UNIVERSITY OF MINNESOTA BLOOD AND MARROW TRANSPLANTATION PROGRAM

Total Body Irradiation Dose De-Escalation Study in Patients with Fanconi Anemia Undergoing Alternate Donor Hematopoietic Cell Transplantation

MT2006-05

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Revision History

Revision #	Revision date	Summary of Changes
	7/26/2006	Eligibility Change: High risk Fanconi anemia patients will be included in this study if they have one or more of the following high risk features: History at any time of systemic fungal or gram negative infection Severe renal disease with a creatinine clearance <40 mL/min Recipient age > 18 years Patients with advanced MDS (.i.e. RAEB or RAEBt or acute leukemia) will be excluded from this study.
1	3/30/2007	Section 9.2 processing, added specific CD3 dose patients will receive Section 11.1, stopping rules, clarified that it is primary graft failure that will determine de-escalation
2	4/14/2008	TBI dose de-escalation trial terminated early due to secondary graft failure in 2 of 2 patients. Aim of study is to evaluate secondary endpoints with additional patients enrolled at TBI 300 cGy.
-	7/27/09	Synopses corrected to match the eligibility criteria
-	9/8/2009	Added additional high risk diagnoses to the eligibility
3	03/24/10	Section 8.2, MMF will now be used for GVHD prophylaxis per current institutional standards; Section 9.1 Cell Processing – replace Isolex 300i System with CliniMACS® Cell Selection System (Miltenyi Biotec), add protocol to current CliniMACS IDE (D. Weisdorf sponsor); add section SAE reporting and DSMP
4	09/29/10	Revisions in conjunction change to CliniMACS® Cell Selection System to be used for bone marrow and peripheral blood processing. Establish study arms based on cell source as follows: arm 1: bone marrow and peripheral blood processed using Isolex 300i (for patients enrolled through April 2010), arm 2: UCB (no processing), arm 3: bone marrow and peripheral blood processed using CliniMACS (for patients enrolled beginning with this protocol version); section 7 – update to reflect change in cell processing device. Other revisions: Section 6.2 - update MMF administration; Section 6.4 update cell infusion guidelines Revision of primary endpoint and accrual plans based on expanding TBI cohort of 300 cGy to a total of at least 41 patients (synopsis, section 1 and 10) Updates: section 5.3 add patient registration information; Section 6 add standard care language (delete supportive care appendix, add drug formulation and availability appendix); Section 9 – update to current definition of UPRITSO and AE reporting table; add data collection and record retention sections; add appendix VI – expected toxicities and appendix VII – study drug formulation; update header and footer; edits throughout for clarity.
5	1/5/2011	• Add stopping rule for remaining (10) subjects to be treated on current TBI dose to monitor for primary graft failure. • Update section 5.2 Exclusion Criteria to be consistent with wording from protocol summary on pages 3-4. • Add language to reflect that study is conducted under IND 14536 (cover page, sections 9.1.1 and 9.2). • Clarify primary graft failure as an 2nd endpoint vs secondary graft failure • Updated schema
-	2/7/11	• Clarification of cell doses in sections 7.2 and 7.3.

Revision #	Revision date	Summary of Changes
6	11/23/11	• Add treatment Arm 4 for very high risk subjects (as defined in section 4.3). excluding thymic shielding and eliminating T cell depletion of donor stem cells regardless of their source. • Update accrual goal at the accepted dose to a total of 52 patients in section 10.1 • Include information regarding the unmanipulated CB IND • Update format and standard language throughout.
-	04/17/12	Correct ATG scheduling inconsistency in schema to match protocol and orders – should read 30 mg/kg/day not 15 mg/kg/day every 12 hours, also adjust MP pre-med to 2 mg/kg
7	05/09/12	• Expand eligibility to include patients requiring a second HSCT for graft failure • Delete ATG and CSA, add rapamycin (sirolimus) while retaining MMF Resolve data collection inconsistencies: • Clarify the secondary objective/endpoint of regimen related toxicity is based on transplant outcomes through day 100 (engraftment, infections, treatment related mortality, etc.) • Patients will be monitored for reportable events through day 100 according to the table in section 8.3, decrease reporting to the DSMC
8	9/14/12	• Correction of typographical error in schema • Correction of typographical error in eligibility criteria section to reflect that High Risk population may include patients requiring a second HSCT for graft failure (change was made in previous revision, but eligibility section was inadvertently not updated) • Correction of typographical error in section 5, Treatment Plan (methylprednisone and ATG were inadvertently not deleted from the list of drugs, although these treatments were removed in the previous protocol revision)
9	11/16/12	• Restore methyl prednisone to the preparative regimen • Section 5.2 update sirolimus (rapamycin) administration section • Section 5.3 update anti-fungal medication section • Sections 7.1 and 7.2 add lipid panel at baseline and post-transplant to monitor for changes in triglyceride and cholesterol level (risks of sirolimus) • Appendix VII Drug Formulation and Availability – Delete – all drugs are commercially available and administered according to current institutional guidelines unless otherwise indicated in individual drug sections • Other minor updates and edits
10	1/11/13	• Increase sample size to 100 subjects
11	09/29/14	• Restore CSA and remove rapamycin as GVHD prophylaxis • Update to current IRB language
12	10/31/16	• Remove references to IND, update to include references to HUD protocol MT2015-31 • Update IRB reporting language • Related donor consents archived as related donors will now be enrolled on BMT protocol MT2012-14C • Antifungal therapy updated to current standard of care (section 5.3) • Updated required observations to remove standard of care evaluations (section 7, appendix II) • Removed Day 21 bone marrow biopsy • Increased accrual goal to 120 subjects
13	11/16/2018	• Revised to clarify that post transplant bone marrow aspirates and biopsies will only be performed as clinically indicated

Table of Contents

SYNOPSIS

Title	**MT2006-05: Total Body Irradiation Dose De-Escalation Study in Patients with Fanconi Anemia Undergoing Alternate Donor Hematopoietic Cell Transplantation**
Primary Objective	To determine the lowest dose of total body irradiation (TBI) required to achieve consistent neutrophil engraftment after alternate donor HSCT in patients with Fanconi anemia. (TBI dose de-escalation was terminated in December 2007 due to secondary graft failure in 2 of 2 patients.) After December 2007 - To evaluate the primary graft failure rate with enough precision to show that it is less than 15% of evaluable patients transplanted with TBI 300 cGy.
Secondary Objectives	To assess risks of primary graft failure, regimen related toxicity, acute and chronic GVHD and survival.
Study Design	Single center, single arm, TBI dose de-escalation trial.
Primary Endpoint and Secondary Endpoints	Primary: Incidence of neutrophil recovery (absolute neutrophil count ≥500/μL for three consecutive days) by day 42. Secondary: Incidence of serious regimen related toxicity based on transplant outcomes at day 100. Incidence of secondary graft failure at 100 days. Incidence of acute graft-versus-host disease (GVHD) at 100 days. Incidence of chronic GVHD at one year. Probability of survival at one year. Incidence of infections at 100 days, 6 months, and one year. Immune reconstitution at 100 days, 6 months, and one year.
Sample Size	35 to 120 patients with at least 91 patients enrolled at the acceptable TBI dose level of 300 cGy

