Randomized Phase II Trial of Bulk versus Fractionated Stem Cell Infusions in Patients with Hematologic Malignancies Undergoing Stem Cell Transplantation

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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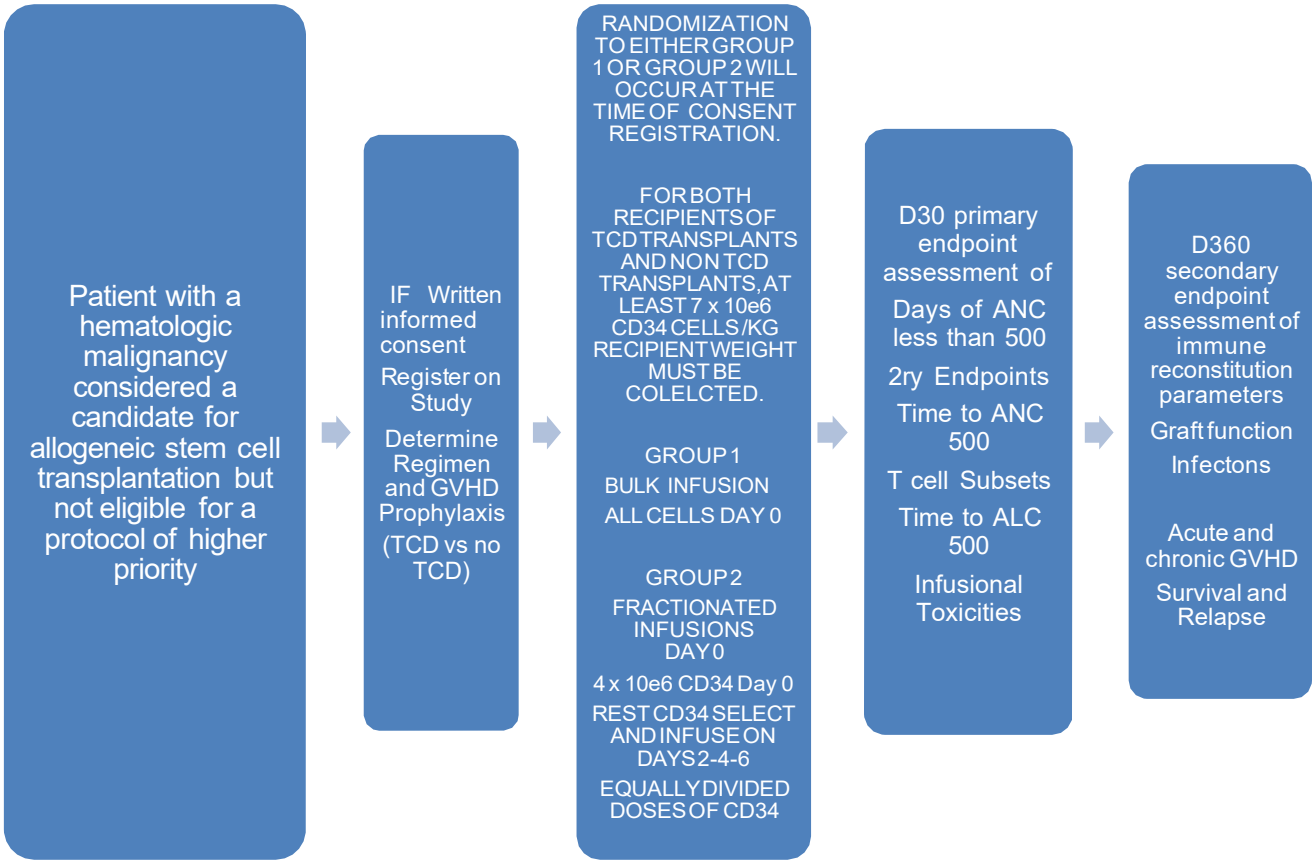
1.0 PROTOCOL SUMMARY AND/OR SCHEMA

In this randomized phase 2 trial we propose to enhance neutrophil recovery, immune reconstitution and graft function post stem cell transplant by giving fractionated stem cell infusions of between 0.5-2.0 x 10^6 CD34+ selected donor cells on days +2, +4, and +6 post SCT and compare them to bulk infusion.

Patients with any hematologic malignancy undergoing an allogeneic hematopoietic stem cell transplant (SCT) with a matched or mismatched related or unrelated donor, and not eligible for a protocol of higher priority, will be considered eligible. Patients agreeing to participate will sign written informed consent. Once consent is obtained and the patient is registered, participants will be randomized to either receive all cells in bulk on day 0 (GROUP 1) or receive 4 x 10e6 CD34+ cells/kg on day 0 with the rest of the cells being infused on days 2, 4, 6 in equally distributed aliquots (GROUP 2). Patients will be stratified by type of transplant (T cell depletion vs no T cell depletion).

For both recipients of T cell depleted transplants and non T cell depleted transplants randomized on to the fractionated or bulk arm, at least 7 x 10e6 CD34 cells/kg recipient weight must be collected. There will be 36 patients in each group. Patients whose donor fails to collect the appropriate number of cells will receive all their cells as a bulk infusion. These patients will continue to receive a transplant on protocol but will be replaced until both arms have reached the target accrual.

STUDY SCHEMA



2.1 OBJECTIVES AND SCIENTIFIC AIMS

Primary Objective

1. Determine the effects of fractionated vs. bulk stem cell infusions on neutrophil recovery as defined by number of days with an absolute neutrophil count of less than 500 neutrophils per micro liter and time to an absolute neutrophil count (ANC) of 500.

Secondary Objective

1. Determine the safety of fractionated vs bulk stem cell infusions in allo SCT recipients.
2. Determine the effect of fractionated vs bulk stem cell infusions on other parameters of immune- reconstitution. (ALC Day 15 and D30, time to ALC 500, CD4 and CD8 count recovery.
3. Determine the effect of fractionated vs bulk CD34 selected stem cell infusion on bacterial, viral, and fungal infections during the first 100 days post transplant
4. Determine the effect of fractionated vs bulk CD34 selected stem cell infusions on hematopoietic function as determined by platelet counts and cumulative platelet transfusion requirements on days 30, 60 and 100, 6 and 12 months post transplant.

3.0 BACKGROUND AND RATIONALE

Allogeneic stem cell transplantation has become a well established curative approach for a variety of malignant and non malignant hematologic disorders. (1) Transplant related mortality has been reduced significantly over the last decade due to improvements in supportive care and HLA typing. (2) Despite these advances the risk of dying from transplant related causes is still around 10-15% at 1 year. A third of those deaths are due to infectious complications. (3) Thus enhancing immune reconstitution post allogeneic transplantation could theoretically improve transplant outcomes by reducing the risk of infectious complications. (4,5) Current strategies being explored include the use of cytokines (such as IL-7), improvements of thymic function using androgen ablation, or addition of cytotoxic T lymphocytes targeting specific viral, bacterial or fungal antigens.(4) However, most of these strategies have yet to find wide application due to costs or logistic reasons. Two other potential strategies to enhance immune reconstitution post allogeneic transplant could be increasing CD34 cell dose or giving fractionated stem cell infusions. Historically stem cell dose as measured by total nucleated cell dose has been a well established prognostic factor for transplant outcome (6,7). The hypothesis underlying this observation was that a higher total nucleated cell dose was associated with higher stem cell doses that would promote a more rapid hematologic and immune recovery. However, due to logistic considerations it will be difficult to explore modulating stem cell dose after marrow transplantation outside of the context of unrelated donor transplants for small children although multiple retrospective analysis suggest that higher CD34+ cell doses are associated with improved outcomes (8-19). With the advent of filgrastim mobilized peripheral blood stem cell transplantation (PBSCT) and the use of CD34 enumeration as a surrogate for hematopoietic stem cell numbers the potential role of stem cell dose (as measured by CD34+ cells on transplant outcomes) can be explored. However, as of today no prospective trials looking at CD34+ cell dose as a variable has actually been performed.

The other potential strategy that could enhance immune-reconstitution post stem cell transplantation could be fractionation of the stem cell dose.(20-22) Donor stem cell engraftment after hematopoietic stem cell transplantation is a complex biological procedure. After the stem cells are infused (usually through a central venous catheter, a significant number of stem cells are sequestered within the lungs where it is believed that they slowly are released into the circulation. Through a complex network of adhesion molecules and their ligands, these stem cells migrate and eventually deposit in appropriate niches for their growth and proliferation. The practice of bulk stem cell infusion as currently done has been adopted because of historical and financial reasons (easier to infuse all cells at once rather than separate them into aliquots and infuse in fractions). However, data is emerging from animal models as well as better understanding of stem cell biology and trafficking suggest that fractionating the stem cell infusion may be beneficial, because it could increase the chance of a hematopoietic stem cell to deposit into a viable hematopoietic niche to successfully grow and proliferate, rather than finding itself sequestered in a damaged niche which could serve as a "stem cell sink". In a murine sickle cell disease transplant model Felfly et al showed that infusing the same number of cells in two, three or four equal sub doses within 28 hours of myelosuppression the engraftment efficiency increased to greater than 85% compared to the 40% obtained following one-cell dose infusion. Importantly, full engraftment for all recipients was obtained when the same number of cells was administered in three or four equal cell sub doses within the 28 hours for both irradiation levels (Table 1). (20) Other murine models have also suggested a potential improvement in engraftment when stem cells are given in fractions over a period of time rather than in bulk. (21,22)

Table 1: Effect of Fractionating Stem Cell Infusions on Engraftment (20 Felfly et al,)

One Cell Dose	Cell Dose e7	Number of Doses	Time to infusion	engraftment	Wbc chimerism
XRT Level 2	2	1	4	4/21	37
	4	1	4	5/12	48
	5	1	4	5/8	76
	2	1	52	2/13	38
	5	1	52	3/8	27
Multi Dose					
XRT Level 2	4	2		6/7	73
	4	3		5/5	51
	4	4		6/6	72
XRT Level 1	6	2		1/7	34
	6	3		7/7	29
	6	4		7/7	27

In the autologous setting multiple infusions of stem cells have been performed but at relatively low CD34 doses with no definitive impact on outcome but no toxicities either, however, the doses explored were relatively low (Less than 5 x 10e6 CD34+ cells/kg) (23,24). **This proposed trial will be the first attempt to demonstrate the safety and immune reconstitution effects of fractionated stem cell infusions in patients undergoing allogeneic stem cell transplantation.**

The primary endpoint of this trial is neutrophil reconstitution, we hypothesize that fractionated stem cell infusion will reduce the period of absolute neutropenia less than 500 cells per microliter by 30% (7 days for a TCD graft and 9 days for a unmanipulated graft). Fractionated stem cell infusions could enhance neutrophil recovery through recruitment of committed progenitor cells during the subsequent stem cell infusion and through reduction of the number of stem cells that migrate to "ineffective niches".

