A Phase II Trial of Transplants from HLA-Compatible Related or Unrelated Donors with CD34⁺ Enriched, T-cell Depleted Peripheral Blood Stem Cells Isolated by the CliniMACS System in the Treatment of Patients with Hematologic Malignancies and other lethal hematologic disorders

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a Phase II trial testing 4 disease-specific myeloablative conditioning regimens for preparatory cytoreduction of patients receiving allogeneic HLA-compatible related or unrelated transplants of GCSF-mobilized peripheral blood stem cells (PBSC) depleted of T-cells by positive selection of $CD34^+$ progenitor cells using the CliniMACS system. The CliniMACS Fractionation system is an experimental method requiring an IND that positively selects $CD34^+$ progenitor cells from PBSC by immunoadsorption of cells binding on anti CD34 monoclonal antibody to paramagnetic beads which can then be isolated by passage through a magnetized column and released by agitation of beads. Two of the four conditioning regimens have been used successfully with an alternative similar system, Isolex, which is no longer being manufactured. Although an IND is required, the CliniMACS system has been used extensively in Europe and under several INDs in the United States including IRB Protocol 06-005 at MSKCC.

The primary objectives of the trial are 1) to assess whether or not each of these cytoreductive regimens is sufficiently immuno ablative to permit consistent engraftment and hematopoietic reconstitution following allogeneic PBSC transplants depleted of T-cells by fractionation on the CliniMACS device; 2) to confirm the capacity of these transplants to reduce or prevent the development of clinically significant acute GVHD and chronic GVHD, 3) to assess the incidence of transplant-related mortality associated with such transplants when administered after each conditioning regimen at approximately day 100, 6, 12 and 24 months post transplant, and 4) to assess overall and disease free survival (DFS) at 6 months, and yearly through at least 5 years and thereafter as clinically indicated post transplant.

For this trial, patients will be stratified to receive one of four myeloablative conditioning regimens, based on their disease, stage of disease, and dose of radiation accumulated in the course of treatment. The four myeloablative conditioning regimens are: 1) Hyperfractionated total body irradiation to a dose of 1375 cGy-1500cGy (with lung shielding to a dose of 800cGy) administered in 125cGy fractions at 4-6 hour intervals three times a day for a total of 11 or 12 doses depending on age and disease risk (or, if general anesthesia is required, 150 cGy q12h x 8 doses to a total dose of 1375 cGy may be given), followed by Thiotepa 5mg/kg/day x 2 (or 10mg/kg/day x 1) and cyclophosphamide 60mg/kg/day x 2 (or fludarabine 25mg/m^2 x 5 if cyclophosphamide is contraindicated); 2) Busulfan 0.8mg/kg/dose q 6h x 10- 12 doses (depending on disease), Melphalan 70mg/m^2/day x 2, and fludarabine 25mg/m^2/day x 5; 3) Clofarabine 20mg/m^2/day x 5 (or, for children < 18yrs of age, 30mg/m^2/day x 5 if deemed suitable and with PI approval), Melphalan 70mg/m^2/day x 2, and Thiotepa 5mg/kg/day x 2 (or 10mg/kg/day x 1) or 4) Melphalan 70mg/m^2/day x 2, Fludarabine 25 mg/m^2/day x 5, and Thiotepa 5mg/kg/day x 2 (or 10mg/kg/day x 1 day). Patients will also receive anti-thymocyte globulin to eradicate host T-cells that could contribute to graft rejection.

Approximately twenty-four to forty-eight hours after completion of each conditioning regimen, patients will receive a transplant of a $CD34^+$ progenitor cell-enriched, T-cell depleted fraction of GCSF-mobilized PBSC after fractionation on the CliniMACS device, from his/her HLA compatible donor.

Each transplant will be derived from a healthy, consenting HLA compatible related or unrelated donor. The donor will receive mobilization therapy with GCSF administered as per standard practice. Leukapheresis will be performed on a continuous flow cell separator according to institutional standards commencing on approximately Day 5 of GCSF treatment. Daily leukapheresis of the donor with subsequent $CD34^+$ cell selection using the Miltenyi CliniMACS device will continue until a post-selection target of $>5.0 \times 10^6$ $CD34^+$ cells/kg recipient body weight and $< 1.0 \times 10^5$ $CD3^+$ cells/kg

recipient body weight is reached following at least two but not more than three leukapheresis procedures. There is no limit to the number of $CD34^+$ progenitors that can be administered.

No additional GVHD prophylaxis will be administered. Due to stringent T-cell depletion, no significant GVHD is anticipated. Should GVHD occur, standard treatment will be initiated- per The Pediatric and Internal Medicine Transplant Service guidelines.

The sample size is as follows:

Regimen A: TBI/Thiotepa/Cyclophosphamide: 110 patients

Regimen B: Busulfan/Melphalan/Fludarabine: 200 patients

Regimen C: Clofarabine/Melphalan/Thiotepa: 60 patients

Regimen D: Melphalan/Fludarabine/Thiotepa: 30 patients

It is anticipated that the accrual will last six years.

Patients will be followed for two years following transplantation.

2.1 OBJECTIVES AND SCIENTIFIC AIMS

Primary:

1. To assess the incidence of durable hematopoietic engraftment for T-cell depleted transplants fractionated by the CliniMACS system administered after each of the four disease targeted cytoreduction regimens.
2. To assess the incidence and severity of acute and chronic GVHD following T-cell depleted, $CD34^+$ progenitor cell enriched transplants fractionated by the CliniMACS system.
3. To assess the incidence of non-relapse mortality (transplant-related mortality) following each cytoreduction regimen and a transplant fractionated by the CliniMACS system.
4. To estimate the probability of survival and disease-free survival (DFS) at 6 months, and yearly through at least 5 years and thereafter as clinically indicated post transplant for life for each disease-targeted cytoreduction regimen when used with a T-cell depleted graft fractionated by the CliniMACS system.

Secondary:

1. To determine the proportion of patients receiving optimal $CD34^+$ ($> 5x10^6$/kg) and $CD3^+$ ($<1x10^5$/kg) cell doses the proportion recurring suboptimal doses ($< 2x10^6$/kg) $CD34^+$ cells; and the proportion of patients receiving $CD3^+$ T-cell doses $> 1x10^5$/kg.
2. To correlate doses of $CD34^+$ progenitors and $CD3^+$ T cells with engraftment, graft vs. host disease and non-relapse mortality.

3.0 BACKGROUND AND RATIONALE

Allogeneic hematopoietic cell transplantation (HSCT) is an accepted therapy for the treatment of acute lymphoblastic (ALL) and myeloblastic (AML) leukemia, and recognized as the curative treatment of choice for patients who fail to sustain an initial remission (1-4). Such transplants are also a recognized as a potentially curative treatment for myelodysplastic syndromes, advanced non-Hodgkins lymphoma

and multiple myeloma (5-9). For adults with high risk AML in 1°CR or 2°CR, unmodified HLA-matched related HSCTs have led to sustained DFS rates of 45-60% and 40-53% respectively; for patients with high risk ALL in 1° CR or 2° CR, the extended DFS rates are 45-75% and 35-40% respectively (3,4,10,11).While comparative trials of transplants vs chemotherapy in adults with high risk acute AML in first remission have consistently recorded higher DFS rates for HSCT recipients, the differences have been significant only in a minority of studies (12-15). In these studies, the decreased risk of relapse observed following an allogeneic HSCT has been offset by the increased non-leukemic mortality resulting from Graft vs Host disease and its treatment.

For patients lacking an HLA-matched sibling, the prospects of transplantation have greatly improved over the last 10 years, reflecting improvements both in the selection and the rapid mobilization of HLA compatible donors through the National Marrow Donor Program (NMDP) and the computerized worldwide network of donor centers that now includes over 12 million volunteer donors. Currently, results from single centers suggest survival rates for HLA-matched unrelated transplants administered to patients with AML and ALL in early remission are comparable to those recorded for matched sibling grafts (16, 17). Strikingly, relapse rates following unrelated grafts are lower. However, this advantage has been neutralized by the higher incidences of acute and chronic GVHD and the morbidity and mortality associated with their treatment.