Eligible Diseases	Patients must be either standard or high risk with the following: Standard risk patients must be <18 years of age with a diagnosis of Fanconi anemia with aplastic anemia (AA), myelodysplastic syndrome without excess blasts, or high risk genotype as defined below: • Aplastic anemia is defined as having at least one of the following when not receiving growth factors or transfusions: - platelet count <20 x 10^9/L - ANC <5 x 10^8/L - hemoglobin <8 g/dL • Myelodysplastic syndrome (MDS) with multilineage dysplasia (< 5% blasts) with or without chromosomal anomalies • High risk genotype (e.g. IVS-4 or exon 14 FANCC mutations, or BRCA1 or 2 mutations) High risk patients must have one or more of the following high risk features: • Advanced MDS (≥ 5% blast) or acute leukemia • Requiring additional HSCT for graft failure • History of systemic fungal or gram negative infection • Severe renal disease with a creatinine clearance <40 mL/min • Recipient age ≥ 18 years Very high risk patients must have the following features advanced MDS (≥ 5% blast) or acute leukemia

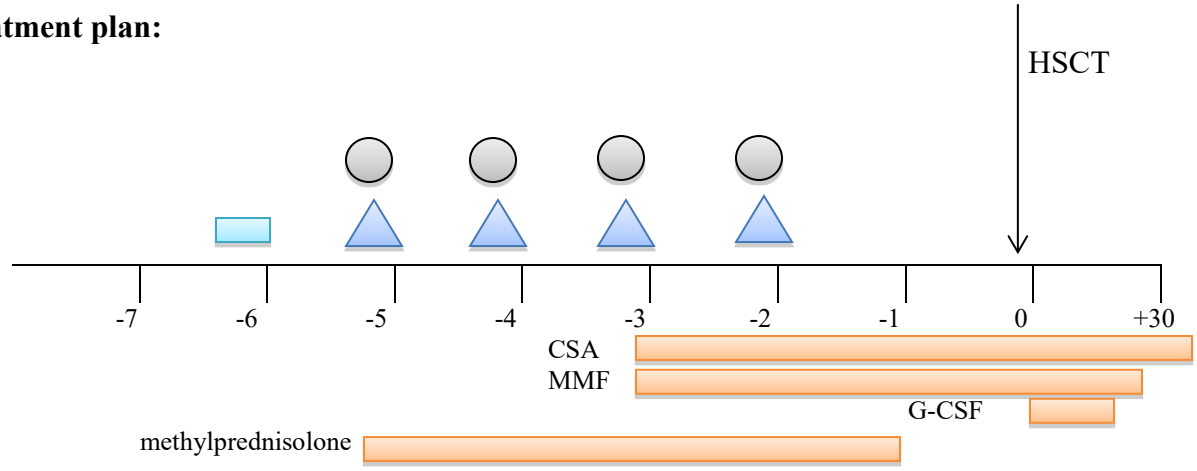
TREATMENT PLAN

TBI dose de-escalation trial was terminated December 2007 due to secondary graft failure in 2 of 2 patients. All patients transplanted after December 2007 will be prescribed TBI 300 cGy with the goal of evaluating the rate of primary graft failure and secondary endpoints.

Revised Schema effective January 2011:

possible stem cell sources: related donor BM, unrelated donor BM, UCB

treatment plan:



Total Body Irradiation (TBI) 300 cGy

Cyclophosphamide 10 mg/kg IV over 2 hours days -5 through day -2

Fludarabine 35 mg/m^2 IV over 30 minutes days -5 through -2

1 OBJECTIVES

1.1 Primary Objective

To determine the lowest possible dose of total body irradiation (TBI) required to achieve consistent neutrophil engraftment after alternate donor hematopoietic stem cell transplantation (HSCT) in patients with Fanconi anemia. (TBI dose de-escalation trial was terminated December 2007 due to secondary graft failure in 2 of 2 patients).

After December 2007 - To evaluate the primary graft failure rate with enough precision to show that it is less than 15% of evaluable patients transplanted with TBI 300 cGy.

1.2 Secondary Objectives

To determine:

- Incidence of serious regimen related toxicity based on transplant outcomes by day 100
- Incidence of secondary graft failure at 100 days
- Incidence of acute graft-versus-host disease (GVHD) at 100 days
- Incidence of chronic GVHD at one year
- Probability of survival at one year
- Incidence of infections at 100 days, 6 months, and one year
- Immune reconstitution at 100 days, 6 months, and one year

2 STUDY DESIGN

This is a single center, single arm, TBI dose de-escalation study designed to determine the lowest dose of TBI required (toxicity endpoint) without impairing the hematopoietic engraftment (safety endpoint) in FA patients. TBI dose de-escalation was terminated in December 2007 due to secondary graft failure in 2 of 2 patients. After December 2007, all patients are to be treated with the fixed dose of TBI 300 cGy. In November 2011 the treatment plan was revised to add "Arm 4," which treats very high risk subjects using TBI without thymic shielding and without T-cell depleting the cell product regardless of source.

3 BACKGROUND

3.1 Fanconi Anemia

Fanconi anemia (FA) is a genetically and phenotypically heterogeneous inherited disorder characterized by congenital malformations, progressive bone marrow (BM) failure and marked predisposition to malignancy. The FA phenotype is extremely variable. Congenital malformations may range from none to many, often involving the major organ system, resulting in dysfunction in some patients[1-3]. Hematologic abnormalities occur in virtually all patients with FA, at a median age of 7 years (range, birth to 41 years).[4] Based on clinical data in the International Fanconi Anemia Registry (IFAR; n = 754 patients), the cumulative incidence of BM failure by age 40 was 90%.[5]

Notably, some patients presented with myelodysplastic syndrome (MDS) or acute myelocytic leukemia (AML) without a prior diagnosis of aplastic anemia (AA). Of the 754 IFAR FA patients, 120 (16%) patients developed MDS or AML with a cumulative incidence of MDS or AML by age 40 years of 33%. Based on a survey of FA patients by Rosenberg et al, the median age of onset of leukemia was 11.3 years.[6]

The fundamental defect in FA cells remains unknown. Defects in DNA repair, cell cycle checkpoints, oxygen metabolism and induction of apoptosis have all been described in FA cells. To date, 11 FA complementation groups have been described and x genes have been identified.[7] These all encode unique proteins, which do not exhibit any functional domains. The heterogenous nature of FA makes an understanding of the correlation between genotype and phenotype important in the clinical management of FA patients. For example, in FA-C patients, patients with intron 4 or exon 14 mutations have an earlier onset of bone marrow failure and poorer survival compared to patients with exon 1 mutations.[8] Patients with biallelic BRCA2 mutations which is the FANCD1 gene, have an extraordinary risk for developing acute leukemia, Wilms tumor and medulloblastoma at a very young age.[9]

3.2 Hematopoietic Stem Cell Transplantation for Fanconi Anemia

Hematopoietic stem cell transplantation (HSCT) from an allogeneic donor is the only treatment with curative potential for patients with the hematological complications of Fanconi Anemia (FA).[1,10-12] Early experiences with HSCT for the treatment of FA were negative. Poor outcomes were primarily the result of excessive regimen-related toxicity (RRT) and severe graft-versus-host disease (GVHD). Conditioning regimens were those used for acquired AA, consisting of cylophosphamide (CY) at a dose of 200 mg/kg with or without irradiation. Severe RRT was noted with a high proportion of patients dying early after transplant with impaired cardiac function, severe mucositis, skin toxicity, infection, gastrointestinal hemorrhage and hemorrhagic cystitis.