Secondary endpoints are immune-reconstitution as defined by ALC on D30 and D15 primarily. This endpoint was derived from a recent analysis performed by Dr. Goldberg in our institution, a retrospective study of 353 consecutive patients (median age 39, range 2-68) who received a fully TCD allo HSCT. The median absolute lymphocyte count at day 30 and 60 post transplant was 500 and 800 per microliter respectively and was associated with improved survival on univariate analysis. On multivariate analysis ALC on day 30 and NK cell count on day 60 were also associated with improved survival. (25)

Other secondary endpoints include incidence of acute GVHD. With current CD34 selection technology the incidence of Grade 2 or greater GVHD is 10-15% for TCD SCT and 40% for unmanipulated stem cells.(26-29) We hypothesize that fractionating the stem cell infusion will not increase the risk of Grade 2 or greater GVHD to over 25% with less than 10% of patients getting Grade 3 or 4 GVHD if they received a TCD transplant and less than 20% if they received an unmanipulated SCT. (26,28) Historical graft failure rates of between 2-5% have been reported with T Cell depletion transplants and less than 2% with an unmanipulated graft. We expect that this rate will not increase with fractionated stem cell infusions.

This trial includes a heterogeneous group of patients, however, since our primary endpoint is neutrophil recovery which is not impacted by diagnosis, disease stage, or conditioning regimen, in this initial trial the patient heterogeneity should not affect the analysis of the primary outcome. The Stem Cell Transplant Trialist Group recently performed a meta-analysis on 9 randomized trials comparing allogeneic peripheral blood to allogeneic bone marrow transplantation. Most of these trials allowed for multiple diagnosis and multiple conditioning regimens. In both individual reports as well as the meta-analysis only stem cell source (peripheral blood versus bone marrow) impacted engraftment kinetics.

Additionally, Pulsipher et al reported on 932 recipients of unrelated donor peripheral blood stem cell hematopoietic cell transplantation (URD-PBSC HCT) for acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia, and myelodysplastic syndrome enrolled on a prospective National Marrow Donor Program trial from 1999 through 2003. Preparative regimens included myeloablative (MA; N = 611), reduced-intensity (RI; N = 160), and nonmyeloablative (NMA; N = 161). For MA recipients, $CD34^+$ counts greater than 3.8×10^6/kg improved neutrophil and platelet engraftment, whereas improved overall survival (OS) and reduced transplant-related mortality (TRM) were seen for all preparative regimens when $CD34^+$ cell doses exceeded 4.5×10^6/kg. Higher infused doses of $CD34^+$ cell dose did not result in increased rates of either acute or chronic graft-versus-host disease (GVHD). Thus, demonstrating that at least for neutrophil recovery patient and disease characteristics did not impact engraftment kinetics.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a randomized Phase II trial of bulk versus fractionated stem cell infusions in patients with hematologic malignancies undergoing allogeneic stem cell transplantation. Patients will be stratified by type of transplant (T cell depletion vs no T cell depletion).

Patients with high risk hematologic malignancies who fulfill eligibility requirements will be randomized at the time of consent registration to either receive their stem cells either as a bulk infusion on day 0 or as fractionated infusions with 4 x 10e6 CD34 cells infused on day 0 and the rest of the cells CD34 selected using the CliniMACS device and cryopreserved in 3 equal aliquots that will be reinfused on days +2, +4, and +6 (+/- 48 hours to avoid infusion during weekend or holidays) with standard premedication. Below is a table of potiential fractionated infusion schedules:

Day 0	Day +2	Day +4	Day +6
Monday	Wednesday	Friday	Monday
Tuesday	Thursday	Monday	Wednesday
Wednesday	Friday	Monday	Wednesday
Thursday	Monday	Wednesday	Friday
Friday	Monday	Wednesday	Friday

Donors will undergo stem cell collection using filgrastim and standard leukapheresis techniques with a collection goal of >7 x 10e6 CD34+ cells per kg recipient weight for T cell depleted (TCD) stem cell transplants and non TCD transplants. The collection target can be reached in up to 2 leukapheresis sessions. Recipients of stem cell transplants from a related donor can have all the product previously collected and cryopreserved prior to beginning conditioning. Stem cell products from an unrelated donor can have all the product cryopreserved. T cell depleted transplants will be capped at a cell dose of 15 million.

Patients will be scored weekly for acute GVHD until day +100 and for chronic GVHD according to MSKCC standard of care guidelines. Hematologic and immunologic parameters will be monitored on a periodic basis as described in Section 10.0.

4.3 Intervention

This is a complex study that involves various interventions, Intervention #1: Donor Initial Stem Cell Collection; Intervention #2: Stem Cell Product Initial Processing Orders; Intervention #3 Patient Admission and Transplantation; Intervention #4: Stem cell infusion; Intervention #5: Post infusion follow up; Intervention #6: Off Study Patient and Donor Evaluation.

4.2.1 : Donor Intervention

4.2.1.1 : INTERVENTION # 1

Donor Stem Cell Collection: will be performed using usual standard institutional protocols.

4.2.1.2 : INTERVENTION # 2: STEM CELL PROCESSING AND RANDOMIZATION

Patients will be randomized to receive either a bulk or fractionated stem cell infusion. Matched related donors CAN HAVE all cells collected and cryopreserved in appropriate aliquots PRIOR to patient being registered on study.

For patients receiving a TCD transplant and randomized to BULK INFUSION: These patients will have their entire stem cells CD34 selected using the CliniMACS device and have ALL cells infused on DAY 0.

For patients receiving a TCD transplant and randomized to FRACTIONATED INFUSION: These patients will have ALL their stem cells CD34 selected using the CliniMACS device. After

CD34 selection 4 x 10e 6 will be infused fresh, the remaining cells will be cryopreserved in 3 equally divided aliquots.

For patients receiving a non TCD transplant and randomized to BULK INFUSION: These patients will receive all collected cells WITHOUT manipulation on day 0 as per the treatment plan.

For patients receiving a non TCD transplant and randomized to FRACTIONATED INFUSION: These patients will have 4 million CD34+ cells / kg infused WITHOUT manipulation on day 0 as per the treatment plan. The remaining cells will undergo CD34 selection using the CliniMACS device and be cryopreserved in 3 equally divided aliquots without manipulation.

4.2.1.3: INTERVENTION # 3: PATIENT ADMIT AND CONDITIONING REGIMEN

All patients will be treated according to extant or standard care protocols as long as they receive any of the following conditioning regimens

TABLE 2: ALLOWABLE CONDITIONING REGIMENS FOR NON T CELL DEPLETED TRANSPLANTS

Reduced Intensity Conditioning		Myeloablative Conditioning	
A	Fludarabine 120-160 mg/m^2 & Busulfan 6.4 mg/kg	C	Fludarabine 120-160 mg/m^2 & Busulfan 9.6 mg/kg
B	Fludarabine 120 mg/m^2 & Melphalan 140 mg/m^2	D	Fludarabine 120 mg/m^2 & Busulfan 12.8 mg/kg
		E	Busulfan 12.8 mg/kg & Cyclophosphamide 120 mg/kg
		F	Cyclophosphamide 120mg/kg & Total Body Irradiation 1200-1420 cGy
		G	Busulfan 12.8 mg/kg & Melphalan 120 mg/m^2

[a] Bu according to institutional guidelines

TABLE 3: ALLOWABLE CONDITIONING REGIMENS FOR T CELL DEPLETED TRANSPLANTS

Myeloablative Conditioning			
H	Busulfan 9.6 mg/kg, Melphalan 140 mg/m^2, & Fludarabine 125 mg/m^2	I	Total Body Irradiation 1200-1420 cGy, Thiotepa 10mg/kg, & Cyclophosphamide 120mg/kg

[a] Bu according to institutional guidelines

TABLE 4: ALLOWABLE GVHD PROPHYLAXIS REGIMENS

	GvHD Prophylaxis
A	Tacrolimus • Blood trough levels 5-15ng/ml • Continue for minimum 6 months (taper may begin 4 months post transplantation Methotrexate • 5mg/m^2 day 1, 3, 6 and 11
B	Cyclosporine • Blood trough levels 200-300 ng/ml • Continue for minimum 6 months (taper may begin 4 months post transplantation Methotrexate • 5 mg/m^2 day 1, 3, 6 and 11
C	Tacrolimus/Sirolimus and mini-methotrexate
D	T cell depletion with CliniMACS device

4.2.1.4 : INTERVENTION # 4: STEM CELL INFUSION:

For patients receiving a TCD transplant and randomized to BULK INFUSION: These patients will have ALL their stem cells infused on DAY 0.