Thus, the early morbidity and mortality associated with acute GVHD is a major factor limiting the success of transplantation as is the long-term morbidity associated with chronic GVHD. The risk of acute GVHD following allogeneic bone marrow transplantation (BMT) from HLA-matched siblings is 20-60% despite the use of immunosuppressive agents like cyclosporine A (CSA), tacrolimus (FK506), methotrexate (MTX), antithymocyte globulin (ATG) and corticosteroids, alone or in combination (18-21). Grades II-IV (moderate to severe) acute GVHD are associated with an increased risk of transplant-related mortality (21, 22). Mortality rates among patients who develop GVHD can be as high as 75% when that disease is unresponsive to therapy (22).

Over the last 35 years, several combinations of immunosuppressive drugs have been evaluated for their potential to prevent acute and chronic forms of GVHD when administered from immediately prior to until 6-12 months post transplant. However, despite prophylaxis with any of these regimens, 18-40% of patients receiving HLA-matched related grafts will develop grade II-IV acute GVHD, and 24-58% of those who survive over 3 months will develop chronic GVHD (18-21). In these trials, lower incidences of acute HVHD have also been invariably counter balanced by higher incidences and sensitivities of chronic GVHD. Higher rates of acute and chronic GVHD are observed following matched unrelated HSCT. Furthermore, rates of GVHD continue to be prohibitive following HLA-non-identical grafts (23, 24). Thus, immunosuppressive agents are only partially effective in reducing the incidence and severity of acute GVHD and have to date failed to decrease the incidence of chronic GVHD. Despite immunosuppressive therapy, engrafted donor T-lymphocytes derived from the hematopoietic cell graft can respond to alloantigens on the cell surface of host cells, initiating the pathologic process leading to GVHD (25).

Over the past twenty years, several techniques for T-cell depletion (TCD) of donor grafts have been introduced and evaluated for their capacity to prevent acute and chronic GVHD. These include techniques involving physical adsorption of T-cells to protein ligands such as lectins, elutriation, and immunoadsorption or immuno deletion with T-cell or lymphocyte-specific monoclonal antibodies (26-30). Comparative analyses have demonstrated that these techniques vary widely in their capacity to

deplete T-cells (1.5-3.5 log_{10} depletion) (31). The relationship between the T-cell dose transplanted and the risk of acute GVHD is complex and varies depending upon the degree of MHC-compatibility, graft source (bone marrow versus peripheral blood), type of GVHD prophylaxis (pharmacological versus T-cell depletion), method of T-cell depletion, conditioning regimen, use of in vivo depleting antibodies (33-36), as well as patient and donor characteristics including underlying disease, age, gender and parity (37-39). Our studies in more than 200 patients have suggested that the threshold dose of T-cells required to induce grade II-IV acute GVHD after HLA-matched sibling transplants is 10^5 clonable T-cells/kg (31). A 2.8-3.0 log_{10} reduction in marrow grafts or a 4.0-4.5 log_{10} reduction in peripheral blood stem cell (PBSC) grafts is required if this threshold is not to be exceeded. This likely explains the variable reductions in GVHD and inconsistent reductions in chronic GVHD observed with several techniques despite the concomitant use of CSA and MTX prophylaxis.

Certain methods of T-cell depletion are effective in reducing or preventing acute GVHD in both HLA-matched and HLA-disparate transplant recipients without co-administration of CSA and/or MTX. The first is: T-cell depletion of a marrow allograft by soybean lectin agglutination and E-rosette depletion (SBA), an approach reported by Memorial Sloan-Kettering Cancer Center (MSKCC) (26,40) and the University of Perugia (41) to be associated with a 0-5% incidence of acute GVHD in HLA-matched transplant recipients and a 0-8% incidence in HLA haplotype transplant recipients. The second is: depletion of T-cells by positive selection of $CD34^+$ hematopoietic cells from GCSF mobilized peripheral blood leukocytes (PBSC) and transplantation of these $CD34^+$ cell-enriched, T-cell depleted cell fractions (43, 44).

Two methods of positive selection of $CD34^+$ progenitors have thus far been found to yield populations of $CD34^+$ progenitor cells adequate to secure engraftment that are also sufficiently depleted of T-cells to prevent GVHD. One approach, extensively studied at MSKCC, requires two steps: a) initial isolation of $CD34^+$ cells on the Isolex device which treats the cells with a murine CD34-specific MoAb and subsequently adsorbs the antibody-coated cells to anti-mouse IGG coated paramagnetic beads. Following separation of the cells adsorbed to the beads, the cells are eluted from the beads. Thereafter, residual T-cells are depleted by removal of cells forming sheep red cell rosettes. This procedure yields a progenitor-enriched cell fraction that provides $CD34^+$ cell doses of 0.7-29.6 (median 6.6) x 10^6/kg and $CD3^+$ T-cell doses of 0.2-4.1 (median 1.4) x 10^3/kg. Such transplants, administered after total body irradiation, Thiotepa and either cyclophosphamide or fludarabine have provided consistent engraftment with rates of grade II-IV acute GVHD of 8% and 9% in recipients of HLA-matched related or unrelated donors respectively, and rates of extensive chronic GVHD of 6% and 11% respectively (44). **However, this method, while effective, will no longer be available as of April 1 2010, because Baxter is terminating the manufacturing of their Isolex system and its reagents.**

The other technique, which has been extensively employed in Europe (43, 45) but has only recently been studied in the United States, is the CliniMACS system (Miltenyi Biotec). In this system, GCSF-mobilized PBSC are introduced into the closed device, which treats the cells with anti CD34 MoAB coated paramagnetic beads. The $CD34^+$ progenitor cells are then separated from other cells by passage through an electromagnetic field, and then washed and eluted. In the large Perugia series, recipients of HLA haplotype disparate $CD34^+$ PBSC isolated on the CliniMACS device had only a 10% probability of developing acute or chronic GVHD (42). In a multicenter trial conducted by the BMT Clinical Trials Network under an FDA IND, for which Dr. O'Reilly was principal investigator, this method yielded a progenitor-enriched cell fraction that provided doses of progenitor cells ranging from 2.4 –

31.3 (med 7.9) x 10^6 $CD34^+$ cells kg and T-cell doses of 1.1-84.9 (median 6.6) x 10^3 $CD3^+$ cells/kg. HLA matched related HSCT fractionated by this approach were administered after cytoreduction with our protocol of hyperfractionated TBI, thiotepa and cyclophosphamide. These transplants provided consistent engraftment (1 late graft failure in 44 patients transplanted), and were associated with incidences of 20.5% grade 2-4 acute GVHD and 7.6% extensive chronic GVHD. Overall survival for patients with AML transplanted in 1º and 2º remission was 74% (46). Overall risk of relapse for patients is 1° or 2° CR was 18%, which compares favorably with results of unmodified transplants for these disease indications.

The highly favorable results of the BMT CTN trial, which were presented at ASH, December 2009, have now been submitted for publication and form the basis for an application to the FDA for licensure as a device to prevent GVHD in patients transplanted for AML. **As we are waiting for approval, trials of T-cell depleted $CD34^+$ PBSC transplants fractionated on the CliniMACS device will continue to require an IND.**

As noted above, over the last 9 years our transplantation program has accrued extensive experience with $CD34^+E^-$ T cell depleted PBSC transplants isolated by positive selection of $CD34^+$ progenitors on the FDA approved Isolex device followed by E. rosette depletion. Under IRB #01-070, 108 patients received transplants of HLA-matched related or HLA compatible unrelated $CD34^+E^-$ PBSC transplants fractionated by this approach after cytoreduction with TBI/THIO/Fludarabine only 2 patients had late graft failure (1.7%) (44). The incidence of acute grade 2-4 GVHD was 9% for recipients of HLA-matched related grafts and 16% for recipients of unrelated or HLA disparate transplants. Chronic GVHD of any severity developed in only 8% of the matched related and 21% of the matched or non-identical unrelated recipients. Of the patients transplanted for acute leukemias in 1º and 2º remission, over 65% are surviving disease-free at up to 9 years of follow-up.