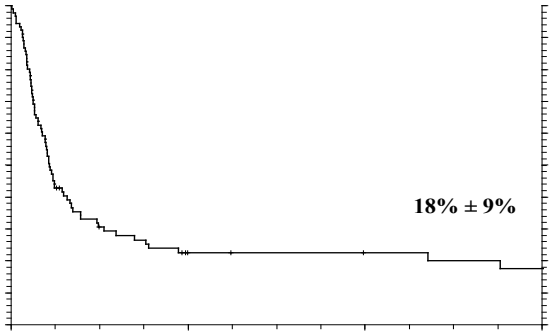


Figure 1. Overall Survival at 2 Years after Unrelated Donor HSC Transplantation in FA Patients

As a result of in vitro tests demonstrating hypersensitivity to radiation and alkylating agents in FA patients, Gluckman et al proposed the use markedly lower doses of CY and lower dose unfractionated irradiation to reduce toxicity and improve survival, with

improved results. Subsequently, survival markedly improved in recipients of HLA matched sibling donor BMT. Incidences of RRT, GVHD and graft failure, however, remained particularly high after unrelated donor BMT with very poor survival (18%) at 3 years (Figure 1).[13] While RRT was less, reduced doses of conditioning were insufficient to achieve consistent donor cell engraftment and GVHD remained a major obstacle.

3.3 University of Minnesota Experience

Over the past 11 years a number of systematic protocol modifications have been made at the University of Minnesota to address the problems of graft failure, RRT and infections after alternate donor HSCT in FA patients.

In 1995, we started to T cell deplete bone marrow from unrelated donors in an attempt to further reduce RRT and overcome the obstacle of GVHD in FA patients.[14] GVHD prophylaxis consisted of cyclosporine A (CSA) for six months, and short course methylprednisolone (2 mg/kg/d) between day 5 and 19. For the entire cohort of 29 FA patients treated from 1995-1998, the incidence of grade 2-4 acute GVHD was 8% with no patient having chronic GVHD.

In 1999, we added fludarabine (FLU) to the CY-TBI-ATG preparative regimen to overcome the high risk of graft failure, particularly in patients with somatic mosaicism. Between April 1999 and August 2003, 45 FA patients were treated with FLU 175 mg/m^2, CY 40 mg/kg, TBI 450 cGy and ATG. All recipients received T cell depleted marrow (or umbilical cord blood if no marrow donor could be identified). As in the prior studies, GVHD prophylaxis consisted of CSA for six months and short course methylprednisolone. Of 45 FA patients who underwent alternate donor HSCT in this manner from 1999-2003, 44 engrafted, resulting in a cumulative incidence of 97% (95% CI, 91-100). The incidence of acute and chronic GVHD was low being 21% (95% CI, 9-33%) and 10% (95% CI, 1-19%).

Despite the advances in engraftment and reducing the incidence of GVHD, regimen related toxicity (RRT) and opportunistic infections remained major obstacles to successful HSCT. In this most recent cohort of patients, significant RRT occurred in all patients with everyone exhibiting significant mucositis requiring narcotics and 48% having significant hemorrhagic cystitis. Other severe toxicities included pulmonary hemorrhage (n=9), interstitial pneumonitis (n=4), GI hemorrhage (n=6), renal failure (n=9), erythroderma (n=3), and severe hepatic veno-occlusive disease (n=2). While RRT was common, RRT was a direct cause of death in 4 patients. Infectious disease complications were also common, with 77 events documented by pathogen isolation in 36 patients with a complete dataset. Bacterial and viral pathogens accounted for most events (34 and 30, respectively) with fungal pathogens (n=12) and other pathogens (n=1) observed less frequently.

In 2003, we initiated a study to determine the effect of thymic shielding as a strategy to improve immune reconstitution and in turn reduce the risk of opportunistic infection. Between October 2003 and January 2006, 15 FA patients were treated with FLU 175 mg/m^2, CY 40 mg/kg, TBI 450 cGy and ATG as previously described for the prior study

with the addition of thymic shielding at the time of radiation therapy. All recipients received T cell depleted marrow (or umbilical cord blood if not marrow donor could be identified). Thirteen of 15 patients achieved neutrophil engraftment at a median of 12 days (range, 10-38 days), suggesting that thymic shielding has no deleterious effect on hematopoietic recovery. Five of 15 patients developed severe acute GVHD and none has developed chronic GVHD thus far. For this group of patients, the probability of survival at 1 year is 66% (95% CI 41-91%). Significant RRT occurred in all patients with all patients exhibiting significant mucositis requiring narcotics and more than 50% having significant hemorrhagic cystitis. Other severe toxicities included pulmonary hemorrhage/interstitial pneumonitis, GI hemorrhage, renal failure and severe hepatic veno-occlusive disease. While RRT was common, RRT was a direct cause of death in 2 patients. Of the 5 patients that have died, two died from graft failure, one from ARDS, one from renal failure and one from fungal infection.

3.4 Non-TBI Based Regimens for Fanconi Anemia Patients

Long-term follow-up studies of FA patients indicate that approximately 40% of FA patients develop a malignancy within 15 – 20 years after HSCT.[15] Reported risk factors include exposure to irradiation and the development of chronic GVHD.[6,15-19] Eliminating irradiation will also potentially reduce the risk of regimen related toxicity and GVHD. In addition, the risks for cataracts, endocrinopathies and potentially infertility in women, will be reduced (the majority of FA males are infertile).

In non-FA patients with aplastic anemia, a multicenter prospective trial was conducted from 1994-1999 to determine the minimum dose of TBI sufficient to achieve sustained engraftment and to determine the tolerability and toxicity of the regimen.[20] All patients received an unrelated donor BMT after 3 cycles of 30 mg/kg ATG and 4 cycles of 50 mg/kg cyclophosphamide (CY) and TBI. The starting dose of TBI was 3 x 200 cGy and was decreased over time in the trial such that the lowest possible dose (200 cGy) in combination with CY/ATG was sufficient to allow engraftment without inducing prohibitive toxicity. The follow up to this trial is the current Blood and Marrow Transplant Clinical Trials Network trial in which fludarabine has been added to CY, TBI 200 cGy and ATG as a means to reduce the dose of CY required to achieve adequate engraftment.

HLA-matched sibling donor HCT. At the University of Minnesota, we developed a non-irradiation based preparative regimen using fludarabine (FLU), CY and anti-thymocyte globulin (ATG) followed by infusion of T-cell depleted (TCD) bone marrow (BM) or unmanipulated umbilical cord blood (UCB) for FA patients with HLA-matched related donors. The goals of this trial were to potentially reduce the risks of malignancy, GVHD and other late effects, such as endocrinopathy and infertility. GVHD prophylaxis consisted of cyclosporine and short course methylprednisolone.[21] Between April 2000 and January 2006, 12 patients with FA (11 AA, 1 MDS) underwent related donor HCT using this regimen. Stem cell sources were BM and UCB in 9 and 3 patients, respectively. All patients demonstrated primary engraftment. Median days to neutrophil and platelet engraftment were 11 days (range 9 – 13) and 38 days (range 19 – 381), respectively. No patient developed GVHD after primary HCT. The patient with MDS

relapsed with AML and a maternal donor recipient experienced secondary graft failure. The Kaplan-Meier estimate of survival at 2 years is 100% at a median follow-up of 2.5 years (range 1.6 – 4.5). The results of this study demonstrate that a FLU-based, non-irradiation approach is effective for FA patients with AA undergoing genotypically HLA-identical sibling HCT.[21]

3.5 Study Rationale

Over the last decade, major advances have been made to improve survival of patients with FA undergoing alternate donor HSCT. We have shown that fludarabine based preparative regimens have drastically improved engraftment rates from 66% to 98%. As well, T cell depletion of bone marrow has decreased the rates of GVHD from 50-70% to 20-30%. We have also shown that thymic shielding during TBI is well tolerated with no deleterious effect on engraftment or other transplant outcomes. However, is too early to determine if thymic shielding speeds immune recovery.