For patients receiving a TCD transplant and randomized to FRACTIONATED INFUSION: These patients will have 4 x 10 e 6 CD34 selected cells /kg infused fresh on Day). The remaining cells will be cryopreserved in 3 equally divided aliquots on days +2, +4, and +6.

For patients receiving a non TCD transplant and randomized to BULK INFUSION: These patients will receive all collected cells on day 0.

For patients receiving a non TCD transplant and randomized to FRACTIONATED INFUSION: These patients will have 4 million CD34+ cells / kg on day 0. The remaining cells will be CD34 selected using the CliniMACS device and cryopreserved in 3 equally divided aliquots without manipulation and infused on day +2, +4, and +6.

All patients will be premedicated according to current institutional guidelines.

4.2.1.5 : INTERVENTIONS #5 and #6: POST INFUSION FOLLOW UP AND OFF STUDY EVALUATION.

All patients will receive post transplant care according to MSKSCC BMT Guidelines or current extant protocols. Study specific laboratory and assessments are summarized in Section 10.0.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1. Drugs Related to Conditioning Regimens Are the Following:

Hyperfractionated total body irradiation, cyclophosphamide, thiotepa, busulfan, melphalan, and fludarabine are standard antineoplastic agents that will be employed in the three cytoreductive regimens as detailed in the treatment plan.

5.1.1 Total Body Irradiation: Hyperfractioned TBI is administered by a linear accelerator at a dose rate of < 20 cGy/minute. Doses of 125 cGy/fraction are administered at a minimum interval of 4 hours between fractions, three times/day for a total of 11 or 12 doses (1375 or 1500 cGy) over 4 days (Day -9, -8, -7, and -6). If general anesthesia is required, 200cGy q12h x 7 doses to a total dose of 1400 cGy may be given Sequential doses are administered in an anterior/posterior or lateral orientation. Compensators and lung blocks are used to shield the lung, so that the lung receives 800 cGy. The blocked areas of the chest will be boosted with high-energy electrons so that the cumulative chest wall dose is approximately 1500 cGy. This insures that marrow sites in the ribs are adequately treated.

5.1.2 Thiotepa (Thioplex®) Formulation: 15 mg vial lyophilized powder; must be diluted prior to infusion.
Reconstitution: Add 1.5 ml of Sterile Water for injection to 15mg vial to yield 10mg/ml. Solutions which are grossly opaque or contain a precipitate, should not be used. In order to eliminate haze, solutions should be filtered through a 0.22-mcron filter prior to administration.
Storage and Stability:
Store vials in refrigerator and protect from light.
Refrigerated: Prepare Infusion in NSS; stable for 14 days.
Room temperature: Prepare Infusion in NSS; stable for 7 days.
Preparation:
Standard IV fluid: NSS
Final concentration range up to: 5mg/ml.
IV piggyback volume: 500 cc.
Spike infusion bag with IMED 2200 tubing, primed with non-chemo containing fluid (i.e. NSS).
Clinical Considerations:
Hydration: NA
Emetic Potential: High
Incompatibilities: Cisplatin, filgrastim (G-CSF), vinorelbine.

5.1.3 Cyclophosphamide (Cytoxan®, Neosar®)
Supplied As: 200 mg, 500 mg, 2000 mg vials
Reconstitution Directions: Add Sterile Water for injection to yield a final concentration of 20 mg/ml.
Storage and Stability:
Store vials at room temperature.
Refrigerated: Prepare infusion in D5W, stable for 28 days.
Room Temperature: Prepare infusion in D5W; stable for 48 hours.
Preparation:
Standard IV fluid: D5W.
Final concentration range up to: 20mg/ml.
IV piggyback volume: For doses <1200mg/m_2, infuse in 25cc D5W; for doses >1200mg, infuse as straight drug.
Clinical Considerations:
Hydration: As per MSKCC guidelines.

Emetic Potential: High and Delayed.

Supportive Medications: None.
Incompatibilities: Do not administer with other drugs.

5.1.4 Busulfan (busulfex®)
Source and Pharmacology: Supplier: Otsuka Pharmaceuticals; Busulfan is a bifunctional alkylating agent known chemically as 1,4-butanediol, dimethanesulfonate. BUSULFEX® (busulfan). This is an agent in which two labile methanesulfonate groups are attached to opposite ends of a four carbon alkyl chain. In aqueous media, busulfan hydrolyzes to release the methanesulfonate groups. This produces reactive carbonium ions that can alkylate DNA. DNA damage is thought to be responsible for much of the cytotoxicity of busulfan.
Formulation and Stability: It is supplied as a clear, colorless, sterile, solution in 10 mL single use ampoules. Each ampoule of BUSULFEX contains 60 mg (6 mg/mL) of busulfan, the active ingredient, a white crystalline powder with a molecular formula of $CH_3SO_2O(CH_2)_4OSO_2CH_3$ and a molecular weight of 246 g/mole. Busulfan is dissolved in N,N-dimethylacetamide (DMA) 33% wt/wt and polyethylene glycol 400, 67% wt/wt. Busulfans solubility in water is 0.1 g/L and the pH of a >0.5% solution in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP as recommended for infusion reflects the pH of the diluent used and ranges from 3.4 to 3.9.
Solution Preparation: BUSULFEX is supplied as a sterile solution in 10 mL single-use clear glass ampoules each containing 60 mg of busulfan at a concentration of 6 mg/mL for intravenous use. BUSULFEX must be diluted prior to use with either 0.9% Sodium Chloride Injection, USP (normal saline) or 5% Dextrose Injection, USP (D5W). The diluent quantity should be 10 times the volume of BUSULFEX, ensuring that the final concentration of busulfan is approximately 0.5 mg/mL.
Storage and Stability: Unopened ampules of BUSULFEX must be stored under refrigerated conditions between 2° -8° C (36° -46° F).
Administration: Intravenous, over 2 hours.

5.1.5 Melphalan (Alkeran®)
Source and Pharmacology: Supplier: Glaxo Wellcome. A derivative of nitrogen mustard, an analog of mustard gas. It is a polyfunctional alkylating agent that causes miscoding, cross-linkage of DNA, and single-strand breakage of DNA. It inhibits cellular glycolysis, respiration, and protein synthesis. It is cell cycle-phase non-specific.
Formulation and Stability: A lyophilized powder of 50 mg melphalan and 20 mg povidone per vial. Also provided is 10 ml of sterile diluent for use in reconstituting the product and a 0.45 micron filter. The special diluent has the following composition: Sodium citrate 0.2 g, Propylene glycol 6.0 ml, Ethanol (95%) 0.5 ml, and sterile water 10 ml.
Solution Preparation: Vial/50 mg: Reconstitute by rapidly injecting 10 ml of the supplied diluent into the vial to yield a final concentration of 5 mg/ml. Shake vigorously until the solution is clear. Immediately dilute the dose to be administered in 0.9% Sodium Chloride, USP, to a concentration no greater than 0.45 mg/ml
Storage and Stability: The intact packages should be stored at room temperature (15-30°C) protected from light. Shelf-life surveillance of the intact dosage form is ongoing. Constitution with the special diluent as directed results in a solution that retains at least 90% potency for about three hours at 30°C. Storage at 5°C results in precipitation.
Administration: Intravenous, over 30 minutes. Complete infusion within 60 minutes of preparation.

5.1.6 Fludarabine (FLUDARA®)
Source and Pharmacology: Supplier: Berlex Laboratories, Inc. FLUDARA FOR INJECTION contains fludarabine phosphate, a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-ß-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination by adenosine deaminase. The chemical name for fludarabine phosphate is 9H-Purin-6-amine, 2-fluoro-9-(5-O-phosphono-ß-D-arabinofuranosyl). Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-

fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.
Formulation and Stability: Each vial of sterile lyophilized solid cake contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Reconstitution with 2 mL of Sterile Water for Injection USP results in a solution containing 25 mg/mL of fludarabine phosphate intended for intravenous administration. FLUDARA FOR INJECTION is supplied in a clear glass single dose vial (6 mL capacity) and packaged in a single dose vial carton in a shelf pack of five
Solution Preparation: FLUDARA should be prepared for parenteral use by aseptically adding Sterile Water for Injection USP. When reconstituted with 2 mL of Sterile Water for Injection, USP, the solid cake should fully dissolve in 15 seconds or less; each mL of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. The pH range for the final product is 7.2-8.2. In clinical studies, the product has been diluted in 100 cc or 125 cc of 5% Dextrose Injection USP or 0.9% Sodium Chloride USP
Storage and Stability: FLUDARA is supplied as a white, lyophilized solid cake. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Store under refrigeration, between 2°-8° C (36°-46° F).
Administration: Intravenous, over thirty minutes.