In a separate protocol (IRB 01-055), we also evaluated T cell depleted $CD34^+E^-$ PBSC transplants fractionated on the Isolex device administered after a chemotherapeutic myeloablative cytoreduction regimen consisting of Busulfan, 0.8mg/kg/dose, every 6 hours x 10 doses, melphalan 70 mg/m²/day x 2 and Fludarabine 25 mg/m²/day x 5. The patients enrolled had advanced AML, ALL, MDS or NHL. They received transplants from matched related (N=23) and unrelated (N=34) donors or HLA non-identical related or unrelated (N=35). Of 91 patients evaluable, 88 (97%) achieved durable engraftment (graft failure rate=3%). Acute grade II-IV acute GVHD was observed in 8%; chronic GVHD in 7%. The non-relapse mortality in this heavily pre-treated patient population was 20% at 2 years. DFS at 2 years was 55%, a result which compares very favorably with published results of unmodified grafts applied to patients in similarly advanced stages of leukemia and MDS. This regimen was particularly promising for patients transplanted for advanced MDS and secondary AML, and forms the basis of a trial being conducted by Dr. Castro-Malaspina for patients with these disease indications (IRB 08-008). <u>This trial also demonstrated for the first time, that engraftment of extensively T-cell depleted hematopoietic progenitor cells could be consistently achieved, both in HLA-matched and HLA disparate patients, after a myeloablative cytoreduction regimen that did not include total body irradiation</u>.

While modifications of these protocols were planned for subsequent trials, such as the adjunctive use of KGF to reduce radiation and chemotherapy-induced injury to the thymus and thereby foster earlier immune reconstitution, the decision of Baxter Bioscience to terminate manufacture of the Isolex device and the reagents required for positive selection of $CD34^+$ progenitor cells has temporarily

closed these options, and created the urgent need to further evaluate the CliniMACS system in our trials of T-cell depleted HSCT.

As a first and needed step, we propose this trial which will evaluate the potential of T-cell depleted HSCT fractionated by the CliniMACS system, when administered after each of four, disease targeted cytoreductive regimens, to secure consistent engraftment and hematopoietic reconstitution in HLA-compatible related or unrelated hosts, and to prevent or abrogate acute and chronic forms of GVHD. We also seek to validate that these pre-transplant conditioning regimens, when administered with a $CD34^+$ progenitor cell enriched, T-cell depleted graft, fractionated in the CliniMACs system, will be associated with an acceptably low incidence of non-leukemic mortality.

The four cytoreduction regimens to be tested in this trial are:

1) The combination of 1375cGy (adults) or 1400-1500 cGy (Pediatrics) hyperfractionated total body irradiation followed by thiotepa (5 mg/kg/day x 2 or 10 mg/kg/day x 1) and cyclophosphamide (60 mg/kg/day x 2) (or fludarabine 25mg/m^2/day x 5 if Cyclophosphamide is contraindicated), which we have previously evaluated with SBA^-E^- T-cell depleted marrow grafts (IRB 89-119 and 01-070), and which has been associated with consistent engraftment, a low incidence of acute and chronic GVHD, and incidences of post transplant relapse in patients transplanted for ALL and AML that are equal to or lower than those reported following unmodified grafts in patients with the same diseases and stages of disease (40). We have also tested this cytoreduction regime together with CliniMACS fractionated T-cell depleted PBSC transplants in HLA-matched related patients with AML in 1° or 2° remission, as part of the multicenter BMT CTN trial (IRB 06-005). As previously noted, the results of this trial are among the best reported (44, 46). However, this regimen has not yet been evaluated with HLA-compatible unrelated T-cell depleted grafts fractionated by the CliniMACS system.

2) The combination of Busulfan 0.8- 1.0mg/Kg/dose q 6 hours x 10- 12 doses (with doses adjusted based on Busulfan pharmacokinetics), melphalan 70 mg/m^2/day x 2 and Fludarabine 25 mg/m^2/day x 5. We have previously studied this regimen in combination with Isolex separated T-cell depleted grafts (IRB 01-055) from both related and unrelated donors. This regimen is applicable to patients with MDS, AML and multiple myeloma and particularly to patients with advanced myeloid malignancies or non-malignant disorders of hematopoiesis who, by virtue of young age or prior radiation therapy, cannot receive a cytoreductive regimen containing total body irradiation.

3) The combination of clofarabine 20 mg/m^2/day x 5 (or, for children < 18 yrs of age, 30mg/m^2/day x 5 if deemed suitable and with PI approval), melphalan 70 mg/m^2/day x 2 and thiotepa 5 mg/kg/day x 2 (or 10mg/kg/day x 1). This regimen is myeloablative and immunoablative. This regimen used with unmodified HLA compatible related or unrelated HSCT, has shown particular promise in the treatment of patients with high risk and advanced staged ALL (IRB 06-125). We have transplanted a series of 19 evaluable patients after cytoreduction with this regimen, of whom 12 had ALL in 2° or greater remission or relapse. All patients achieved full engraftment. Of the 19 patients, 15 are alive, 14 disease-free at a median follow-up of 10 months. We will continue to give priority to IRB 06-125 for patients with advanced ALL. However, graft vs. host disease has been a significant cause of morbidity; five patients developed grade II-IV acute GVHD, which was lethal in 1 case; 3 patients have chronic GVHD. To address this complication, we now propose to evaluate this regimen for preparatory cytoreduction of patients with lymphoid malignancies who will then receive a T-cell depleted HLA compatible related or unrelated HSCT fractionated by the CliniMACS system.

4) The combination of fludarabine 25mg/m^2/day x 5, melphalan 70 mg/m^2/day x 2 and thiotepa 5 mg/kg/day x 2 (or 10mg/kg/day x 1). This reduced intensity, myeloablative regimen was employed as preparative conditioning for 7 CD34+ T cell depleted Isolex transplants and, under single patient use INDs, 3 T cell depleted CD34+ (CliniMACS) PBSC transplants to treat 10 patients: refractory leukemia (n=2), aplastic anemia, (n=3), PNH or refractory autoimmune cytopenia (n=2), lethal congenital immune deficiencies (n=3). The regimen induced only moderate GI toxicity. However, in each of 9 evaluable patients, we observed rapid reconstitution with full donor chimerism and without GVHD. This regimen is proposed for use in (1) patients heavily sensitized by transfusions for whom busulfan, melphalan and fludarabine may not be adequate to ensure engraftment, (2) patients who, because of prior hepatic and/or pulmonary injury are at high risk of severe and life threatening toxicity if treated with regimen A, B and C and, (3) patients with life threatening nonmalignant acquired and genetic disorders of hematopoiesis and immunity.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a four-armed phase II trial designed to evaluate the potential of an HLA compatible related or unrelated GCSF-mobilized peripheral blood progenitor cell (PBSC) transplants depleted of T-cells by positive selection of CD34+ progenitors, using the CliniMACS system to secure consistent engraftment, a low rate of acute and chronic GVHD with an acceptably low incidence of non-relapse mortality when administered after one of four disease-targeted myeloablative conditioning regimens.

4.3 Intervention

Patients with high risk forms of acute leukemia, CML, multiple myeloma, myelodysplastic syndrome (MDS) or other lethal disorders of hematopoiesis who fulfill eligibility requirements and consent to treatment, will be stratified to receive one of four myeloablative conditioning regimens, based on their disease and/or stage of disease, and clinical parameters including age, prior history of radiation therapy, prior exposure to specific chemotherapies or the presence of comorbidities that could increase risk of severe toxicity or disease relapse in the post transplant period.