We hypothesize that FA patients undergoing alternate donor HSCT can achieve neutrophil engraftment with the use of fludarabine, cyclophosphamide and ATG, without the use of irradiation in a similar fashion as was accomplished in FA patients undergoing HLA-matched sibling donor HSCT. It is anticipated that rates of regimen related toxicity and GVHD will be lower with this non-irradiation based approach, resulting in improved survival. We also anticipate that risk for late malignancies will be reduced. All patients will have immune function tests performed to study the pace of immune recovery.

Our plan is to build upon our sequential modifications in our approach to alternate donor HSCT for FA patients to improve survival without compromising engraftment after HSCT. We will decrease the dose of TBI in each cohort, using FLU, CY and ATG, followed by T cell depleted BM or UCB. The decision to proceed with each stepwise decrease in TBI (300 cGy to 150 cGy to no irradiation) will be based upon achieving adequate neutrophil engraftment in the current cohort of patients.

TBI dose de-escalation was terminated in December 2007 due to secondary graft failure in 2 of 2 patients. After December 2007, all patients are to be treated with the fixed dose of TBI 300 cGy.

In June 2012, ATG was deleted from the preparative regimen as graft failure is no longer a major concern. Patients will continue to receive methylprednisolone for 5 days prior to transplant. Omitting ATG may speed immune recovery after HSCT and reduce the risk for opportunitistic infections. Additionally CSA was replaced by sirolimus to test the effectiveness of this immunosuppressive agent. The future plan for FA patients undergoing alternative HSCT is to give T regulatory cells (Tregs) at the time of HSCT to help promote engraftment and reduce the risk of GVHD. CSA inhibits the growth of T regs whereas sirolimus promotes their growth. Therefore sirolimus has replaced CSA in this trial in anticipation of the future trial with T regs.

In September 2014, the protocol was revised to restore CSA as GVHD prophylaxis as it is better tolerated by the patient. Sirolimus (rapamycin) was deleted as future trials with T regs are on hold indefinitely (see previous paragraph).

4 PATIENT SELECTION/REGISTRATION

4.1 Patient Inclusion Criteria

4.1.1 Disease Criteria

- **Standard risk patients** must be <18 years of age with a diagnosis of Fanconi anemia with aplastic anemia (AA), myelodysplastic syndrome without excess blasts, or high risk genotype as defined below:
  - Aplastic anemia is defined as having at least one of the following when not receiving growth factors or transfusions:
    - platelet count $<20 \times 10^9$/L
    - ANC $<5 \times 10^8$/L
    - Hemoglobin <8 g/dL
  - Myelodysplastic syndrome with multilineage dysplasia with or without chromosomal anomalies
  - High risk genotype (e.g. IVS-4 or exon 14 FANCC mutations, or BRCA1 or 2 mutations)

- **High risk patients** must have one or more of the following high risk features:
  - Advanced MDS (≥ 5% blast) or acute leukemia
  - Require additional HSCT for graft failure
  - History at any time of systemic fungal or gram negative infection
  - Severe renal disease with a creatinine clearance <40 mL/min
  - Age ≥ 18 years

- **Very high risk patients** (Arm 4, described in section 4.3) must have Advanced MDS (≥ 5% blast) or acute leukemia after initial HSCT

4.2 Patients must have an appropriate source of stem cells (as detailed in section 6.1). Patients and donors will be typed for HLA-A, B, C and DRB1 using high resolution molecular typing. Related donors will be evaluated under the University of Minnesota donor protocol MT2012-14C

4.2.1 Adequate organ function including:

- Cardiac: ejection fraction >45%
- Hepatic: bilirubin, AST or ALT, ALP <5 x normal

4.2.2 Karnofsky performance status >70% or Lansky >50 (if < 16 years of age)

4.2.3 Women of child-bearing age must be using adequate birth control and have a negative pregnancy test

4.2.4 Written consent

4.3 Patient Exclusion Criteria

4.3.1 Available HLA-genotypically identical related donor in standard risk patients.

4.3.2 Active CNS leukemia at time of study enrollment

4.3.3 History of squamous cell carcinoma of the head/neck/cervix within previous 2 years

4.3.4 Prior radiation therapy that prevents further TBI

4.4 Patient Registration with the Clinical Trials Office

Upon completion of the screening evaluation, eligibility confirmation and obtaining written consent, a designated study staff person will enroll the patient into OnCore.

At the time of registration, the patient's cell source/processing will be indicated in OnCore as one of the following study arms:

Arm 1: bone marrow or peripheral blood processed using Isolex 300i (for patients enrolled through April 2010)

Arm 2: UCB – no processing. If one or both of the cord blood units used for the graft is unlicensed, the participant will co-enroll on University of Minnesota protocol MT2011-13R "Infusion of Cell Populations from Unlicensed Umbilical Cord Blood Units."

Arm 3: bone marrow or peripheral blood processed using CliniMACS (for patients enrolled beginning with the September 2010 protocol version). Subjects enrolled on the October 31, 2016 version of the protocol will co-enroll on University of Minnesota Protocol MT2015-31 "CliniMACS CD34 Reagent System as a HUD for Obtaining CD34+ Cell-Enriched Products"

Arm 4: TBI with no thymic shielding and no T-cell depletion (processing using CliniMACS device) regardless of stem cell source

TBI dose de-escalation was terminated December 2007 due to secondary graft failure in 2 of 2 patients.

4.5 Patients Who Are Registered and Do Not Receive Study Treatment

If a patient is registered to the study, and is later found not able to begin the preparative regimen (beginning with the first dose of fludarabine), for whatever reason, the patient

will be removed from study and treated at the physician's discretion. The patient will be considered a screen/baseline failure and the reason for removal from study will be clearly indicated in OnCore.

5 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 therapy (i.e. acetaminophen, diphenhydramine, G-CSF, antimicrobials, etc).

Patients will receive antifungal prophylaxis beginning 1 month prior to conditioning therapy, if possible. Refer to section 5.3.

See appendices VI and VII for preparative and prophylaxis drug information including expected toxicities

Day	Treatment
-6	TBI 300 cGy with thymic shielding*
-5	Cyclophosphamide (CY) 10 mg/kg IV Fludarabine (FLU) 35 mg/m2 IV Methylprednisolone 1 mg/kg IV q12h
-4	CY 10 mg/kg IV FLU 35 mg/m2 IV Methylprednisolone 1 mg/kg IV q12h
-3	CY 10 mg/kg IV FLU 35 mg/m2 IV Methylprednisolone 1 mg/kg IV q12h
-2	CY 10 mg/kg IV FLU 35 mg/m2 IV Methylprednisolone 1 mg/kg IV q12h
-1	Methylprednisolone 1 mg/kg IV q12h
0	HSCT
+1	Initiate G-CSF 5mcg/kg per day IV (continue until ANC >2.5 x 10^9/L)

* Shielding of the thymus will not be required in those individuals who, in the opinion of the treating investigator, would be at an increased risk of graft failure or relapse due to non-irradiated marrow in the sternum.

5.1 Preparative Therapies

The preparative cytoreductive therapy will be identical for all patients. Treatment related toxicities are defined in the Appendix VI.

The administration of the preparative regimen will follow the institutional and supportive care guidelines. Dose and/or schedule adjustments consistent with the standard of care may be made on an individual patient basis as needed for safety.

Total Body Irradiation

TBI will be given in a single fraction of 300 cGy administered on day –6 via anterior and posterior fields as detailed in Appendix I.

The thymus will be shielded using 5 HVL anterior and posterior cerrabend thymus blocks designed by determining the position of the thymus by treatment planning CT scan. Shielding of the thymus will not be performed for subjects enrolled in Arm 4 ('very high risk' as defined in section 4.1), as these subjects would be at an increased risk of relapse due to unradiated marrow in the sternum.