5.1.7 Anti-Thymocyte Globulin (Rabbit) (Thymoglobulin®)

Source and pharmacology: Supplier: Genzyme®. Thymoglobulin® [Anti-thymocyte Globulin (Rabbit)] is a purified, pasteurized, gamma immune globulin, obtained by immunization of rabbits with human thymocytes. This immunosuppressive product contains cytotoxic antibodies directed against antigens expressed on human T-lymphocytes.
Formulation and stability: Thymoglobulin is a sterile, freeze-dried product for intravenous administration after reconstitution with sterile Water for Injection, USP (WFI). Each package contains two 7 mL vials: Vial 1: Freeze-Dried Thymoglobulin Formulation Active ingredient: Anti-thymocyte Globulin (Rabbit) 25 mg - Inactive ingredients: Glycine (50 mg), mannitol (50 mg), sodium chloride (10 mg); Vial 2: Diluent Sterile Water for Injection, USP 5 mL. The reconstituted preparation contains approximately 5 mg/mL of Thymoglobulin, of which >90% is rabbit gamma immune globulin (IgG). The reconstituted solution has a pH of 7.0± 0.4. Human red blood cells are used in the manufacturing process to deplete cross-reactive antibodies to non-T-cell antigens. The manufacturing process is validated to remove or inactivate potential exogenous viruses. All human red blood cells are from US registered or FDA licensed blood banks. A viral inactivation step (pasteurization, i.e., heat treatment of active ingredient at 60°C/10 hr) is performed for each lot. Each Thymoglobulin lot is released following potency testing (lymphocytotoxicity and E-rosette inhibition assays), and cross-reactive antibody testing (hemagglutination, platelet agglutination, anti-human serum protein antibody, antiglomerular basement membrane antibody, and fibroblast toxicity assays on every 5th lot).
Solution preparation: Each reconstituted vial contains 25 mg or 5 mg/mL of Thymoglobulin. Transfer the contents of the calculated number of Thymoglobulin vials into the bag of infusion solution (saline or dextrose). Recommended volume: per one vial of Thymoglobulin use 50 mL of infusion solution (total volume usually between 50 to 500 mL). Mix the solution by inverting the bag gently only once or twice.
Storage and stability: Store in refrigerator between +2° C to +8° C (36° F to 46° F). Protect from light. Do not freeze. Do not use after the expiration date indicated on the label. Reconstituted vials of Thymoglobulin should be used within 4 hours. Infusion solutions of Thymoglobulin must be used immediately. Any unused drug remaining after infusion must be discarded.
Administration: Infuse through a 0.22-micron filter. Set the flow rate to deliver the dose over 12 hours.

5.2 Drugs or Devices Related to Graft vs Host Prevention are the Following:

5.2.1 Tacrolimus

Source and Pharmacology: Prograf (Astellas Pharmaceuticals) Absorption of tacrolimus from the gastrointestinal tract after oral administration is incomplete and variable. The absolute bioavailability of tacrolimus was 17 ± 10% in adult kidney transplant patients (N=26), 22 ± 6% in adult liver transplant patients (N=17), and 18 ± 5% in healthy volunteers (N=16). The plasma protein binding of tacrolimus is approximately 99% and is independent of concentration over a range of 5-50 ng/mL. Tacrolimus is bound mainly to albumin and alpha-1-acid glycoprotein, and has a high level of association with erythrocytes. The distribution of tacrolimus between whole blood and plasma depends on several factors, such as hematocrit, temperature at the time of plasma separation, drug concentration, and plasma protein concentration.
Formulation and Stability: Tacrolimus injection must be diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection to a concentration between 0.004 mg/mL and 0.02 mg/mL prior to use. Diluted infusion solution should be stored in glass or polyethylene containers and should be discarded after 24 hours. The diluted infusion solution should not be stored in a PVC container due to decreased stability and the potential for extraction of phthalates. In situations where more dilute solutions are utilized (e.g., pediatric dosing, etc.), PVC-free tubing should likewise be used to minimize the potential for significant drug adsorption onto the tubing. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Due to the chemical instability of tacrolimus in alkaline media, Prograf injection should not be mixed or co-infused with solutions of pH 9 or greater (e.g., ganciclovir or acyclovir). Supplied as a sterile solution in 1-mL ampoules containing the equivalent of 5 mg of anhydrous tacrolimus per mL, in boxes of 10 ampoules. Store between 5°C and 25°C (41°F and 77°F). Prograf capsules (1 and 5 mg) are stored at controlled room temperature, 15°C-30°C (59°F-86°F).

5.2.2 Cyclosporine

Source and Pharmacology: Novartis Neoral Supplied as 50 mg/ml – 5 ml ampule or 25-50-100 mg capsules
Reconstitution: N/A
Indications: Immunosuppressant used in the prevention of graft versus host disease.
Preparation:
Dilute in D5W or NS to make a 2.5 mg/ ml solution.
Infuse slowly over approximately 1-4 hours (intermittent infusion) or 24 hours for continuous infusion.
Clinical Considerations:
Patients should be under close observation for possible allergic manifestations including facial flushing, respiratory distress, with dyspnea and wheezing, blood pressure changes and tachycardia.
Prior to infusion solution should be inspected visually for particulate matter and discoloration.
Other nephrotoxic agents will increase the risk of nephrotoxicity (amphotericin B, aminoglycosides, and acyclovir).
Plasma concentrations of cyclosporine may be affected by the following drugs:
Increased cyclosporine levels: ketoconazole, erythromycin, cimetidine, calcium channel blockers, fluconazole, itraconazole, norfloxacin, imipenem/ cisplatin.
Decreased cyclosporine levels: rifampin, phenytoin, phenobarbital, imipenem/ cisplatin.
The IV to oral dose conversion is 1:3. The target serum level of 200- 400 is desirable; 800 is considered toxic.
Renal and hepatic parameters should be monitored routinely with dosage adjustments in the case of serum creatinine or LFT elevations.
Incompatibilities: Do not co-administer with any drug.

5.2.3 Methotrexate
Reconstitution: Dilute powder with D_5W or NS to a concentration ≤25 mg/mL (20 mg and 50 mg vials) and 50 mg/mL (1 g vial). Intrathecal solutions may be reconstituted to 2.5-5 mg/mL with NS, D_5W, lactated Ringer's, or Elliott's B solution.
Storage and Stability: Store tablets and intact vials at room temperature (15°C to 25°C). Protect from light. Solution diluted in D_5W or NS is stable for 24 hours at room temperature (21°C to 25°C). Reconstituted solutions with a preservative may be stored under refrigeration for up to 3 months, and up to 4 weeks at room temperature. Intrathecal dilutions are stable at room temperature for 7 days, but it is generally recommended that they be used within 4-8 hours.
Toxicities: see section 11.0
Incompatibility: Chlorpromazine, gemcitabine, idarubicin, ifosfamide, midazolam, nalbuphine, promethazine, propofol.

5.2.4 The CliniMACS System for Positive Selection of CD34+ Progenitor Cells and Depletion of T-Cells

The CliniMACS System (Miltenyi Biotec, Auburn, CA) including the CliniMACS$_{plus}$ Instrument, a CliniMACS Tubing Set, the CliniMACS CD34 Reagent and the CliniMACS PBS/EDTA Buffer is intended for the selection and enrichment of human CD34 positive hematopoietic progenitor cells from a leukapheresis product. The CD34 antigen is a cell membrane glycoprotein expressed by early hematopoietic stem and progenitor cells. The CD34 positive cell separation process may be useful in several areas of clinical stem cell transplantation, including purging of tumor cells, T-cell depletion, *ex vivo* cell expansion and gene therapy. When re-infused after myeloablative chemotherapy, CD34 positive peripheral blood progenitor cells have been shown to reconstitute all hematologic lineages and exhibit both short and long term repopulating capacities. The ClinMACS System uses selective CD34 monoclonal antibodies conjugated to superparamagnetic particles. The CD34 positive target cells are selected in an automated continuous flow separation system. The CD34 positive cells are specifically labeled for selection by incubation with the CliniMACS CD34 Reagent. After unbound reagent is washed from the suspension, the cells are ready for the automated separation process. The CliniMACS System passes the antibody-labeled suspension; the cells are ready for the automated separation process. The CliniMACS System passes the antibody-labeled suspension through a column in which strong magnetic gradients are generated. The Selection Column retains the magnetically labeled CD34 positive cells, while unwanted cells flow through the Selection Column and are collected in the Negative Fraction Bag. The system performs several washing steps, disposing most of the liquid into the Buffer Waste Bag. The Separated CD34 positive cells are released from the column by removing the column from the magnetic field and collecting the cells into the Cell Collection Bag.

The components of the CliniMACS System include:

5.2.4.1 The CliniMACS Instrument

The CliniMACS Instrument is a bench-top instrument consisting of a supporting structure to hold the column/tubing assembly and various bags, a series of valves through which the tubing set is fitted, a magnet between the poles of which the separation column is placed, a peristaltic pump through which a section of tubing is placed, software to control the instrument and user interface and a computer touchpad with a display window. The instrument is operated at ambient temperature and it is intended to be multi-use item.

The software for the CliniMACS Instrument controls the function of the electromechanical

components of the instrument and the user interface. Two separate computers, one a microcontroller located on a control board of the CliniMACS Instrument and the second a PC compatible computer which operates the user interface are incorporated with the instrument. Software Version 2.31, the current version of software is directly traceable to the version of software utilized in pre-clinical testing and European Safety trials, and has been inspected and approved by TÜV product services with the CE Mark.

5.2.4.2. CliniMACS Tubing Set

The CliniMACS Tubing Set consists of a tubing element combined with a pair of proprietary cell selection columns. These form a closed, sterile system for processing the cells. The separation column is a proprietary component of the CliniMACS System consisting of a plastic column housing with polypropylene frits in each end. The interior of the column housing is filled with a matrix of sub-millimeter iron beads coated with a heat-cured biocompatible resin. The columns are placed at appropriate locations in the CliniMACS Tubing Set to facilitate the cell selection process. The first column serves as a device to remove components that bind non-specifically to the column. The second column which is placed within a magnetic field performs the actual cell selection. The columns are incorporated sterile as part of the tubing set and are intended for single use only.

The tubing element consists of a series of tubes, connectors, spikes, Luer locks, and collection bags. The tubing of the tubing element is comprised of materials that have been qualified for use in this application by testing to ISO 10993. The principal constituents are polyvinyl chloride (PVC) and silicone. The connectors are made of various polymers (e.g., ABS and PVC) suitable for use in a blood contact environment. They are solvent bonded to the PVC tubing. The silicone pump tubing is softened with petroleum ether for manufacturing and mechanically fixed to connectors. The cell wash bags are composed of PVC.