The four cytoreduction regimens to be evaluated are:

A. TBI/Thiotepa/Cyclophosphamide:
   a. Hyperfractionated total body irradiation to a dose of 1375-1500 cGy (depending on age, stage of disease and requirement of general anesthesia) with lung shielding)
   b. Thiotepa (5 mg/kg/day x 2 or 10 mg/kg/day x 1)
   c. Cyclophosphamide (60 mg/ kg/day x 2) (or fludarabine 25mg/m^2 x 5 if cyclophosphamide is contraindicated).
B. Busulfan/Melphalan/Fludarabine:
   a. Busulfan (0.8 mg/kg every 6 hours x 10 or 12 doses), (depending on disease) with dose modified according to pharmacokinetics
   b. Melphalan (70mg/m^2/day x 2)
   c. Fludarabine (25mg/m^2/ day x 5)
C. Clofarabine/Melphalan/Thiotepa:
   a. Clofarabine (20mg/m^2/ day x 5) (or, for children <18 years of age, 30mg/m^2/day x 5 if deemed suitable and with PI approval),
   b. Melphalan (70 mg/m^2/day x 2)

c. Thiotepa (5 mg/kg/day x 2 or 10mg/kg/day x1)

D. Melphalan/Fludarabine/Thiotepa

a. Melphalan (70 mg/m^2/day x 2)
b. Fludarabine (25mg/m^2/ day x 5)
c. Thiotepa (5 mg/kg/day x 2 or 10mg/kg/day x1)

All patients (with the exception of patients ≤ 18 who receive transplants from HLA-matched related siblings in Arm A) 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 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.

Following preparative cytoreduction, all patients will receive a GCSF mobilized PBSC transplant depleted of T-cells by positive selection of CD34+ progenitor cells with the CliniMACS system. The targeted dose progenitor cells will be $\geq$5 x 10^6 $CD34^+$ cells/kg with the dose of T-cells limited to $\leq$1.0 x $10^5 CD3^+$ cells/kg.

Following transplantation, the patients will receive transfusions and supportive care according to the guideline of the Transplant Service in Pediatrics and Medicine. These guidelines will also be invoked for prophylaxis and treatment of infectious complications.

The patients will then be evaluated sequentially for toxicities, engraftment, acute and/or chronic GVHD, the kinetics and quality of hematopoietic and immune reconstitution, and both relapse-free survival and overall survival.

Historically, under the multicenter BMTCTN trial 0303 (IRB 06-005) which employed the CliniMACS device, GCSF mobilized PBSC, after washing, were suspended in buffered saline only and then incubated with antiCD34 coated paramagnetic beads prior to separation of the $CD34^+$ cells bound to the beads by adherence to an electromagnet in the CliniMACS device. A provision recommended as an adjunct but not required or known to be necessary was that the diluent in which the washed GCSF-mobilized PBMC were incubated with the antiCD34 coated beads also include 30% autologous plasma or intravenous gamma globulin at a concentration of 1.5 mg/ml. This provision was not used by the centers participating in the BMTCTN trial, including our own. That trial, as initially noted in the Background for Protocol 10-050 recorded an incidence of acute grade II-IV GVHD of 20.5% and a 7.6% incidence of extensive GVHD. While these findings were significantly better than any results recorded with drug prophylaxis or methods of T-cell depletion developed at other centers, the incidence of acute GVHD was higher than what we had published using SBA^-E^- T-cell depleted marrow or $CD34^+$ (ISOLEX) E^- T-cell depleted PBSC. Since we did not see significant GVHD in the small number of patients that we contributed to the trial, I ascribed the higher incidence to limited experiences with the CliniMACS device and to recent changes in the grading system used for acute GVHD. Accordingly, no change in procedures were made to protocol 10-050.

However, as accrual to 10-050 proceeded, we also saw acute GVHD in up to 19% of HLA-matched cases, and severe acute GVHD in some patients who received partially HLA-matched transplants. Again, the data were not different from the BMT CTN trial results. However, the rate of acute GVHD was higher than that reported by 2 European groups using the CliniMACS device for HLA disparate grafts. We subsequently learned that the largest of these centers, Perugia U., did incorporate IVIG in the incubation step.

Considering the possibility that in the absence of plasma or IVIG, cells can non-specifically adsorb to paramagnetic beads, the cytotherapy laboratory validated separation of $CD34^+$ cells on the CliniMACS in the presence or absence of IVIG in the incubation period, demonstrating comparable yields of $CD34^+$ cells and similar levels of T-cell depletion. Thereafter, on 7/25/12, the procedure was modified to include the IVIG. In February 2013, we evaluated the incidence of acute grade II-IV GVHD and chronic GVHD in 19 patients treated since then. The incidence of acute grade II-IV GVHD has been 5% in the whole group and 0% in HLA matched recipients. Because of the size of the group, this reduction in GVH incidence does not reach significance.

In order to be able to meaningfully compare the incidence of acute GVHD in patients receiving transplants fractionated in the presence or absence of IVIG, we wish to continue to enroll patients to all four arms. To insure appropriate representation of patients conditioned on Arm B, we request that enrollment on Arm B continue for an additional 20 patients. Enrollment to Arm B is nearly complete. In order to further evaluate the efficacy of patients treated on Arm B, an additional120 patients will be enrolled onto Arm B, 60 in the poor risk group and 60 in the standard risk group. In order to have comparative numbers for Arms B and C, an additional 30 patients will also be enrolled onto the standard risk group of Arm A.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Hyperfractionated total body irradiation, cyclophosphamide, thiotepa, Busulfan, Melphalan, fludarabine and clofarabine are standard antineoplastic agents that will be employed in the four 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, 150 cGy q12h x 8 doses to a total dose of 1375 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:

1. Store vials in refrigerator and protect from light.
2. Refrigerated: Prepare Infusion in NSS; stable for 14 days.
3. Room temperature: Prepare Infusion in NSS; stable for 7 days.

Preparation:

1. Standard IV fluid: NSS
2. Final concentration range up to: 5mg/ml.
3. IV piggyback volume: 500 cc.
4. 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:

1. Store vials at room temperature.
2. Refrigerated: Prepare infusion in D5W, stable for 28 days.
3. Room Temperature: Prepare infusion in D5W; stable for 48 hours.

Preparation:

1. Standard IV fluid: D5W.
2. Final concentration range up to: 20mg/ml.
3. IV piggyback volume: For doses <1200mg/m^2, infuse in 25cc D5W; for doses >1200mg, infuse as straight drug.

Clinical Considerations:

1. Hydration: As per MSKCC guidelines.
2. Emetic Potential: High and Delayed.
3. Supportive Medications: None.

Incompatibilities: Do not administer with other drugs.

5.1.4 ***Busulfan (busulfex®)***

a. **Source and Pharmacology:** Supplier: Orphan Medical Company; 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.

b. **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. Busulfan's 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.

c. **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.

d. **Storage and Stability:** Unopened ampules of BUSULFEX must be stored under refrigerated conditions between 2° -8° C (36° -46° F).

e. **Administration:** Intravenous, over 2 hours.

5.1.5 ***Melphalan (Alkeran®)***

a. **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.

b. **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.

c. **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

d. **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.

e. Administration: Intravenous, over 30 minutes. Complete infusion within 60 minutes of preparation.

5.1.6 ***Fludarabine (FLUDARA®)***

a. 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.

b. 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

c. 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

d. 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).

e. Administration: Intravenous, over thirty minutes.

5.1.7 ***Clofarabine (Clolar™)***

a. Formulation and Stability:

Clofarabine is formulated at a concentration of 1 mg/mL in sodium chloride (9 mg/mL), United States Pharmacopeia (USP) and water for injection, USP, qs to 1 mL. Clofarabine is supplied in a 1 mg/ml, 20 mL vial. The pH range of the solution is 4.0 to 7.0. The solution is clear with color ranging from colorless to yellow and is free from visible particulate matter. Genzyme will be supplying the drug for this study.