If necessary because of decreased attenuation through the lungs, lung compensators will be designed to keep the dose to the lungs 300 cGy (+/-5%). The position of these lung compensators will be determined by treatment planning CT scan. Dose is prescribed at the midplane of the patient at the midpelvis. The dose rate will be 26 cGy/minute.

Cyclophosphamide
Cyclophosphamide 10 mg/kg is to be given as a 2 hour infusion for 4 days for a total dose of 40 mg/kg. Strict attention should be made to vigorous hydration, fluid balance and maintenance of good urine output. Mesna (10 mg/kg/day) in divided doses will be given on the same days as cyclophosphamide for 4 days for a total dose of 40 mg/kg per institutional guidelines.

Fludarabine
Fludarabine 35 mg/m^2 will be given IV over 30 minutes daily for 4 days for a total dose of 140 mg/m^2. Preparation, administration and monitoring will be according to standard practice procedures.

Methylprednisolone
Methylprednisolone 1 mg/kg will be given IV over 30 minutes every 12 hours for 5 days on days -5 to -1. Preparation, administration and monitoring will be according to standard practice procedures.

5.2 GVHD Prophylaxis

Patients will receive cyclosporine A (CSA) and mycophenylate mofetil (MMF) as GVHD prophylaxis.

Cyclosporine A (CSA)
All patients (regardless of allograft source) will receive CSA therapy beginning on day –3.

Dosing of CSA will be per the University of Minnesota BMT Program guidelines. The target CSA trough level will be 200 mg/L to 400 mg/L.

CSA taper begins at day +180 or 1 month after control of GVHD. Taper to zero by 10% weekly dose reduction over approximately 10 weeks.

MMF

Patients will receive MMF therapy beginning on day –3 until day +30 or for 7 days after engraftment, whichever day is later. Engraftment is defined as 1st day of 3 consecutive days of absolute neutrophil count [ANC] > 0.5 x 10^9/L.

MMF will be given at a dose of 15 mg/kg/dose every 8 hours PO (to a maximum dose of 1 gram). The MMF dose may be adjusted to tablet sizes of 250 mg and 500 mg or administered using the suspension. MMF may be given IV at the same dose if PO not tolerated. MMF dosing is to be monitored and altered as clinically appropriate based on Pharmacist orders. Patients will be eligible for pharmacokinetic studies.

If the patient has acute GVHD requiring systemic therapy, MMF may be stopped 7 days after initiation of systemic therapy for acute GVHD (e.g. resolution of skin rash, vomiting, and diarrhea).

5.3 Antifungal Therapy

Patients will receive anti-fungal prophylactic therapy with itraconazole, posoconazole, caspofungin or micofungin beginning up to 1 month prior to conditioning therapy. Antifungal prophylaxis will be given until at least 100 days after HSCT.

6 STEM CELL COLLECTION, PROCESSING AND INFUSION

6.1 Stem Cell Source Selection

The following will be the preferential order of stem cells to be used, provided appropriate cell doses are available as detailed in sections 6.2, 6.3 and 6.4:

1. Related donor matched at HLA-A, B, C and DRB1. Related donors will be evaluated and collected per MT2012-14C.
2. Related marrow donor mismatched at 1 HLA-A, B, C and DRB1 antigen. Related donors will be evaluated and collected per MT2012-14C.
3. Unrelated marrow donor matched at HLA-A, B, DRB1. Use unrelated donor PBSC if marrow not available.
4. Unrelated UCB single or double units matched or 1 locus mismatch at HLA-A, B, DRB1 with adequate cell doses per institutional guidelines.
5. Unrelated marrow donor mismatched at 1 HLA-A, B, DRB1. Use unrelated PBSC if marrow not available.

If and when a marrow donor has been selected on the basis of HLA-A, B, C and DRB1 typing as above, preference will be made for donors matched at the HLA-C locus.

Bone marrow, peripheral stem cells, and UCB grafts will be evaluated for sterility, cell viability, nucleated cell count, CD34 and CD3 counts.

Preference will be made for the use of bone marrow BM cells as the stem cell source for patients from either related donors or unrelated donors. Peripheral blood stem cells will only be used if a donor is unwilling or unable to undergo bone marrow cell collection. If a suitable donor is not available in a timely fashion, unrelated donor umbilical cord blood will be used as the stem cell source.

6.2 Bone Marrow Collection and Processing

Unrelated donor bone marrow will be collected in the usual sterile manner using established parameters determined by the National Marrow Donor Program.

Refer to MT2012-14C for related donor collection guidelines.
A goal of $\geq 5.0 \times 10^8$/kg (based on patient ideal body weight) nucleated bone marrow cells will be collected.

With the September 2010 amendment, CD34+ cells will be isolated from the bone marrow using the CliniMACS® Cell Selection System (Miltenyi Biotec). Patients will be given all isolated CD34+ cells. Prior to activation of the September 2010 amendment, cell selection was done using the Isolex®300i System (Nexell Therapeutics, Inc.). "Very high risk (see section 4.1)" patients who are treated on Arm 4 (see section 4.3) will receive cell products which are not T-cell depleted using the CliniMACS or other cell selection device. In August 2016, the University of Minnesota received approval to use the CliniMACS CD34 Reagent System as a Humanitarian Use Device (HUD) as protocol number MT2015-31. With the October 2016 amendment, we will be using MT2015-31 to co-enroll subjects needing CD34+ cell isolation.

Infusion will be according to the current University of Minnesota guidelines.

Each patient is to receive 1.0×10^5/kg CD3+ cells. An add back infusion of CD3+ cells may be required to achieve this prescribed cell dose if an insufficient number are present in the CD34+ isolated cell fraction given to the patient.

Peripheral blood stem cells will be used only if bone marrow cannot be collected as BM and UCB are the preferred stem cell source. PBSC will be collected and processed per institutional guidelines.

6.3 Umbilical Cord Blood

The UCB graft may consist of one or two UCB units to provide an adequate cell dose per institutional guidelines. Cord Blood units should be selected according to current University of Minnesota Umbilical Cord Blood Graft Selection Algorithm.

Cord blood products are thawed and filtered (170-micron) in the Molecular and Cellular Therapeutics (MCT) Lab.

Infusion will be according to the current University of Minnesota guidelines.

7 SCHEDULE OF STUDY ACTIVITIES

See Appendix II and Appendix III. Scheduled evaluations prior to engraftment (day 30) may be performed +/-3 days from the targeted date; assessments to be performed between engraftment and day 100 may be done +/-7 days of the targeted date; assessments after day 100 may be performed +/-30 days of the targeted date. In addition, targeted days may be altered as clinically appropriate.

7.1 Schedule of Activities – refer to appendix II

7.2 Pre Study Screening Procedures

- Immune function testing (30 cc [10 cc in 3 green top tubes] to Wagner lab – appendix III)
- Immunoglobulin levels
- Urinalysis and 24 hour urine creatinine clearance or GFR
- Pregnancy test (urine) – for age appropriate females
- Chest radiograph
- High resolution CT chest, abdomen and pelvis
- CT of sinuses
- Ultrasound of liver and kidneys
- Bone marrow aspirate and biopsy
- Karnofsky or Lansky performance status (Appendix I)
- Pulmonary functions tests (children >6 years, adults)
- ECG; echocardiography with left ventricular ejection fraction
- Other radiographic studies (as clinical indicated)

7.3 Evaluation During Therapy Until Engraftment

- CBC with platelet count daily until one week after ANC $\geq 5 \times 10^8$/L for 3 consecutive days, then weekly, platelets to be monitored daily until the count is $\geq$ 50,000 μL for 7 consecutive days without transfusion, then weekly thereafter
- Review for events/toxicity and for the BMT database per section 8
- After BM infusion, GVHD evaluation weekly and as clinically indicated