The CliniMACS Tubing Set is packaged in a thermoformed tray and heat sealed with a Tyvek® lid. The CliniMACS Tubing Set is sterilized by ethylene oxide gas in a validated sterilization cycle and supplied as a single-use component for the CliniMACS Instrument.

5.2.4.3 CliniMACS CD34 Reagent

The CliniMACS CD34 Reagent is a dark amber, nonviscous, colloidal solution containing the antibody conjugate in buffer. The conjugate consists of a monoclonal antibody towards the human CD34 antigen. The murine monoclonal IgG1 antibody is covalently linked to dextran beads having an iron oxide/hydroxide core. The concentration of the conjugate is equivalent to 20 micrograms (µg) per mL of antibody protein, 800 µg/mL of dextran and 800 µg/mL of iron. The colloid is buffered in a phosphate-buffered saline (PBS) containing
ethylenediaminetetraacetic acid (EDTA) and Poloxamer 188. The nominal concentrations of its components are 0.0095 M phosphate, 0.004 M potassium, 0.163 M sodium, 0.139 M chloride, 0.005 M EDTA and 0.03 % (w/v) Poloxamer 188. The pH is 7.4 - 7.7. Poloxamer 188 is added to the CliniMACS CD34 Reagent to stabilize it during shipping, handling and storage. The CliniMACS CD34 Reagent is supplied sterile and pyrogen-free in glass vials containing 7.5 mL and is intended for single use and in vitro use only.

5.2.4.4 The CliniMACS PBS/EDTA Buffer

The CliniMACS PBS/EDTA Buffer is an isotonic and isohydric buffer solution with a pH-value of 7.2 and osmolarity of 290 mosmol/L. Its formulation is shown in the following table.

Table 1 Formulations of the CliniMACS PBS/EDTA Buffer

Ingredient	Compendial	Amount
NaCl	Ph. Eur.	8.0 g/L

KCl	Ph. Eur.	0.19 g/L
Na2HPO4 anhy.	Ph. Eur.	1.15 g/L
KH2PO4	Ph. Eur.	0.19 g/L
Na2EDTA	Ph. Eur.	0.37 g/L
Water for Injection	Ph. Eur.	ad 1L

The CliniMACS PBS/EDTA Buffer is used as external wash and transport fluid for the in vitro preparation of human heterogeneous cell populations intended to be separated with the CliniMACS Cell Selection System

5.3 Other Drugs

5.3.1 Filgrastim/ Granulocyte-Colony Stimulating Factor (Neupogen®)
Supplied as: 300 mcg/ml; 1 ml vial (300 mcg) and 1.6 ml vial (480 mcg); 300 mcg/0.5 ml pre-filled syringe; 480 mcg/0.8 ml pre-filled syringe.
Storage and Stability: Store in a refrigerator (2-8°C). Do not freeze. If inadvertently the filgrastim is exposed to freezing temperatures for up to 24 hours, it may be thawed and refrigerated for use. Avoid shaking. Filgrastim may be allowed to reach room temperature for 24 hours prior to use.
Preparation: For IV infusion, dilute filgrastim in 25-50 ml D5W.
The minimum concentration must not be less than 5 mcg/ml.
If the final concentration of filgrastim in solution is between 5-15 mcg/ml, albumin 2 mg/ml must be added to the solution prior to addition of the drug.
Stability (IV) once diluted in 25-50 ml of D5W, filgrastim is stable for 7 days.
Stability (plastic syringe) filgrastim is stable for two weeks in BD 1 ml plastic TB syringes at 2-8°C.
For the prevention/treatment of chemotherapy induced neutropenia, the dose of filgrastim is standardized per body weight: ≤ 60 kg = 300 mcg daily subcutaneously; > 60 kg = 480 mcg subcutaneously daily.
Clinical Considerations: If being administered as an intermittent IV infusion, it should be administered via an infusion control device and administered over a 15- 30 minute period.
Incompatibilities: The drug may precipitate in the presence of Normal Saline. Do not mix with any other drugs.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

1) Patients who are considered candidates for an allogeneic stem cell transplantation as treatment for any of the following hematologic disorders:

a) Acute Leukemia

b) Myelodysplastic syndrome

c) Other myeloproliferative disorder (i.e. myelofibrosis, chronic myelomonocytic leukemia, or chronic myelogenous leukemia)d) Non Hodgkins Lymphoma

e) Hodgkins Disease

f) Multiple Myeloma

2) Age includes from birth to < 75 years old.

3) Patients must have a Karnofsky (adult) or Lansky (pediatric) Performance Status ≥ 70%

4) Patients must have adequate organ function measured by:
Cardiac: asymptomatic or if symptomatic then LVEF at rest must be > 40%
Hepatic: < 5x ULN ALT and < 1.5 total serum bilirubin, unless there is congenital benign hyperbilirubinemia.
Renal: serum creatinine <1.5 mg/dl or if serum creatinine is outside the normal range, then CrCl > 40 ml/min (measured or calculated/estimated)
Pulmonary: asymptomatic or if symptomatic, DLCO > 40% of predicted (corrected for hemoglobin).

6.3 Subject Exclusion Criteria

1) Female patients who are pregnant or breast-feeding.

2) Active viral, bacterial or fungal infection

3) Patient seropositive for HIV-I/II; HTLV -I/II

4) Presence of leukemia in the CNS

5) Candidate for a protocol of higher priority. For the purpose of this study, the following protocols will be considered of higher priority: 10-051.

6.4 Donor Inclusion Criteria

1) HLA compatible related or unrelated donor, (i.e. a fully matched unmanipulated grafts or 1-2 HLA allele disparate donor for CD34 selected grafts).

2) Meets criteria outlined in the FACT-approved SOP for "DONOR EVALUATION AND SELECTION FOR ALLOGENEIC TRANSPLANTATION" in the Blood and Marrow Transplant Program Manual, document E-1 (see attached, or link to URL: http://mskweb5.mskcc.org/intranet/html/80312.cfm.)

3) Donor must have adequate peripheral venous catheter access for leukapheresis or must agree to placement of a central catheter.

4) Wt >25kg.

6.5 Donor Exclusion Criteria

1) Evidence of active infection (including urinary tract infection, or upper respiratory tract Infection) or viral hepatitis exposure (on screening), unless only HBS Ab+ and HBV DNA negative.

2) Medical or physical reason which makes the donor unlikely to tolerate or cooperate with growth factor therapy and leukapheresis.

3) Factors which place the donor at increased risk for complications from leukapheresis or G-CSF therapy (e.g., autoimmune disease, sickle cell trait, symptomatic coronary artery disease requiring therapy).

4) Pregnancy (positive serum or urine β-HCG) or breastfeeding. Women of childbearing age must avoid becoming pregnant while on the study.

7.0 RECRUITMENT PLAN

Eligible patients will be identified through the weekly BMT review meeting. Once transplant dates and collection dates are set they will be offered this trial and informed of the rationale for this trial as well as the logistic implications and risks and benefits. Related donors of patients identified for this protocol can have their cells cryopreserved prior to conditioning. Patients randomized into the fractionated arm will be advised that if the donor fails to collect the appropriate number of cells they will not be randomized but continued to be treated on protocol and receive all their cells as a bulk infusion.

Those patients who were randomized into either the bulk or fractionated arm and failed to collect the appropriate number of cells will be replaced until both arms have reached the target accrual.

Patients who are enrolled on study and are not subsequently transplanted on protocol will be removed from the study and replaced.

8.0 PRETREATMENT EVALUATION

8.1. Pretransplant evaluation of the patient:

The patient will receive an extensive medical evaluation within approximately 45 days prior to starting treatment. Tests outside of the 45 day window only need to be repeated if clinically indicated. This evaluation includes:

- Complete physical exam and medical history
- Dental evaluation (not required within 45 day window).
- CBC PT/PTT/INR
- Blood Type and screen (not required within 45 day window).
- Serum chemistries including BUN, creatinine, electrolytes, glucose, total protein, albumin, liver function tests (AST, ALT, bilirubin, alkaline phosphatase). Infectious disease markers will be performed as per each department"s guidelines or at the discretion of the treating attending.
- Pregnancy test for women of childbearing age
- Bone marrow aspirate (biopsy if clinically indicated)
- Urinalysis
- Electrocardiogram, echocardiogram or a gated pool scan if needed
- Pulmonary function test for patients older than 7 years
- Chest X-ray and other types of scans (CT scan and PET scan, if needed)
- Samples of bone marrow and/or peripheral blood cells will be obtained to define donor/host genetic differences and to determine engraftment of donor cells (not required within 45 day window).

9.1 TREATMENT/INTERVENTION PLAN

9.2 Selection of Cytoreduction Regimen

Patients eligible for this protocol include individuals with a variety of hematologic disorders who fulfill eligibility requirements and consent to treatment. Since the primary endpoint of this study is safety of fractionated stem cell infusion and neutrophil recovery the selection of the conditioning regimen and GVHD prophylaxis will be left to the discretion of the transplant attending in consultation with the

Adult BMT Transplant Planning Meeting. Patients will be eligible as long as the conditioning regimen and GVHD prophylaxis regimen are among the regimens considered acceptable for this protocol. Refer to Appendix 3 for regimen roadmaps.

Note (for all regimens): If scheduling issues arise, 0, 1, or 2 days of rest are allowable. Schedules for each regimen are delineated in appendix 3. Preferred schedule is per appendix.

Acceptable Regimens for Recipients of NON TCD Grafts

9.1.2 Reduced Intensity Conditioning Regimens

A. Fludarabine and busulfan (Flu/Bu)

The recommended Flu/Bu regimen is the following:

- Days -5 to -2: Flu (30-40 mg/m^2/day, total dose of 120-160 mg/m^2)
- Days -5 to -4: Busulfan (3.2mg/Kg IV, total dose of 6.4mg/Kg)

The sequence of fludarabine and busulfan administration in RIC regimens will be done according to institutional standards as long as the prescribed doses are the same as the recommended regimen above.