Vials containing undiluted Clofarabine for injection should be stored at controlled room temperature (15 to 30°C). Shelf-life studies of intact vials are currently ongoing. Clofarabine for injection should be filtered through a sterile 0.2 μm syringe filter and then further diluted with 5% dextrose injection USP or European

Pharmacopoeia (EP) (D5W) or 0.9% sodium chloride injection USP or EP (normal saline [NS]) prior to IV infusion. The resulting admixture may be stored at room temperature, but must be used within 24 hours of preparation.

b. Administration:
Clofarabine will be administered by IV infusion over 2 hours daily for 5 consecutive days. To prevent drug incompatibilities, no other medications should be administered through the same IV line.

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

a. Source and pharmacology: Supplier: Sangstat, The Transplant Company®.
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.

b. 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).

c. 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.

d. 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.

e. Administration: Infuse through a 0.22-micron filter.

5.3 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 CliniMACS System uses selective CD34 monoclonal antibodies conjugated to super-paramagnetic 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 cluting the cells into the Cell Collection Bag.

The components of the CliniMACS System include:

5.3.1 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 micro-controller 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.

5.3.2. 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.

5.3.2. 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.

5.4. 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. Prior to and during incubation of the antiCD34 beads with the mobilized PBSC, intravenous gammaglobulin is added to the incubation fluid at a concentration of 1.5 mg IVIG/ml.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

Malignant conditions or other life threatening disorders correctable by transplant for which CD34+ selected, T-cell depleted allogeneic hematopoietic stem cell transplantation is indicated such as:

1) AML in 1st remission - for patients whose AML does not have 'good risk' cytogenetic features (i.e. t 8;21, t15;17, inv 16).

2) Secondary AML in 1st remission

3) AML in 1st relapse or $\geq$ 2nd remission

4) ALL/CLL in 1st remission clinical or molecular features indicating a high risk for relapse; or ALL/CLL $\geq$ 2nd remission

5) CML failing to respond to or not tolerating Imatinib or dasatinib in first chronic phase of disease; CML in accelerated phase, second chronic phase, or in CR after accelerated phase or blast crisis.

6) Non-Hodgkins lymphoma with chemoresponsive disease in any of the following categories:

   a) intermediate or high grade lymphomas who have failed to achieve a first CR or have relapsed following a 1st remission who are not candidates for autologous transplants.

   b) any NHL in remission which is considered not curable with chemotherapy alone and not eligible/appropriate for autologous transplant.

7) Myelodysplastic syndrome (MDS): RA/RARS/RCMD with high risk cytogenetic features or transfusion dependence, as well as RAEB-1 and RAEB-2 and Acute myelogenous leukemia (AML) evolved from MDS who are not eligible for transplantation and/or unable to enroll onto protocol IRB 08-008.

8) Chronic myelomonocytic leukemia: CMML-1 and CMML-2.

9) Multiple Myeloma with disease in the following categories:

   a) Patients with relapsed multiple myeloma following autologous stem cell transplantation who have achieved at least partial response following additional chemotherapy.

b) Patients with high risk cytogenetics at diagnosis must have achieved at least a partial response following autologous stem cell transplantation. Patients must have complex karyotype, del17p, t4;14 and/or t14;16 by FISH and/or del13 by karyotyping.

10) Other rare lethal disorders of Hematopoiesis and Lymphopoiesis for which a T-cell depleted transplant is indicated (e.g. hemophagocytic lymphohistiocytosis; refractory aplastic anemia or congenital cytopenias; non-SCID lethal genetic immunodeficiencies such as Wiskott Aldrich Syndrome, CD40 ligand deficiency or, ALPS, as well as refractory autoimmune cytopenias, PNH, metabolic storage diseases or heavily transfused congenital hemoglobinopathies.)

Accrual to each treatment arm will include standard risk and poor risk patients, except for Regimen D. All patients on Arm D will be poor risk by virtue of risks of relapse and/or transplant related mortality.

Standard risk patients will include eligible patients, as defined above, who are receiving transplants as treatment for MDS in RA/RCMD,RARS, AML in 1st or 2nd remission, ALL in 1st CR, NHL in 1st remission, MM in 1st remission, Very Good Partial Response, or 1st Partial Response or CML in the first chronic phase or 1st remission.

All other patients, including those with treatment related malignancies and/or those who have AML derived from MDS, will have received extensive prior chemo/radiotherapy and, therefore, will be considered to be at poor risk of conditioning and transplant related morbidities, and potentially transplant related mortality. Patients with life threatening non-malignant genetic and acquired disorders will also, by virtue of their history of, optional transfusions and/or infection be considered poor risk. Stopping rules for non-relapse related mortality in these heavily treated patients are, therefore, slightly less stringent than patients in the poor risk transplant groups. Stopping rules for the principal endpoints of graft failure and GvHD are the same for all groups.

The following inclusion criteria are also required:

- Patient's age includes from birth on to < 70 years old.
- Patients may be of either gender or any ethnic background.
- Patients must have a Karnofsky (adult) or Lansky (pediatric) Performance Status ≥70%
- Patients must have adequate organ function measured by:

  Cardiac: asymptomatic or if symptomatic then LVEF at rest must be ≥50% and must improve with exercise.

  Hepatic: < 3x ULN AST and ≤1.5 total serum bilirubin, unless there is congenital benign hyperbilirubinemia or if the hyperbilirubinemia is directly caused by the disease in which the patient is receiving a transplant (e.g. AML Chloroma obstructing the biliary tree). Patients with higher bilirubin levels due to causes other than active liver disease are also eligible with PI approval e.g. patients with PNH, Gilbert's disease or other hemolytic disorders.

  Renal: serum creatinine ≤1.2 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 ≥50% of predicted (corrected for hemoglobin).

Each patient must be willing to participate as a research subject and must sign an informed consent form.

6.3 Subject Exclusion Criteria

- Female patients who are pregnant or breast-feeding
- Active viral, bacterial or fungal infection
- Patient seropositive for HIV-I/II; HTLV -I/II
- Presence of leukemia in the CNS.

6.3 Donor Inclusion Criteria

- Each donor must meet criteria outlined by institutional guidelines
- Donor should agree to undergo general anesthesia and bone marrow harvest collection if PBSC yield is inadequate or otherwise not transplantable for whatever reason.

6.4 Donor Exclusion Criteria

- If donors do not meet institutional guidelines, exclusion will be considered.

7.1 RECRUITMENT PLAN

Patients who fulfill the eligibility criteria as listed in Section 6.0 will be recruited for this study by an Attending Physician of the Allogeneic BMT services in Medicine or Pediatrics. This protocol will take due notice of NIH/ADAMHA policies concerning inclusion of women and minorities in clinical research populations.

8.0 PRETREATMENT EVALUATION

8.1. Pretreatment evaluation of the patient

The patient will receive an extensive medical evaluation within approximately 45 days prior to starting preparatory cytoreduction. This evaluation may include the below tests:

- Complete physical exam and medical history
- Dental evaluation (may be completed outside of 30 day window).
- CBC
- PT/PTT/INR (if clinically indicated)
- Blood Type and screen
- 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
- Disease Evaluation (including bone marrow aspirate and/or biopsy, when indicated)
- Urinalysis (if clinically indicated)
- Electrocardiogram, echocardiogram or a gated pool scan, if clinically indicated
- Pulmonary function test for patients older than 7 years, if clinically indicated
- Chest X-ray and/or 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 (may be completed outside of 45 day window).

9.1 TREATMENT/INTERVENTION PLAN

9.2 Selection of Cytoreduction Regimen

Patients eligible for this protocol include individuals with ALL, AML, CML, multiple myeloma, MDS or other lethal disorders of hematopoiesis or immunity who fulfill eligibility requirements and consent to treatment. Stratification of patients to one of the four disease-targeted cytoreductive regimens will be based on the patient's disease and stage of disease as well as clinical parameters that could increase risk of severe toxicity or disease relapse in the post transplant period. Examples of disease indications for each of the four cytoreductive regimen are summarized in Table _1_. The basis for selection of a specific regimen can be briefly described.