7.4 Evaluation Post Engraftment To Discharge

- Physical examinations weekly until discharge
- CBC with platelet count weekly until discharge
- GVHD evaluations as clinically indicated
- Test peripheral blood and/or bone marrow for chimerisms as clinically indicated
- Review for events/toxicity and for the BMT database per section 8

7.5 Evaluation Post Engraftment at Day 60, 100 and 180, 365 and then Once Yearly until 2 Years after HSCT

- CBC, differential and platelet

- GVHD score
- Physical exam
- Bone marrow aspiration and biopsy - if clinically indicated at day 100, 180 and 365 days.Additional biopsies performed more often as clinically indicated
- Peripheral blood chimerism at day 60
- Patient characteristics evaluated at each visit (Karnofsky or Lansky scale, weight, age)
- Review for events/toxicity and for the BMT database per section 8
- Clinical Summary at close of study 2 years after BMT or, at death (obtain autopsy report if available)
- Lipid panel

7.6 GVHD Assessments

Acute GVHD

Acute GVHD will be evaluated daily during inpatient hospitalization, at all follow-up visits and at Day 100. Evaluation will include descriptive characteristics of rash and estimates of body surface area involved; extent of any wet dermal/epidermal separation; identification of concomitant causes of rash other than GVHD; peak serum bilirubin; concomitant causes of increased bilirubin other than GVHD; presence or absence of nausea, vomiting or anorexia persistent after engraftment; peak diarrhea volume with annotations concerning the presence or absence of urinary mixing and estimates of the true diarrhea volume; presence or absence of abdominal cramps; presence or absence of frank stool blood or melena; concomitant causes of GI symptoms other than GVHD; biopsy results; identification of any agents used for treatment; and autopsy results (if applicable). The diagnosis and staging of acute GVHD is detailed in Appendix IV.

Chronic GVHD

At Day 100 and at all evaluations thereafter, patients will be evaluated for chronic GVHD. Symptoms to be monitored include skin disorders (rash, dermatitis, hardening), liver damage (ALT and total serum bilirubin), difficulty swallowing, dry eyes and mouth, increased susceptibility to infections, and hair loss. The diagnosis and staging of chronic GVHD is detailed in Appendix V.

7.7 Immune Reconstitution Evaluation

Immune recovery will be measured as detailed in the Appendix III. All patients will have a systematic evaluation of 1) lymphocyte subset recovery, and 2) immunoglobulin levels. Patients will be studied before HSCT as baseline (before preparative therapy) and at day 100, 6 months, and 1 year after HSCT.

8 ADVERSE EVENT MONITORING AND REPORTING

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 4.0 (CTCAE). A copy of the CTCAE can be downloaded from the CTEP home page:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

FOR UNLICENSED UCB UNITS ONLY: Selected expected adverse reactions determined to be caused by or at least possibly caused by the UCB units based on objective evidence will be reported in an expedited manner to the FDA under University of Minnesota IND BB-14797 (C. Brunstein, MD, PhD –sponsor/investigator).

FOR CD34+ SELECTED CELLS ONLY: Any clinically significant safety issues associated with cell processing failure or device malfunction regarding the CliniMACS® system must be reported to the PI (Dr. Margaret MacMillan) and Miltenyi (boston@miltenyibiotec.com) per institutional procedures under protocol MT2015-31.

8.1 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.

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.

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, adverse events that occur as part of the disease process or underlying medical conditions are considered unexpected; however, they will not be reportable per section 8.3.

FOR UNLICENSED UCB UNITS ONLY: These patients will be co-enrolled on University of Minnesota protocol MT2011-13R. **Selected expected adverse reactions** determined to be caused by or probably caused by the UCB unit based on objective evidence will be reported in an expedited manner to the FDA under University of Minnesota IND BB-14797 (C. Brunstein, MD, PhD – sponsor/investigator). Included are the following:

- The unit is mislabeled or failure to pass local lot release
- Serious infusion reaction within first 24 hours after infusion
- Recipient bacteremia with clinical signs and symptoms related to a contaminated UCB within 24 hours after infusion

FOR CD34+ SELECTED CELLS ONLY: These patients will be co-enrolled on University of Minnesota protocol MT2015-31. Adverse events determined to be caused by or probably caused by the CD34+ enriched cell product for hematopoietic reconstitution based on objective evidence will be reported to the IRB and Miltenyi Biotech per institutional procedures under protocol MT2015-31.

8.2 Adverse Event Monitoring

Patients will be monitored for excessive treatment related toxicity through day 100 and reported as applicable per section 8.3. After day 100 and upon knowledge, only those events meeting the definition of expedited reporting will be submitted to the appropriate entity per section 8.3

8.3 Reporting Requirements

After day +100, only those events requiring expedited reporting will be reported upon knowledge.

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/ fax numbers	Copy AE to:
U of MN IRB	**UPIRTSO**: any event which is unanticipated, involved new or increased risk to subjects, and was at least possibly related to study procedures **Other Problems or Events** meeting the definition of UPIRTSO in section 8.1	5 Working Days	MCC SAE UPIRTSO Form	University Of Minnesota IRB MMC 820	Masonic Cancer Center SAE Coordinator mcc-saes@umn.edu
	Non-UPIRTSO: events that do *not* meet the IRB's definition : For a complete list refer to http://www.research.umn.edu/irb/guidance/ae.html#.VC7xral0-sh	Annually	Summary Format		
MCC SAE Coordinator	Any event that counts toward a study stopping rule (see section 10.3)	Upon reporting	Study stopping rule form	SAE Coordinator mcc-saes@umn.edu	Not applicable

The SAE Coordinator will provide the Masonic Cancer Center's Data and Safety Monitoring Council (DSMC) with the SAE in an appropriate format depending on the individual SAE (as reported or in a summary format).

9 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 moderate risk. Therefore the following requirements will be fulfilled:

- The Masonic Cancer Center Data and Safety Monitoring Council (DSMC) will review the trial's progress twice yearly
- 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 8.3 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 Cancer Protocol Review Committee (CPRC).

10 STATISTICAL CONSIDERATIONS

10.1 Study Design

To date, TBI 300 cGy has shown that primary graft failure is no longer a concern. We will no longer dose-escalate or de-escalate but instead try to give a precise estimate of donor engraftment with this preparative therapy.

Approximately 91 patients will be treated at the accepted dose.

The TBI dose de-escalation trial was terminated December 2007 due to secondary graft failure in 2 of 2 patients. All patients transplanted after December 2007 will be prescribed TBI 300 cGy with the goal of evaluating the primary endpoint with more precision. As of January, 2013, there were 42 evaluable patients enrolled at the accepted dose level with one non-evaluable patient due to early death. Our goal is to reach 91 evaluable subjects. One or two patients may be considered high risk and two patients considered very high risk (and treated) after October 2011; these patients will not be included in the evaluable population. With the addition of 49 patients at the accepted dose, the 3 patients in cohort 2 and the 6 potential patients in the high risk arm, our total enrollment on the protocol will be approximately 120.

10.2 Sample Size Considerations

All patients transplanted after December 2007 will be prescribed TBI 300 cGy with the goal of evaluating the primary endpoint with more precision. Specifically we would like to show that with the current estimated graft failure rate of 5.0%, we can show with 95% confidence that the true graft failure rate is less than 10%. To do this we will need to enroll an additional 49 patients or more to the current 43 evaluable patients for a total of at least 91 patients at the accepted dose of TBI 300 cGy.