B. Fludarabine and melphalan (Flu/Mel)

The recommended Flu/Mel is the following:

- Days -5 to -2: Flu (30 mg/m^2/day, total dose of 120 mg/m^2)
- Day -2: Mel (140mg/m^2 or 100 mg/m2 for patients over the age of 65)

The sequence of fludarabine and melphalan administration in RIC regimens will be done according to institutional standards as long as the prescribed doses are the same as the recommended regimen above.

Myeloablative Conditioning Regimens

C. Fludarabine and busulfan (Flu/Bu)

The recommended Flu/Bu regimen is the following:

- Days -5 to -2: Flu (30-40 mg/m^2/day, total dose of 120-160 mg/m^2)
- Days -5 to -3: Busulfan (3.2mg/Kg IV, total dose of 9.6 mg/Kg)

The sequence of fludarabine and busulfan administration in RIC regimens will be done according to institutional standards as long as the prescribed doses are the same as the recommended regimen above.

D. Fludarabine and Busulfan (Flu/Bu)

The recommended Bu/Flu regimen is the following:

- Days -5 to -2: Busulfan (Targeted Bu according to institutional guidelines IV doses of 3.2 mg/Kg/day; total dose of 12.8 mg/Kg)
- Days -5 to -2: Flu (30-40 mg/m^2/day, total dose of 120-160 mg/m^2)

The sequence of busulfan and fludarabine administration in MAC regimens will be done according to institutional standards as long as the prescribed doses are the same as the recommended regimen above.

E. Busulfan and cyclophosphamide (Bu/Cy)

The recommended Bu/Cy regimen is the following:

- Days -7 to -4: Busulfan (Targeted Bu according to institutional guidelines or 3.2mg/Kg/day IV, total dose of 12.8mg/Kg).
- Days -3 to -2: Cy (60 mg/Kg/day, total dose of 120mg/Kg).

F. Cyclophosphamide and total body irradiation (Cy/TBI)

The recommended Cy/ TBI regimen is the following:

- Days -7 to -4: TBI (1200-1420 cGy)
- Days -3 to -2: Cy (60 mg/Kg/day, total dose of 120 mg/Kg)
- Palifermin per institutional standards

The sequence of cyclophosphamide, TBI and TBI administration practices in MAC regimens will be done according to institutional standards as long as the prescribed doses are the same as the recommended regimen above.

G. Busulfan and Melphalan (Bu/Mel)

The recommended Bu/Mel regimen is the following:

- Days -8 to -5: Busulfan (Targeted Bu according to institutional guidelines or 3.2mg/Kg/day IV, total dose of 12.8mg/Kg).
- Days -4 to -2: Melphalan (40 mg/m^2/day, total dose of 120mg/m^2).

Recommended Regimens for Recipients of T cell depleted Grafts

Myeloablative Conditioning Regimens

H. Busulfan, Melphalan and Fludarabine (Bu/Mel/Flu)

The recommended Bu/Mel/Flu regimen is the following:

- Days -9 to -7: Busulfan (Targeted Bu according to institutional guidelines or 3.2mg/Kg/day IV, total dose of 9.6mg/Kg).
- Days -6 to -5: Melphalan (70 mg/m^2/day, total dose of 140mg/m^2).
- Days -6 to -2: Fludarabine (25 mg/m^2/day, total dose 125 mg/$m^{2)}$.

The sequence of busulfan and fludarabine administration in MAC regimens will be done according to institutional standards as long as the prescribed doses are the same as the recommended regimen above.

I. Total Body Irradiation, Thiotepa, and Cyclophosphamide (TBI/Thio/Cy)

The recommended TBI/Thio/Cy regimen is the following:

- Days -9 to -6: TBI (1200-1420 cGy)
- Days -5 to -4: Thiotepa (5 mg/kg/day, total dose of 10mg/kg).
- Days -3 to -2: Cylophosphamide (60 mg/kg/day, total dose 120 mg/kg$^{)}$.
- Palifermin per institutional standards

Thiotepa may be given as one dose of 10 mg/kg if scheduling requires.
1200-1420 cGy hyperfractionated total body irradiation (depending on age, stage of disease and requirement of general anesthesia) with lung shielding, followed by thiotepa (5 mg/kg/day x 2 or 10 mg/kg/day x 1) and cyclophosphamide (60 mg/kg/day x 2).

Antithymocyte globulin (ATG): All recipients of a T cell depleted transplant or an unmodified transplant from a mismatched sibling or unrelated donor will also receive antithymocyte globulin

(ATG) (thymoglobulin 2.5 mg/kg/day x 2 or equine ATG 15 mg/kg/day x 2 or 30mg/kg/day x 1 on Days -3 and -2 if thymoglobulin is not tolerated) during pre-transplant conditioning to deplete radiation or chemotherapy resistant host T-cells that could hamper engraftment.Recipients of HLA-non-identical transplants will receive an additional dose of ATG. If patient is receiving a second transplant from the same donor, ATG administration will be at the discretion of the physician.

Conditioning Regimen Administration

Ideal Body Weight Formulas:

Males IBW = 50 kg + 2.3 kg/inch over 5 feet
Females IBW = 45.5 kg + 2.3 kg/inch over 5 feet

Adjusted Ideal Body Weight Formula:

AIBW = IBW + [(0.4) x (ABW - IBW)]

Busulfan administration

RIC Regimens:
Busulfan can be administered intravenously once daily. Pharmacokinetic analysis with the intent of dose adjustment is not required for busulfan with total doses lower than 9 mg/kg.

Adjusted body weight should be used for calculating the doses if patient"s actual weight is > 125% of ideal body weight.

Intravenous Bu is administered at a total dose of 3.2 mg/kg/day or 130 mg/m^2/day on Days -7, -6, -5 and -4 either in four divided doses (0.8 mg/kg) or once daily (3.2 mg/Kg or 130 mg/m^2). Target dosing through pharmacokinetic assays for Bu intravenous administration is not required under the protocol. Adjusted body weight should be used for calculating the doses if patient"s actual weight is > 125% of ideal body weight.

Melphalan administration

Melphalan will be infused once daily intravenously to a total dose not greater than 150 mg/m^2. Dose of melphalan might be reduced to 100 mg/m^2 at the discretion of the treating physician, in the setting of renal insufficiency or other comorbidities. Adjusted body weight should be used for calculating the doses if patient"s actual weight is > 125% of ideal body weight.

Cyclophosphamide administration

Cyclophosphamide (Cy) will be administered on Day -3 and Day -2 at a dose of 60 mg/kg per day IV. Adjusted body weight should be used for calculating the doses if patient"s actual weight is > 125% of ideal body weight.

Fludarabine administration

Fludarabine will be administered intravenously at a minimum total dose of 120 mg/m^2 divided into three or more daily doses according to institutional practices. Since patients with creatinine clearance < 50 ml/min/1.73m^2 are not eligible for this trial, dose adjustment for renal function will not be performed.

Total body irradiation administration (TBI)

Fractionated TBI will be administered according to the schedules utilized by the participating clinical centers. Radiation sources, dose rates, details of lung shielding, and sites receiving boost radiation will also be defined by the institution. TBI may be delivered from either linear accelerator or Cobalt sources.

Additional Drugs

Allopurinol

Allopurinol is recommended for patients with high tumor bulk. Eligible patients in this protocol are on remission or have low burden of disease. Thus the utilization of allopurinol will be done according to institutional guidelines. A common regimen employs allopurinol at the daily dose of 300 mg, beginning at least six hours before the start of conditioning and until the day before marrow or PBSC infusion.

GVHD Prophylaxis Regimen

Only the following GVHD prophylaxis regimens are allowable on this study.

a) Calcineurin inhibitor (tacrolimus or cyclosporine) that is continued for a minimum of 6 months (therapeutic blood levels for a minimum of 4 months) in addition to mini dose methotrexate or sirolimus.
b) Ex-vivo T-cell depletion with CD 34 selection using the CliniMACS device.

Recommendations for the administration of the GVHD agents are listed as follows:

Tacrolimus treatment regimen

Tacrolimus is administered beginning at least one day before transplantation for a minimum of six months. The initial dose should be based on the ideal body weight of the recipient. Subsequent doses are based on blood levels. Determinations of blood levels should be performed at least once weekly for the initial three months. Dose reductions should be made if toxicity is present or whole blood levels are above the recommended range, in the absence of toxicity. Dose reductions for high levels without toxicity should be conservative, e.g. 25%, to avoid inadequate immunosuppression.

The tacrolimus regimen for GVHD prophylaxis will initially employ an intravenous total daily dose of 1.3 mg/kg/day. Subsequent tacrolimus doses are adjusted to target whole blood levels between 5 and 15 ng/mL. When a patient is switched from intravenous to oral tacrolimus, the dose is increased by 3-4 fold to adjust for the lower bioavailability of oral compared to intravenous tacrolimus

If there is nausea and vomiting, the drug should be given intravenously. Patients with severe intolerance of tacrolimus may be placed on cyclosporine.

Drugs that may affect tacrolimus levels are:

1. Caspofungin, phenobarbital, phenytoin, rifampin, carbamazepine, rifabutin, St. John"s Wort (lowers levels);
2. Glucocorticoids, fluconazole, voriconazole, ketoconazole, itraconazole, grapefruit juice, amprenavir, bromocriptine, chloramphenicol, cimetidine, cisapride, clarithromycin, clotrimazole, danazol, diltiazem, erythromycin, ethinyl estradiol, metoclopramide, metronidazole, mibefradil, nefazodone, nelfinavir, nifedipine, omeprazole, quinupristin/dalfopristin, ritonavir, saquinavir, theophylline, troleandomycin, verapamil (increases levels). For patients who are taking both tacrolimus.