Table 1: Examples of Disease Indications for Each Cytoreductive Regimen (NOT EXCLUSIVE)

DISEASE INDICATION	REGIMEN A TBI/THIOTEPA/CTX	REGIMEN B BU/MEL/FLU	REGIMEN C CLO/MEL/THIO	REGIMEN D MEL/THIO/FLU
ALL 1°CR	+	-	+	+
ALL 2°CR	+	-	+	+
ALL>CR2	-	-	+	+
AML CR1	+	+	**+ (infant AML)**	-
AML CR2	+	+	-	+
AML>CR2	-	+	+	+
MDS RA/CR	-	+	-	+
MDS>RA/CR	-	+	+	+
MULTIPLE MYELOMA	-	+	-	+

DLBCL $\geq$2° CR	+	-	+	+
OTHER (E.G HLH, ALPS)	+	+	-	+

Regimen A which combines total body irradiation, thiotepa and cyclophosphamide (TBI/THIO/CTX) is the standard cytoreduction regimen employed for patients with high risk ALL or AML in 1° or 2° CR. It is also the standard preparation used for patients with diffuse large B cell lymphoma in 2° CR or greater as well as for patients with rare malignant disorders such as hemophagocytic lymphohistiocytosis (HLH) treated with a T-cell depleted graft. Selection of an alternate cytoreduction regimen for these disease indications will be based on clinical parameters which preclude TBI or suggest that this conditioning would be too toxic or less likely to induce durable remissions.

Regimen B which combines busulfan, melphalan and fludarabine, is a regimen that has been tested with T-cell depleted grafts fractionated on the Isolex device (IRB 01-055). It is a chemotherapeutic regimen that secures consistent engraftment and has been particularly effective for patients with AML and MDS as well as patients with multiple myeloma. It should be the cytoreduction regimen of choice for multiple myeloma and for AML > 2° CR and for MDS, including patients with advanced MDS who are not eligible for transplantation under protocol 08-008. It should also be the regimen employed for those patients who, by virtue of very young or advanced age, prior radiation exposure, or prior cardiac, renal or gastrointestinal disorders cannot receive total body irradiation or cyclophosphamide.

Regimen C, which combines clofarabine, melphalan and thiotepa has been tested with unmodified HLA-compatible transplants (IRB 06-125) and has shown particular promise in the treatment of high risk and advanced forms of ALL, including infant ALL. It should be the regimen of choice for patients with ALL referred after a first relapse, for infants and young children with high risk ALL in 1° CR or others with ALL or DLBCL who are at undue risk of early and/or late toxicities associated with TBI or cyclophosphamide.

Regimen D, which combines fludarabine, melphalan and thiotepa has been employed successfully for cytoreduction of heavily transfused patients with congenital acquired cytopenias, aplastic anemia, genetic immune deficiencies, and acquired auto immune disorders resulting in severe autoimmune cytopenias and lymphoproliferative disease and refractory leukemia. The immunosuppressive activity of thiotepa and fludarabine, coupled with the myeloablation induced by melphalan and thiotepa, has generated consistent full engraftment with only moderate gastrointestinal toxicities.

Please note: patients weighing < 10 kg may have medications calculated per kg instead of per m^2, when indicated.

9.3 Admission for Transplantation

Patients will be treated as inpatient on the Memorial Hospital Allogeneic Transplant Services in Pediatrics or Medicine. All orders will be administered upon admission as per institutional standard of practice.

9.4 Cytoreduction Regimens in Preparation for Transplantation

Please note: prior to transplant, an additional day of rest may be added to the treatment schedule, due to unforeseen scheduling issues.

9.4.1. Regimen A: Hyperfractioned Total Body Irradiation, Thiotepa and Cyclophosphamide.

Hyperfractionated TBI is administered 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).

1500 cGy will be administered to children ≤ 18 years of age with ALL > 1CR, since the higher dose has greater anti-leukemic activity and is well tolerated. Older patients, but ≤ 21 years of age, with ALL types seen predominantly in children and adolescents (e.g. T-cell leukemia/lymphoma syndrome) may also receive a dose of 1500cGy TBI, if the attending physician considers it appropriate and likely to be well tolerated. Sequential doses are administered in an anterior/posterior or lateral orientation. Lung blocks will be used to reduce the lung dose to 800 cGy. The blocked chest wall areas will be boosted with high-energy electrons so that the cumulative chest wall dose is approximately 1500 cGy, so as to insure that marrow sites in the ribs are adequately treated.

In addition, male patients receiving transplants for ALL or AML will receive an additional dose of 400 cGy to the testes to reduce the risk of relapse from leukemia cells in this privileged site.

If general anesthesia is required for TBI administration (e.g. young children), a dose of 150 cGy q12h x 8 doses to a total dose of 1375 cGy may be given.

9.4.1.b Thiotepa

Thiotepa: 5mg/kg/day IV over approximately 4 hr daily x 2 (Day-5 and Day -4). If scheduling of transplant harvests requires, the dose of Thiotepa may be administered as a single dose of 10mg/ kg/ day x 1. On days when Thiotepa and ATG are both administered, Thiotepa will be administered first, followed by ATG. In select cases in which the peripheral blood stem cells must be harvested a day later than requested due to a scheduling issue with the donor or Stem Cell Processing Laboratory, there may be a day of rest between the last day of radiation and the first day of thiotepa (Donor can only donate on Friday). Alternatively, if the peripheral blood stem cells must be handled a day earlier than requested, the doses of Thiotepa may be given approximately 12 hours apart on Day -4/-5. Thiotepa dosing will be adjusted if patient is > 125% of ideal body weight and will be calculated based on adjusted ideal body weight, as per MSKCC standard of care guidelines.

9.4.1.c Cyclophosphamide

Cyclophosphamide: 60 mg/kg/day IV over approximately 30 min daily x 2 days (d –3 and –2). On days when Cyclophosphamide and ATG are both administered, Cyclophosphamide will be administered first, followed by ATG. If a patient cannot receive cyclophosphamide due to a prior history of hemorrhagic cystitis or exposure to high doses of cyclophosphamide or ifosfamide, the patient may receive fludarabine 25mg/m^2/day x 5 days. Cyclophosphamide dosing will be adjusted if patient is > 125% of ideal body weight and will be calculated based on adjusted ideal body weight, as

per MSKCC standard of care guidelines. If Fludarabine is given, dose may be adjusted in the case of renal toxicity.

9.3.1d Rabbit antithymocyte globulin (thymoglobulin) and Methylprednisolone (MPD)

Rabbit ATG will be given to all transplant recipients > 18 years old. Rabbit ATG will also be given to patients ≤ 18 years of age who receive transplants from donors other than HLA-matched related siblings on this arm. Rabbit antithymocyte globulin (Genzyme) will be administered on day -4 and -3 at a dose of 2.5 mg/kg/day. If the patient has a history of allergy or intolerance to rabbit ATG, equine antithymocyte globulin at a dose of 15 mg/kg x 2 or 30 mg/kg x 1 may be used. If the clinical condition of the patient requires it, ATG may be administered on days -3 and -2. Furthermore, if severe reaction is encountered after the first dose of ATG, the second dose can be delayed until day +5. Methylprednisolone 1 mg/kg will be given as premedication. Additional medications to prevent or treat reactions will be administered as indicated according to institutional guidelines. Patients receiving transplants from HLA-mismatched donors should receive an additional dose of ATG on day -2 or -1, depending on when the doses are administered. If patient is receiving a second transplant from the same donor, ATG administration will be at the discretion of the physician. ATG dosing will be adjusted if patient is > 125% of ideal body weight and will be calculated based on adjusted ideal body weight, as per MSKCC standard of care guidelines.

Because Acetaminophen depletes glutathione and the alkylators require glutathione transferase for their metabolism, Acetaminophen administration is discouraged during the course of cytoreduction.