10.3 Analysis

The primary endpoint of graft failure will be estimated by a simple proportion with confidence limits constructed from the standard errors. Any patients dying prior to day 28 post transplant without an evaluable bone marrow assessment will be considered un-evaluable. The primary objective of this study will be answered by the design of the trial. The secondary endpoints of engraftment, acute GVHD and chronic GVHD will be estimated by cumulative incidence treating early deaths as a competing risk. Survival

will be estimated by Kaplan-Meier methods. Regimen-related toxicity will be summarized by simple proportions.

10.4 Monitoring Guidelines

Patients will be monitored for excess 100 day transplant-related mortality (>50%). With the change back to CSA from Rapamycin 09/29/2014, we will only be monitoring among the additional cohort of 20 patients.

We hypothesize that the rate of TRM will be less than 25% based on current experience. A rate that exceeds 50% is considered unacceptable. The power for TRM is preset at 90% with a type-I error of 5%. Therefore, the trial will be stopped if 5 events occur in the first 6 patients, 6 in the first 9, 7 in the first 11, 8 in the first 14, 9 in the first 17 or 10 in the first 19.

Following completion of the TBI dose de-escalation phase of the study at the selected dose of TBI 300 cGy and the change in regimen on November, 2012 stopping rules will be monitored for the cohort of 49 patients receiving Cy/Flu/TBI (300)/Methyprednisone:

This study will be monitored for primary graft failure by day 42. The study will be stopped (and reviewed) if there is an excess incidence of graft failure. Based on our prior experience with this therapy in this population, we expect that the rate of primary graft failure will be approximately 5%. Our maximum tolerated level will be 20%.

The stopping rule has been developed using the sequential probability ratio test with the level of significance and power preset at 5% and 80% respectively. With these stipulations, the trial will be stopped if there is graft failure in 2 out of 2 or 3 out of 11, 4 out of 20, 5 out of 29, 6 out of 38 or7 at any time.

10.5 Data Collection

Data collection will include those data that are routinely recorded for related donor transplants with special attention to neutrophil engraftment and donor chimerism. Protocol summaries will be available to the protocol chair when requested and as needed for standard Cancer Center safety monitoring procedures.

11 ETHICAL AND REGULATORY CONSIDERATIONS

11.1 Inclusion of Women and Minorities

While there will be every effort to seek out and include women and minority patients, the patient population is dependent upon the referral pattern and the ability to locate a suitable unrelated marrow donor. Women and minority patients are eligible for all aspects of the study and their participation will be actively encouraged. It is recognized that certain minority groups are less likely to find suitably compatible unrelated BM donors through the NMDP registry. Formal efforts to increase minority donor

recruitment are being made by the NMDP in order to enhance the availability of this treatment option to all minorities. All minority patients with suitable unrelated BM donors will be actively encouraged to enroll in this pilot study.

11.2 Monitoring

The sponsor-investigator will permit study-related monitoring, audits, and inspections by the IRB, government regulatory bodies, and University of Minnesota compliance groups in addition to the monitoring requirements of the Cancer Center's Data and Safety Monitoring Plan (section 9). 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.

11.3 Informed Consent

The principles of informed consent described in FDA Regulations (21 CFR Part 50) will be followed to comply with Food and Drug Administration regulations. A patient must give written consent prior to study participation which must be witnessed and dated. The original consent will be retained by the investigator as part of the study records. A copy of the consent form will be given to the patient.

Once a suitable donor has been identified for an individual patient, the patient will be completely evaluated by a physician who subsequently outlines the course of therapy. Assuming the donor and patient meet the medical requirements for undergoing the stem cell donation and transplant procedure, respectively, the patient will then be admitted to the inpatient unit where the course of therapy will again be reviewed. The risks of the procedures to the patient will be discussed in detail. The plan of the pilot study, including the potential risks and benefits will be presented as objectively as possible.

11.4 Record Retention

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

In addition, the Clinical Trials Office (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.

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APPENDIX I - TOTAL BODY IRRADIATION

Dose Calculations for the Prescription Point and Off-Axis Points

The calculation of the treatment time or the meter set for the prescribed dose can be carried out using standard techniques. However, TBI presents special problems relative to the routine treatment situation in that the field sizes are much larger and treatment distances are much longer. Therefore, it is recommended that checks be performed to guarantee the correct delivery of the prescribed dose. For example, the calculation method should be compared to a measurement using a unit density phantom with dimensions equal to the trunk size of an average patient. The skin dose should be >90 % of the prescription point dose. Beam spoilers may be used, if necessary, to ensure this.

Provided an agreement between measurements and calculations is established, the following technique is used to find the meter set or treatment time. If differences are detected, modified terms or additional correction factors should be introduced. For example, modified values for percentage depth dose, tissue-air-ratios and tissue-phantom-ratios are necessary for some treatment units when the patient is positioned at a long distance from the photon source and near the floor or one wall of the room. Also, some deviation from an exact inverse square decrease with distance has been demonstrated for certain room geometries.

Example calculation (central axis prescription point)

MS=	meter set for linear accelerator
D=	prescribed dose per field
PR=	tissue-phantom ratio for area, A and depth, d
A=	the area of the entrance surface presented by the patient's trunk
D=	depth to midline at level of the umbilicus
FSCF=	field size correction factor for field length, L, and field width, W
U=	the treatment unit calibration in units of cGy/meter unit
ISCF=	inverse square correction factor

Similar calculations can be written for time treatment machines (Co 60 units) or for fixed SSD set ups. In order to obtain uniformity among centers using TBI, the Percentage Depth Dose (PDD), Tissue Air Ratio (TAR) must be determined for a depth equal to the half-separation at the level of the umbilicus and for a unit density scattering volume having dimensions equal to the top-of-shoulder to bottom-of-pelvis measurement in one direction and equal to the width at the umbilicus in the other direction. This second dimension will be equal to the AP thickness at the umbilicus for lateral field irradiation, and will correspond to the lateral width at this same level for AP/PA treatment.

Prescription Point and Dose Rate

A midplane dose rate of 26 cGy per minute is required. The prescription point is the midplane at the low pelvis at the level of the mid-hip. If the patient is short, he/she will be treated with a

single anterior and posterior field. If the patient is too tall to fit into the single field, the patient will either be treated standing on a specially designed stand or treated with 2 abutting fields lying prone and supine on the floor. If abutting fields are used, they will be angled away from each other so as to prevent overlap. The match point will be below in the mid femur area to avoid matching over the pelvis. Total body irradiation will be delivered with a linear accelerator using 6, 18, or 24 MV X-rays.

Lung Compensators

A treatment planning CT scan will be performed and a isodose computer plan generated using lung inhomogeneity correction factors. If needed aluminum compensators or thin cerrubend blocks will be fabricated to attenuate the beam so as to keep the lung dose no higher than the prescription dose of 300 cGy. Customized bolus may be necessary to compensate for the thickness of the breast tissue, or if there is a large variation in the chest wall thickness over the field.

Since patients need to be supine for the treatment planning CT and are upright for the chest wall boost, if there is uncertainty about the chest wall thickness with the patient in an upright position, an ultrasound evaluation of the chest wall thickness with the patient upright may be performed.

Thymus blocking

The treatment planning CT scan will be used to determine the location of the thymus. If it is poorly visualized, the radiation oncologist and radiologist will block the area that best corresponds to the usual thymus position. A 5HVL cerrubend blocks will be fabricated and placed so as to block the thymus from both the anterior and posterior TBI fields. The thymus blocks will be attached to the special TBI stand brackets that secure the lung compensators.

At the discretion of the treating investigator(s), thymic shielding will not be done in a small subset of patients who, based on their disease status at study entry and/or previous treatment history, would be at an increased risk of relapse due to the unradiated marrow in the sternum.

A beam “spoiler” or bolus will be used to ensure a full skin dose. Testicular boosts (used for leukemia patients) will not be utilized.