Per the tacrolimus package insert, when initiating therapy with voriconazole in patients already receiving tacrolimus, it is recommended that the tacrolimus dose be reduced to one-third of the original dose and followed with frequent monitoring of the tacrolimus blood levels. Increased tacrolimus levels have been associated with nephrotoxicity. When voriconazole is discontinued, tacrolimus levels should be carefully monitored and the dose increased as necessary.
Dose adjustments by age are recommended as follows:

< 6 yr: 0.04 mg/kg/24 hr
≥ 6 yr: 0.03 mg/kg/24 hr

Cyclosporine treatment regimen
The cyclosporine regimen for GVHD prophylaxis will initially employ an intravenous total daily dose of 3 mg/kg/day. Subsequent cyclosporine doses are adjusted to target whole blood levels between 150 and 450 nano (n)g/mL.

Oral formulations of cyclosporine have variable bioavailability (intestinal absorption), and Neoral appears to have a higher and more predictable bioavailability than other formulations. When a patient is switched from intravenous cyclosporine to Neoral (preferred) or other oral formulation, the dose is increased by 2.5-3 fold to adjust for the lower bioavailability of Neoral compared to intravenous cyclosporine.

Drugs that may affect cyclosporine levels:

1. Caspofungin, phenobarbital, phenytoin, rifampin, carbamazepine, rifabutin, St. John"s Wort (**lowers levels**)
2. Glucocorticoids, fluconazole, voriconazole, ketoconazole, itraconazole, grapefruit juice, acetazolamide, amiodarone, amlodipine, amprenavir**,** bromocriptine, chloramphenicol, cimetidine, cisapride, clarithromycin, clotrimazole, danazol, diltiazem, erythromycin, ethinyl estradiol, metoclopramide, metronidazole, mibefradil, nefazodone, nelfinavir, tacrolimus, nifedipine, omeprazole, quinupristin/dalfopristin, ritonavir, saquinavir, theophylline, troleandomycin, verapamil (**increases levels**)
3. Per the voriconazole package insert, when initiating therapy with voriconazole in patients already receiving cyclosporine, it is recommended that the cyclosporine dose be reduced to one-half of the original dose and followed with frequent monitoring of the cyclosporine blood levels. Increased cyclosporine levels have been associated with nephrotoxicity. When voriconazole is discontinued, cyclosporine levels should be frequently monitored and the dose increased as necessary.([i])

Methotrexate
The regimen of methotrexate for GVHD prophylaxis will employ intravenous doses of 5 mg per m^2 on Day 1 post-transplant, and days 3, 6, and 11 post-transplant according to institutional standards. Third space syndromes with large accumulation of ascites or pleural effusions are a contraindication to the use of methotrexate. Dose reductions should be made for renal, hepatic and mucosal toxicity. Determinations of blood levels are indicated 24-72 hours after administration in patients with impaired renal function. Leucovorin rescue should be considered in patients with decreased clearance, severe toxicity or fluid accumulation/effusions.

Drugs that may increase methotrexate levels are:

1. Non-steroidal anti inflammatory drugs
2. Penicillins
3. Diuretics

Post transplant supportive care will follow institutional and BMT guidelines

Tacrolimus/Sirolimus and mini-methotrexate

Adults: *Sirolimus* will be given in a loading dose of 12 mg orally on Day –3 followed by a daily oral dose of 4 mg per day. Doses may be repeated if the subject vomits within 15 minutes of an oral dose.

Children: Children weighing < 40.0 kg will be given an oral loading dose of sirolimus of 3 mg/m2 followed by a daily oral dose of 1 mg/m2, rounded to the nearest full milligram.

Adults and Children: *Tacrolimus* will be given at a dose of 0.02 mg/kg every 24 hours as a continuous intravenous infusion beginning on Day –3. Convert the tacrolimus to oral dosing at 2-3 times the total 24-hour intravenous dose, split into 2 doses given every 12 hours as soon as clinically feasible.

Methotrexate will be given at a dose of 5 mg/m2 intravenously on days +1, +3, +6, and +11 to recipients of related or unrelated donors. The day +1 dose should not be administered until 24 hours after the BM or PBSC infusion. All doses of methotrexate should be administered unless life-threatening complications prevent administration. The BMT attending will determine if any MTX dose adjustments are necessary. IV folinic acid is NOT given after mini-methotrexate.
Methotrexate dose adjustment will be as follows:

HOLD: for Creatinine that doubles from admission AND is >2.0 mg/dl OR Creatinine >3.0 mg/dl (regardless of baseline

HOLD: for total Bilirubin > 3.0 mg/dl
MTX DOSE ADJUSTMENTS FOR WEIGHT: Use actual body weight for patients with BSA < 2.5 m2, and adjusted body weight for BSA > 2.5 m2.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

HSCT evaluations are summarized in the following table. Scheduled evaluations for D30 may be performed +/-7 days, for D60 and D90 may be done on +/-7 days, for 6 months may be done +/-14 days, and for 12 months may be performed +/-30 days of the targeted date. Evaluations may be withheld if the treating physician feels that there is a strong contra-indication to perform the study (e.g. patient has relapsed and is terminally ill). Also, additional tests will be performed as clinically indicated.

ACTIVITY		**D0-D30**	**D60, D90, D180 EVALUATION**	**D360 (12 MONTHS)**
Karnofsky score			D60, D90, D180	X
History and physical			D60, D90, D180	X
Toxicity Assessments		Weekly	D60, D90, D180	X
Comprehensive Metabolic Panel		Weekly	D60, D90, D180	X
Counts/differential		Weekly	D60, D90, D180	X
Disease evaluation, Bone marrow aspirate with chimerism		D30 (core if clinically indicated)		X
Chimerism: blood			D60, D90,	X

			D180	
Chimerism: T cells (ARC)		D30	D90	
GVHD evaluation		Weekly	D60, D90, D180	X
Imaging studies for disease evaluation, when clinically indicated (CT Scan, PET Scan)				X
Immune Recovery		D30	D90, D180	X

10.1 Post-transplant evaluation

Post transplant care will follow the Adult BMT Service standard of care guidelines. In brief:

1) Laboratory Evaluations and Protocol Specific Evaluations. The table above shows the approximate dates for tests and procedures performed after transplant for this protocol.

10.2 Assessment of Immune-recovery

For this study immune-recovery will be assessed by absolute lymphocyte count on Day 15 (+/- 24 hrs), day 30, and time to lymphocyte count of 500.

Quantitative recovery of T cell subpopulations will be assessed by flow cytometry, through 2 panels of markers, which are currently used in the clinical laboratory performed on D30, 90, 180 and at 12 months post stem cell transplant: (ref)[83]

Per BMT SOC

1) CD3+ T cells and 2 subsets CD3+CD4+ and CD3+CD8+.
2) CD16+56+ NK cells.
3) CD19+ B cells.

11.1 TOXICITIES/SIDE EFFECTS

Toxicities will be graded on a scale of 0 to 4 as described by the NCI-Common Terminology for Adverse Events (CTCAE), version 4.0.

Side effects common to all transplant procedures include:

<u>Likely</u>

- Nausea and vomiting
- Infusion reactions including fever and rash
- Mouth sores
- Diarrhea
- Change in taste
- Poor appetite.

- Weight loss
- Low red cell count requiring red cell transfusions
- Low platelet count increasing risk of bleeding and requiring platelet transfusions.
- Weakened immune system with risk of viral, bacteria or fungal infection
- Some degree of graft versus host disease: Graft-versus-host disease (GVHD) is a frequent problem after unrelated donor transplantation. GVHD can cause skin rashes, nausea, vomiting, severe diarrhea and liver problems.
- GVHD can occur as an early (acute form) or late chronic form. Chronic GVHD involves problems with the eyes, mouth, lips, throat and liver.

Possible

- Recurrence of the original malignancy
- Reversible damage to heart, lung, liver and kidney

Unlikely

- Poor graft function and failure of the donor cells to work properly.
- Severe irreversible liver, heart, kidney or lung failure.
- Serious uncontrolled infection.

Infusional Toxicities Include:

Likely

- Changes in heart rate and/or rhythm
- changes in blood pressure
- fever
- chills
- sweats
- headache

Unlikely

- Nausea
- vomiting,
- diarrhea
- abdominal cramping

Extremely Rare

- Hemoglobinuria (blood in the urine)
- acute renal failure
- allergic reactions
- respiratory dysfunction

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Definition of events in the post-transplant course important for analysis and treatment:

12.1 Engraftment and Chimerism

Engraftment will be documented by analysis of blood cells, T cells and bone marrow cells for chimerism by standard cytogenetic studies at about 1 month and as stated in Section 10.0

12.2 Graft failure or rejection
Primary non-engraftment is diagnosed when the patient fails to achieve an ANC ≥500/㎕ at any time in the first 28 days post-transplant. (30,31)

Patients who suffer graft failure will be considered for a secondary transplant. The need for additional immunosuppression or treatment for viral infection prior to the secondary transplant will be determined by the results obtained from chimeric and viral studies.

For patients who are engrafted with donor cells but have severe cytopenia affecting one or more blood cell lineages, secondary transplants of CliniMACS fractionated CD34+ T-cell depleted PBSCs may be administered to booster and replenish donor hematopoietic cells without conditioning or after treatment with anti-thymocyte globulin. If at any time, a patient receives a secondary transplant they will be taken off study. These patients will be followed for survival.

12.3 Graft-versus-host disease
Standard BMT-CTN and IBMTR systems clinical criteria as defined by Rowlings, et al will be used to establish and grade acute GvHD.(32)

Patients will be observed for acute and/or chronic GvHD as long as they have not received donor derived leukocytes infusions (DLI) for the treatment of relapse or infections. If at any time, a patient receives DLI they will be taken off study. These patients will be followed for survival.