9.3.1 e Schema of conditioning regimen

EXAMPLE OF ROAD MAP OF PREPARATION FOR TRANSPLANT

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
					-13	-12
-11	-10	-9 TBI	-8 TBI	-7 TBI	-6 TBI	-5 Thiotepa 5 mg/kg/day IV X 1
-4 **Thiotepa** 5 mg/kg/day IV X 1 **Rabbit ATG*** 2.5 mg/Kg/d	-3 **Cyclophosphamide** 60 mg/kg/day IV X 1 **Rabbit ATG** 2.5 mg/Kg/d	-2 **Cyclophosphamide** 60 mg/kg/day IV X 1 **Rabbit ATG**** 2.5 mg/Kg/d For HLA-mismatched SCTs only	-1	0 **T-cell depleted Stem cell transplant**	+ 1	+2
+3	**+4**	+5	+6	+7 Start **G-CSF**		

* If the clinical condition of the patient requires it, ATG may be administered on days -3 and -2.
** Patients receiving transplants from HLA-mismatched donors will receive an additional dose of ATG on day -2 or -1, depending on when the doses are administered.

9.4.2. Regimen B Busulfan, Melphalan and Fludarabine

9.3.2a Busulfan

For patients with hematologic malignancies other than multiple myeloma:

Busulfan* 0.8 mg/Kg/dose Q6H X 12 doses/3 days IV If ≥ 4 years of age over 2 hours

(Busulfex®) 1.0 mg/Kg/dose Q6H X 12 doses/3 days IV If < 4 years of age over 2 hours

For patients with multiple myeloma: only .8 mg/Kg/dose Q6H X 10 doses/3 days IV will be administered.

busulfan dosing and administration:

Patients will have busulfan levels drawn whenever possible after the first dose on day 1, with adjustments in dosing based on the pharmacokinetics of the first dose according to institutional standard clinical practice, as indicated. The doses administered on days -9 and -8 should be adjusted if patient is > 125% ideal body weight and should be calculated on adjusted ideal body weight per MSKCC standard of care guidelines. Once pharmacokinetics are available, subsequent dosing will be adjusted as per institutional guidelines.

Keppra (Levetiracetam) will be administered as per institutional guidelines to all research participants prior to starting busulfex for the prevention of busulfan-associated seizures. In case of allergic reactions to Levetiracetam, alternative anti-seizure medications will be used as clinically indicated.

9.4.2.b Melphalan

Melphalan 70 mg/m^2/day x 2 days IV over 30 minutes

melphalan dosing and administration:
Dose should be adjusted if patient is > 125% ideal body weight and should be calculated on adjusted ideal body weight per MSKCC standard of care guidelines. Melphalan will be administered on Days -7 and -6 for multiple myeloma patients.

9.3.2 c Fludarabine

Fludarabine 25 mg/m^2/day x 5 days IV over 30 minutes

fludarabine dosing and administration:
Fludarabine may be adjusted in the case of renal toxicity.

In select cases in which the peripheral blood stem cells must be harvested a day later than requested due to a scheduling issue with the donor or Stem Cell Processing Laboratory, there may be a day of rest between the last day of busulfan and the first day of melphalan and fludarabine.

9.3.2.d Rabbit Anti-thymocyte Globulin (Thymoglobulin®) and Methylprednisolone (MPD)

Rabbit ATG will be administered in the pre-transplant period for all research participants on this arm. Research participants will receive rabbit ATG (Thymoglobulin®) at 2.5 mg/Kg/day x 2 days on days -3 and -2. If the patient has a history of allergy or intolerance to rabbit ATG, equine antithymocyte globulin at a dose of 15 mg/kg x 2 or 30 mg/kg x 1 may be used. If severe reaction is encountered after the first dose of ATG, the second dose can be delayed until day +5. Methylprednisolone will be given at 1 mg/Kg/day x 2 days with the ATG administration and will be discontinued thereafter. Patients receiving transplants from HLA-mismatched donors should receive an additional dose of ATG on day -1. If patient is receiving a second transplant from the same donor, ATG administration will be at the discretion of the physician. ATG dosing will be adjusted if patient is > 125% of ideal body weight and will be calculated based on adjusted ideal body weight, as per MSKCC standard of care guidelines.

Because Acetaminophen depletes glutathione and the alkylators require glutathione transferase for their metabolism, Acetaminophen administration is discouraged during the course of cytoreduction.

9.3.2.e SCHEMA OF CYTOREDUCTION AND PREPARATION FOR ALLOGENEIC PBSCT

EXAMPLE OF ROAD MAP OF PREPARATION FOR TRANSPLANT

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
					-13	-12
-11	-10 *Levetiracetam*	-9 Busulfan IV 0.8-1.0 mg/Kg/dose IV Q 6H X4 *Levetiracetam*	-8 Busulfan IV 0.8-1.0 mg/Kg/dose IV Q 6H X4 Busulfan dose will be adjusted per institutional guidelines *Levetiracetam*	-7 Busulfan IV dose per PK IV Q 6H X2 Busulfan IV dose per PK IV Q 6H X2 *Levetiracetam*	-6 Fludarabine 25 mg/m2/day IV X 1 Melphalan 70 mg/m2/day IV X 1 *Levetiracetam*	-5 Fludarabine 25 mg/m2/day IV X 1 Melphalan 70 mg/m2/day IV X 1
-4 **Fludarabine** 25 mg/m2/day IV X 1	-3 **Fludarabine** 25 mg/m2/day IV X 1 **Rabbit ATG** 2.5 mg/Kg/d	-2 **Fludarabine** 25 mg/m2/day IV X 1 **Rabbit ATG** 2.5 mg/Kg/d	-1 **Rabbit ATG** 2.5 mg/Kg/d For HLA-mismatched SCTs only	0 **T-cell depleted Stem cell transplant**	+ 1	+2
+3	**+4**	+5	+6	+7 Start **G-CSF**		

9.3.3 Regimen C: Clofarabine, Thiotepa, Melphalan

9.3.3a Clofarabine

Clofarabine will be administered via approximately a 2 hour intravenous infusion at a dose of 20mg/m^2/day for 5 doses (days -9, -8, -7, -6. -5). Patients < 18 years of age deemed suitable may receive Clofarabine at 30mg/m^2/day with PI approval.

Clofarabine will be administered after premedication with hydrocortisone. Hydrocortisone was added to the amended protocol in an attempt to prevent hepatic inflammation and elevation of transaminases. The timing of conditioning may be lengthened by 2 days based if deemed clinically necessary and approved by the PI.

Hydrocortisone will be dosed at 50 mg/m2 dose IV once daily pre-clofarabine (maximum 100 mg).

9.3.3b Thiotepa

Thiotepa will be administered via approximately a 4 hour intravenous infusion at a dose of 10mg/kg/day for 1 dose (day –4). Thiotepa may also be administered at 5mg/kg/day IV over approximately 4 hr daily x 2 days (Day-5 and Day -4), depending on scheduling of transplant harvests. In select cases in which the peripheral blood stem cells must be harvested a day later than requested due to a scheduling issue with the donor or Stem Cell Processing Laboratory, there may be a day of rest between the last day of clofarabine and the first day of thiotepa. Thiotepa dosing will be adjusted if patient is > 125% of ideal body weight and will be calculated based on adjusted ideal body weight, as per MSKCC standard of care guidelines.

9.3.3c Melphalan

Melphalan will be administered via approximately a 30 minutes infusion at a dose of 70mg/m^2/day for 2 doses (days -3, and -2). Melphalan dosing will be adjusted if patient is > 125% of ideal body weight and will be calculated based on adjusted ideal body weight, as per MSKCC standard of care guidelines.

9.3.3d Rabbit Anti-thymocyte Globulin* (Thymoglobulin®) and Methylprednisolone (MPD)

Rabbit ATG will be administered in the pre-transplant period for all research participants on this arm. Research participants will receive rabbit ATG (Thymoglobulin®) at 2.5 mg/Kg/day x 2 days on days -3 and -2. If the patient has a history of allergy or intolerance to rabbit ATG, equine antithymocyte globulin at a dose of 15 mg/kg x 2 or 30 mg/kg x 1 may be used. If severe reaction is encountered after the first dose of ATG, the second dose can be delayed until day +5. Methylprednisolone will be given at 1 mg/Kg/day x 2 days with the ATG administration and will be discontinued thereafter. Patients receiving transplants from HLA-mismatched donors should receive an additional dose of ATG on day -1. If patient is receiving a second transplant from the same donor, ATG administration will be at the discretion of the physician. ATG dosing will be adjusted if patient is > 125% of ideal body weight and will be calculated based on adjusted ideal body weight, as per MSKCC standard of care guidelines.

