper GI (UGI). (Equivalent to Seattle Grade II, III).

Severe GVHD Any stage IV along with severe clinical illness.

Late Acute and Chronic GVHD

Late acute and chronic GVHD will be assessed using the National Institutes of Health (NIH) Consensus Criteria.

Appendix V – Targeted Toxicities

MT2018-06 Refer to section 9.2.2 for time points

Patient Initials: _____ Date of Assessment: _____ Assessment Time Point: _____

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Infusion Related Reaction	None	Mild transient reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < 24 hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; prolongation of hospitalization for clinical sequelae	Life-threatening consequences; urgent intervention indicated
Bronchospasm	None	Mild symptoms; intervention not indicated	Symptomatic; medical intervention indicated; limiting instrumental ADL	Limiting self-care ADL; oxygen saturation decreased	Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated
Dyspnea/ Wheezing	None	Shortness of breath with moderate exertion	Shortness of breath with minimal exertion; limiting instrumental ADL	Shortness of breath at rest; limiting self- care ADL	Life-threatening consequences; urgent intervention indicated
Hypoxia	None	Decreased oxygen saturation with exercise (e.g., pulse oximeter < 88%); intermittent supplemental oxygen	Decreased oxygen saturation at rest (e.g., pulse oximeter < 88% or PaO2 < =55 mm Hg)	Life-threatening airway compromise; urgent intervention indicated (e.g., tracheotomy or intubation	Life-threatening airway compromise; urgent intervention indicated (e.g., tracheotomy or intubation
Fever	None	38.0 - 39.0 degrees C (100.4 - 102.2 degrees F)	>39.0 - 40.0 degrees C (102.3 - 104.0 degrees F)	>40.0 degrees C (>104.0 degrees F) for < 24 hrs	>40.0 degrees C (>104.0 degrees F) for >24 hrs
Chills	None	Mild sensation of cold; shivering; chattering of teeth	Moderate tremor of the entire body; narcotics indicated	Severe or prolonged, not responsive to narcotics	Life Threatening
Hypertension	None	Prehypertension (systolic BP 120 - 139 mm Hg or diastolic BP 80 - 89 mm Hg)	Stage 1 hypertension (systolic BP 140 - 159 mm Hg or diastolic BP 90 -99 mm Hg); medical intervention indicated; recurrent or persistent (>24 hrs); symptomatic increase by >20 mm Hg (diastolic) or to >140/90 mm Hg if previously WNL; monotherapy indicated; Pediatric: recurrent or persistent (>24 hrs) BP >ULN; monotherapy indicated	Stage 2 hypertension (systolic BP >= 160 mm Hg or diastolic BP >=100 mm Hg); medical intervention indicated; more than one drug or more intensive therapy than previously used indicated; Pediatric: Same as adult	Life-threatening consequences (e.g. malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis); urgent intervention indicated; Pediatric: Same as adult
Hypotension	None	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Medical intervention or hospitalization indicated	Life-threatening and urgent intervention indicated
Sinus Bradycardia	None	Asymptomatic, intervention not indicated	Symptomatic, medical intervention indicated	Severe, medically significant, medical intervention indicated	Life-threatening consequences; urgent intervention indicated
Sinus Tachycardia	None	Asymptomatic, intervention not indicated	Symptomatic; non-urgent medical intervention indicated	Urgent medical intervention indicated	Life Threatening
Vomiting	None	1 - 2 episodes (separated by 5 minutes) in 24 hrs	3 - 5 episodes (separated by 5 minutes) in 24 hrs	>6 episodes (separated by 5 minutes) in 24 hrs; tube feeding, TPN or hospitalization indicated	Life-threatening consequences; urgent intervention indicated
Creatinine	Normal	>1-1.5x baseline; >1.5 x LN	>1.5 - 3.0x baseline; >1.5-3.0x LN	>3.0 baseline; > 3.0 - 6.0 x ULN	>6.0 x ULN
Rash	None	Macules/papules covering < 10% BSA with or without symptoms (e.g., pruritus, burning, tightness)	Macules/papules covering 10-30% BSA with or without symptoms; limiting instrumental ADL	Macules/papules covering >30% BSA with or without associated symptoms; limiting self-care ADL	Life Threatening
Chest Pain	None	Mild pain	Moderate pain; limiting instrumental ADL	Pain at rest; limiting self-care ADL	Life Threatening
Back/Flank Pain	None	Mild pain	Moderate pain; limiting instrumental ADL	Pain at rest; limiting self-care ADL	Life Threatening
Flushing	None	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Moderate symptoms; medical intervention indicated; limiting instrumental ADL	Symptomatic, associated with hypotension and/or tachycardia; limiting self-care ADL	----------
Urticaria(Hives)	None	Urticarial lesions covering < 10% BSA; topical intervention indicated	Urticarial lesions covering 10 - 30% BSA; oral intervention indicated	Urticarial lesions covering >30% BSA; IV intervention indicated	Life Threatening
Neurologic-	None	Mild	Moderate	Severe	Life Threatening
Other-Specify	None	Mild	Moderate	Severe	Life Threatening

Person Completing Form: _____ ADL = activities of daily living

All Events are Expected and Attributable Except: _____