12.4 Regimen-related and transplant-related mortality

Regimen related toxicity (RRT) refers to those toxicities that can be attributed directly to the preparative regimen (including radiation, chemotherapeutic agents and ATG).

Transplant-related mortality (TRM) includes the RRT and other fatal complications resulting from the allogenic transplant such as graft failure, GvHD, hemorrhages, and infections. The grading for monitoring transplant related toxicities will be based on the NCI/CTEP common toxicity criteria (57). This will include assessment of severity and duration of oral mucositis and sequelae specifically parenteral opioid analgesic use, TPN use, febrile neutropenia, hospital days and intubation.

12.5 Infections
The occurrence of life-threatening opportunistic infections will be evaluated according to the criteria established by BMT CTN (see Appendix 1) and will correlate this with the level of immune recovery. The infection-related mortality will be also determined. Patients will be considered to have died from infection if death is attributed to a recent severe infection and/or infection was identified at autopsy. Patients with relapsed disease before death will be excluded from the above definition, even if an infection was the final cause of death.

12.6 Disease relapse
Relapse of MDS, AML and AML will be defined by an increasing number of blasts in the marrow over 5%, by the presence of circulating peripheral blasts, or by the presence of blasts in any extramedullary site. Cytogenetic analysis of the marrow and/or peripheral blood will also be obtained for the diagnosis of relapse. Other disease relapses will be scored by clinical, laboratory and pathologic criteria.

12.7 Disease-free Survival
DFS is defined as the minimum time interval of times to relapse/recurrence, to death or to the last follow-up, from the time of transplant.

12.8 Overall Survival

Overall survival is defined as time from transplant to death or last follow-up.

12.9 $CD34_+$ and $CD3_+$ Cell Doses

Total $CD34_+$ and $CD3_+$ cell doses will be calculated based on results of flow cytometric analysis.

12.10 Immune reconstitution Parameters

For this study immune-recovery will be assessed by absolute lymphocyte count on day 15, 30 and time to lymphocyte count of 500 as noted above in Section 10.2.

13.0 CRITERIA FOR REMOVAL FROM STUDY

If at any time the patient is found to be ineligible for the protocol as designated in the section on Criteria for patient/subject eligibility (e.g. a change in diagnosis), the patient will be removed from the study. Also patients may be removed from the study if requested by the patient. Management will depend on where they are in their treatment course. Such patients will receive appropriate supportive care. The PI may also remove patients from the study for noncompliance.

14.0 BIOSTATISTICS

This randomized phase 2 study is designed to explore the effects of CD34+ dose on hematopoietic recovery for patients with myeloid leukemias undergoing an allogeneic stem cell transplant. The primary endpoint of the study is the time to engraftment. Patients will be randomized to one of two groups.

Group 1: Patients targeted to receive 8 x 10e6 CD34+ cells per kg on the day of the transplant. Group 2: Patients targeted to receive 4 x 10e6 CD34+ cells per kg on the day of the transplant and then receive three supplemental infusions on days 2, 4, and 6 post SCT. The three supplemental infusions will contain a total of 4 x 10e6 CD34+ cells per kg. In addition, patients will be stratified by type of transplant (T cell depletion vs no T cell depletion).

Each treatment group will accrue 36 patients. To test whether the time to engraftment differs between the randomized groups, the times are ranked after pooling the data in the two treatments in each transplant type separately. Patients who have not engrafted by day 43 will be assigned a day 43 time to engraftment. A stratified Wilcoxon rank sum test statistic (van Elteren test) will be used for this comparison. The test will be stratified by the type of transplant received (T cell depletion vs no T cell depletion).

A power function for the rank sum test is computed from the Lehmann family of alternative hypotheses, using a single parameter, the ratio of the expected time to engraftment for patients receiving the single cell dose (group 1) relative to the expected time to engraftment for patients receiving multiple cell doses (group 2). It is projected that the expected time to engraftment is 11 days for patients in the single cell dose group and this projection decreases to 7 days for the multiple cell dose group. Based on 36 patients in each group, the Lehmann alternative power function for a one-sided 0.20 level test has power equal to 0.80 based on these projections (Heller 2006). The choice of a 0.20 significance level is intended to reduce the sample size required for this two group comparative study. A significant outcome does not imply definitive evidence of superiority for one treatment group due to this high type 1 error, but instead provides sufficient evidence that testing of this treatment should proceed to a larger multicenter comparative study (Korn et al, 2005).

To insure patient safety each of the treatment groups will be monitored for grade 3-4 graft versus host disease, graft failure, and non-relapse mortality. The same stopping boundaries will be used for both groups. In the event that the stopping boundary is crossed for one of the two randomized treatments, the study will be terminated.

Failure type	# of failures needed to stop the study	Failure rate in the population	Probability boundary is crossed
One-year NRM	3 in the first 19 patients 4 in the first 31 patients 5 within 36 patients	0.05	0.10
		0.20	0.92
Primary Graft failure	2 within the first 14 patients 3 within the first 31 patients 4 during the study	0.03	0.10
		0.15	0.90
Acute GvHD (grades 3-4)	3 in the first 19 patients 4 in the first 31 patients 5 within 36 patients	0.05	0.10
		0.20	0.92

The following secondary endpoints will be compared between randomized groups:

- Day 30 ANC, CD4 and CD8 counts.
- The area under the hematopoietic recovery curve for the factors: ALC, CD4, CD8, and platelet count. The area under the curve will be computed based on recordings at days 30, 60, 100, 180, 365.
- The time to ALC 500, and the time to first bacterial, viral, and fungal infection will be computed using the cumulative incidence function and a comparison between groups will be undertaken using Gray"s test.
- Kaplan-Meier estimates of overall and disease free survival over time will be computed.
- Secondary Graft Failure

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System..

15.3 Randomization

After eligibility is established and immediately after consent is obtained, patients will be registered in the Protocol Participant Registration (PPR) system and randomized using the Clinical Research Database (CRDB) by calling the MSKCC PPR Office at 646-735-8000 between the hours of 8:30 am and 5:30 pm, Monday - Friday. Randomization will be accomplished by the method of random permuted block and patients will be stratified by the type of transplant (T cell depletion vs no T cell depletion).

At the time of registration, patients will be randomized to either receive all cells in bulk on day 0 (GROUP 1) or receive 4 x 10e6 CD34+ cells/kg on day 0 with the rest of the cells being infused on days 2, 4, 6 in equally distributed aliquots (GROUP 2). At least 7 x 10e6 cells/kg recipient weight must be collected for both recipients of T cell depleted transplants and non T cell depleted transplants randomized on to the fractionated arm. Patients randomized into the fractionated arm will be advised that if the donor fails to collect the appropriate number of cells they will not be randomized but continued to be treated on protocol and receive all their cells as a bulk infusion.

Those patients who were randomized into the fractionated or bulk arm and failed to collect the appropriate number of cells will be replaced until both arms have reached the target accrual.

16.1 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study and will be responsible for both pediatric and adult accruals. The responsibilities of the RSA and the principal investigator include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate activities of the protocol study team.

The data collected for this study will be entered into the Clinical Research Database (CRDB), a secure database. Source documentation will be available to support the computerized patient record.

16.2 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.3 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials” which can be found at: http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: http://mskweb2.mskcc.org/irb/index.htm

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: Data and Safety Monitoring Committee (DSMC) for Phase I and II clinical trials, and the Data and Safety Monitoring Board (DSMB) for Phase III clinical trials, report to the Center"s Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) Will be addressed and the monitoring procedures will be established at the time of protocol activation.

17.1 PROTECTION OF HUMAN SUBJECTS

This study will explore the potential benefits of infusing stem cells over various days instead of in bulk as is currently the standard. The study was conceived based on observations made in animal models and has a rationale basis supported by the potential improvement in engraftment that could occur if stem cells are infused at a time when marrow stem cell niches have more likely recovered from the effects of chemotherapy.

Aside from the increase chances of having an infusional toxicity and the exposure to DMSO we do not foresee any added risk to the patients who are randomized to the fractionated stem cell infusion arm. The potential benefit is a more robust hematologic and immunologic recovery which could potentially have significant impact on transplant morbidity and mortality.

This study protects the rights of human subjects because it has carefully devised stopping and safety rules, the consent form clearly specifies the investigational intervention and describes all potential side effects and toxicities. Adverse event reporting follows the current MSKCC guidelines for transplant studies.

Participation in this study is voluntary. All patients will be required to sign a statement of informed consent which conforms to MKSCC IRB guidelines and explains the risks and potential benefits of this study.

The patient"s health plan/insurance company will need to pay for all of the costs of treatment in this study. The patient will be responsible for the costs of standard medical care, all hospitalizations and any transplant complications. Pre-authorization for the transplant will be cleared with the health plan/insurance company prior to admission. Patients will not be paid for taking part in this study.

17.2 Privacy

MSKCC"s Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.3 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event

- An adverse event that results in 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 (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon 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
-

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant"s last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

If an SAE requires submission to the IRB office per IRB SOP RR-408 „Reporting of Serious Adverse Events", the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office as follows:
For IND/IDE trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org.
All other reports should be sent to saemskind@mskcc.org.

For all other trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org.
All other reports should be sent to sae@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject"s initials
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject"s condition
    - Indication if the subject remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem
-

The PI"s signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

17.2.1

For IND/IDE protocols:
The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

18.1 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

Appendix 1. BMT CTN Definitions of Infection Severity

Appendix 2. Adult and Pediatric BMT Adverse Event Reporting Guide

Appendix 3. Regimen Roadmaps

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[i] Product Package Insert Information. VFEND®, Voriconazole. Pfizer, New York, NY, USA.