APPENDIX II - SCHEDULE OF ACTIVITIES FOR EACH PATIENT

Scheduled evaluations prior to engraftment (day 30) may be performed +/-3 days from the targeted date; assessments to be performed between engraftment and day 100 may be done +/-7 days of the targeted date; assessments after day 100 may be performed +/-30 days of the targeted date. In addition, targeted days may be altered as clinically appropriate.

Activity	Pre-BMT	Day +1 to Engraftment	Post-engraftment to Discharge (days <30 minimum)	Short-term Post-engraftment Follow-up (days 31-100 minimum)	Long-term Post-engraftment Follow-up (>day 100)
Informed consent	x				
Clinical evaluation					
Karnofsky/Lansky score	x	x(2)	x(2)	x(2, discharge home)	x(day 180,360, ,720)
CXR	x				
PFT	x				x(3)
ECG/ECHO	x				
US liver & kidneys	x				
CT chest, abd, pelvis	x				
CT of sinus	x				
Urine pregnancy test (if applicable)	x				
Cr/Cr cl or GFR	x				
Serum chemistries	x	x(1)	x(1)	x(2)	x(day 180,360,720)
Lipid panel				x (day 60)	x(day 100,180,360,720)
CBC/differential	x	x(1)	x(1)	x(2)	x(day 180,360, 720)
PT/INR, serum alpha fetoprotein, CMV titer	x				
BM aspirate/chimerism[1]	x		x(3)	x(day 90-100)	x(day 180,360, 720)
Toxicity Evaluation		x(1)	x(1)	x(3)	x(3)
GVHD evaluation		x(1)	x(1)	x(3)	x(3)
Immunoglobulin levels and Immune Function Evaluation [2]	x			x (day 100)	x (day 180, 360)

x(1)=perform test daily x(2)=perform test weekly
x(3)=perform test as clinically indicated x(day)=perform test on day indicated
1 - to cell repository - $PBL_{patient}$ for DNA; BM_{donor} for DNA
2 – refer to appendix III - three 10 cc green top tubes for Wagner lab per section 7.7

APPENDIX III - IMMUNE RECONSTITUTION: SCHEDULE OF ACTIVITIES

Activity	Pre-HSCT	Day 100	6 months	1 year
*Cellular Immunity • thymopoiesis/peripheral T cell reconstitution - FACS (CD3, CD4, CD8, CD19, CD25, CD27, CD57, CD69, CD45RA, CD45RO, HLA-DR)	X	X	X	X
**Humoral Immunity • immunoglobulin levels	X	X	X	X

*For each time point, send 30cc blood (3 green tops) to John Wagner's lab (call 625-2966). Label tube 'FA:2006-05 Immune Assays'.
** Immunoglobulin levels to chemistry lab.

APPENDIX IV - ACUTE GRAFT-VERSUS-HOST DISEASE

Patients will be considered evaluable for acute GVHD if they demonstrate donor cell engraftment and survive to day 42. 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
(Glucksberg *et al*, 1974; Thomas *et al*, 1975, 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 1000 – 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	

Conversion Chart for Staging the Volume of Diarrhea in Pediatric Patients

Stage	Stool Volume/day
0	< 7 mL/kg
1	7 – 14 mL/kg
2	14 – 21 mL/kg
3	21 – 28 mL/kg
4	≥28 mL/kg

University of Minnesota Acute GVHD Grading

Acute GVHD Grade	Skin Stage	Liver Stage	Lower GI Stage	Upper GI Stage
I	1-2	0	0	0
II	3	1	1	1
III	-	2-4	2-3	
IV	4	-	4	

- Each column identifies <u>minimum</u> criteria for organ grade.
- Each grade is based on maximum stage for each individual organ involved

e.g. Grade II = skin stage 3 and/or liver stage 1 and/or gut stage 1 and/or UGI stage 1

APPENDIX V CHRONIC GRAFT-VERSUS-HOST DISEASE

Limited CGVHD
Localized skin involvement (<50% body surface area)
and/or
Limited hepatic involvement (abnormal LFTS; bilirubin < 3 mg/dl)

Extensive CGVHD
The presence of one or more of the following criteria may be used for the diagnosis of extensive CGVHD:

- Generalized skin involvement (≥ 50% body surface area)
- Liver histology consistent with involvement by CGVHD with bilirubin ≥ 3 mg/dl
- Positive Schirmer's test (< 5 mm wetting)
- Histologically-proven involvement by CGVHD of oral mucosa or salivary glands
- Lung dysfunction with bronchiolitis obliterans with no evidence of viral causation on histology.
- Gastrointestinal involvement: malabsorption and/or weight loss due to anorexia without explanation other than CGVHD

APPENDIX VI - EXPECTED TOXICITIES

Toxicities associated with the Preparative Therapies:

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)

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

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	• low platelet count with increased risk of bleeding • darkening of nail beds • acne • tiredness • infection • fetal changes if pregnancy occurs	• 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

Cyclophosphamide		
Common	**Less Common**	**Rare**
• sores in mouth or on lips • bleeding from bladder, with blood in urine • diarrhea • long-term or short-term infertility (inability to have children) in women and men	during cyclophosphamide	• 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

Toxicities Associated With Immunosuppressive Therapies

CSA	Mycophenolate Mofetil (MMF)
• nephrotoxcity • seizures • hypertension • hirsutism • increased risk of relapse • thrombotic thrombocytopenic purpura • electrolyte imbalances • paresthesias/neuropathy • gingival hyperplasia • increased risk of opportunistic infection •	• pancytopenia • headache • insomnia • electrolyte imbalances • leg cramps/bone pain • hypertension • dizziness • hyperglycemia • rash • nausea/diarrhea

Toxicities Associated With Other Therapies

Methylprednisolone may cause nausea, vomiting, heartburn, headache, dizziness, trouble sleeping, appetite changes, increased sweating, acne, or pain/redness/swelling at the injection site. This medication may make your blood sugar level rise, which can cause or worsen diabetes. Serious, but rare side effects include: unusual weight gain, menstrual period changes, bone/joint pain, easy bruising/bleeding, mental/mood changes (such as mood swings, depression, agitation), muscle weakness/pain, puffy face, slow wound healing, swelling of the ankles/feet/hands, thinning skin, unusual hair/skin growth, vision problems, fast/slow/irregular heartbeat. Very rarely, but possibly fatal, stomach or intestinal bleeding has been reported.

Toxicities associated with Voriconazole

- diarrhea, nausea, vomiting
- fever, headaches
- liver abnormalities
- swelling of extremities
- rash
- visual changes
- allergic reaction

Risks Associated with T cell depletion
The toxicities and complications that could potentially result from the removal of lymphocytes from the donated bone marrow are failure of bone marrow engraftment (due to loss or damage to hematopoietic progenitor cells), an increased risk of relapse, and an increased risk of EBV-associated lymphoproliferative syndrome.

Risks Associated with Hematopoietic Stem Cell Infusion/Overall Transplant Process

- 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)
- graft-versus-host-disease (GVHD)
- veno-occlusive disease
- mucositis,
- infections (sepsis)
- acute hemolytic reactions
- febrile nonhemolytic reactions
- allergic reactions
- anaphylactoid or anaphylactic reactions
- transfusion-related acute lung injury (TRALI)
- DMSO toxicity
- transmission of bacterial, viral or protozoal infection
- fat embolism (marrow)
- bleeding
- transfusion-associated circulatory overload (TACO)
- hypothermia
- non-immunologic hemolysis
- granulocyte-related complications
- cardiotoxicity

G-CSF

- bone pain
- headaches
- body aches
- fatigue
- nausea/vomiting
- insomnia
- dyspnea
- rash
- edema