Because Acetaminophen depletes glutathione and the alkylators require glutathione transferase for their metabolism, Acetaminophen administration is discouraged during the course of cytoreduction.

9.3.3 e Approximate Schema for Regimen C

EXAMPLE OF ROAD MAP OF PREPARATION FOR TRANSPLANT

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
					-13	-12
-11	-10	-9 **Clofarabine** 20 mg/m2/day IV X 1	-8 **Clofarabine** 20 mg/m2/day IV X 1	-7 **Clofarabine** 20 mg/m2/day IV X 1	-6 **Clofarabine** 20 mg/m2/day IV X 1	-5 **Clofarabine** 20 mg/m2/day IV X 1
-4 **Thiotepa** 10 mg/kg/day IV X 1	-3 **Melphalan** 70 mg/m2/day IV X 1 **Rabbit ATG** 2.5 mg/Kg/d	-2 **Melphalan** 70 mg/m2/day IV X 1 **Rabbit ATG** 2.5 mg/Kg/d	-1 **Rabbit ATG** 2.5 mg/Kg/d For HLA-mismatched SCTs only	0 **T-cell depleted Stem cell transplant**	+ 1	+2
+3	+4	+5	+6	+7 Start **G-CSF**		

9.3.4 Regimen D: Fludarabine, Thiotepa, Melphalan

9.3.4a Fludarabine

Fludarabine will be administered via approximately a 30 minute infusion at a dose of 25 mg/m^2/day for 5 days (day -6 through day -2). Fludarabine may be adjusted in the case of renal toxicity.

9.3.4b Melphalan

Melphalan will be administered via approximately a 30 minute infusion at a dose of 70mg/m^2/day for 2 doses (days -8, and -7). Melphalan dosing will be adjusted if patient is > 125% of ideal body weight and will be calculated based on adjusted ideal body weight, as per MSKCC standard of care guidelines.

9.3.4c Thiotepa

Thiotepa will be administered via approximately a 4 hour intravenous infusion at a dose of 5mg/kg/day IV over approximately 4 hr daily x 2 days (Day-6 and Day -5). Thiotepa may also be administered at 10mg/kg/day for 1 dose (day –5) depending on scheduling of transplant harvests. Thiotepa dosing will be adjusted if patient is > 125% of ideal body weight and will be calculated based on adjusted ideal body weight, as per MSKCC standard of care guidelines.

In select cases in which the peripheral blood stem cells must be harvested a day later than requested due to a scheduling issue with the donor or Stem Cell Processing Laboratory, there may be a day of rest between the last day of melphalan and the first day of fludarabine and thiotepa.

9.3.4d Rabbit Anti-thymocyte Globulin (Thymoglobulin®) and Methylprednisolone (MPD)

Rabbit ATG will be administered in the pre-transplant period for all research participants on this arm. Research participants will receive rabbit ATG (Thymoglobulin®) at 2.5 mg/Kg/day x 2 days on days -3 and -2. If the patient has a history of allergy or intolerance to rabbit ATG, equine antithymocyte globulin at a dose of 15 mg/kg x 2 or 30 mg/kg x 1 may be used. If severe reaction is encountered after the first dose of ATG, the second dose can be delayed until day +5. Methylprednisolone will be given at 1 mg/Kg/day x 2 days with the ATG administration and will be discontinued thereafter. Patients receiving transplants from HLA-mismatched donors should receive an additional dose of ATG on day -1. If patient is receiving a second transplant from the same donor, ATG administration will be at the discretion of the physician. ATG dosing will be adjusted if patient is > 125% of ideal body weight and will be calculated based on adjusted ideal body weight, as per MSKCC standard of care guidelines.

Because Acetaminophen depletes glutathione and the alkylators require glutathione transferase for their metabolism, Acetaminophen administration is discouraged during the course of cytoreduction.

9.3.4e Approximate Schema for Regimen D

EXAMPLE OF ROAD MAP OF PREPARATION FOR TRANSPLANT

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
					-13	-12
-11	-10	-9	-8 **Melphalan** 70 mg/m2/day IV X 1	-7 **Melphalan** 70 mg/m2/day IV X 1	-6 **Fludarabine** 25mg/m2/day IV X 1 **Thiotepa** 5 mg/kg/day IV X 1	-5 **Fludarabine** 25 mg/m2/day IV X 1 **Thiotepa** 5 mg/kg/day IV X 1
-4 **Fludarabine** 25 mg/m2/day IV X 1	-3 **Fludarabine** 25 mg/m2/day IV X 1 **Rabbit ATG** 2.5 mg/Kg/d	-2 **Fludarabine** 25 mg/m2/day IV X 1 **Rabbit ATG** 2.5 mg/Kg/d	-1 **Rabbit ATG** 2.5 mg/Kg/d For HLA-mismatched SCTs only	0 **T-cell depleted Stem cell transplant**	+ 1	+2
+3	**+4**	+5	+6	+7 Start **G-CSF**		

9.4 <u>Prophylaxis against acute graft-versus-host disease</u>

No GvHD prophylaxis will be administered other than the CliniMACS fractionated T-cell depleted transplant.

9.5. *<u>Stem Cell Transplantation</u>*

9.5.1. PBSCT

Donor peripheral blood progenitor cells: stimulation, harvesting, isolation and T-cell depletion. For related donors, GCSF will be administered according to MSKCC standard of care. For unrelated donors, the G-CSF will be administered and the leukapheresis obtained according to the National Marrow Donor Program protocol and IND. Mononuclear cell fractions collected on the fourth and fifth days will be pooled.

HLA-compatible donors (9/10 or 10/10 HLA-matched), whenever possible, will be further prioritized based on KIR/HLA genotype combinations between the donor and recipient. Donors who are KIR3DS1 (40% of the population) are prioritized in order to improve transplant-related mortality and to prevent viral reactivation[61]. For patients with AML/MDS/blastic CML, two donor populations are desired: 1) donors who exhibit the HLA-C1 class I KIR ligand group and who are KIR2DS1-positive (34% of all donors) [62], and 2) donors who are negative for KIR2DL3 and homozygous for KIR2DL2 (cenBB compound haplotype, 17% of all donors) [63]. In addition, allele level typing for inhibitory KIR3DL1 may be incorporated into donor selection, where donors with KIR3DL1 alleles with low affinity will be selected over donors with high affinity for the patients HLA-Bw4 allele[64]. For AML/MDS/blastic CML patients whose HLA-C alleles belong to the HLA-C2 class KIR ligand group (HLA-C2C2) and who are at higher risk of relapse[65-67], consideration may be given to choosing an HLA-C1C2 donor. In this situation, the donor will, by definition, be mismatched for one HLA-C locus in order to capture donor NK alloreactivity due to “missing self.”

Isolation of CD34+ hematopoietic progenitor cells with the CliniMACS TM System, Miltenyi Biotec.

The apheresis product is collected from a blood-related or matched unrelated donor. Aliquots of the apheresis product are collected and then tested and screened as per blood banking guidelines. The apheresis product is then prepped for the CliniMACS Cell Selection System. After washing the GCSF mobilized PBSC, the cells are suspended in CliniMACS PBS/EDTA buffer to which is added sterile human intravenous Immune Globulin at a concentration of 1.5mg IVIG/ml. This fluid is then used during incubation of the PBSC with the antiCD34 antibody coated paramagnetic beads.
The mechanism of action of the CliniMACS Cell Selection System is based on magnetic-activated cell sorting, which can select or remove specific cell types depending on the cell-specific immunomagnetic label used. The apheresis product is first co-incubated with the CliniMacs CD34 reagent (antibody-coated paramagnetic particles). After magnetic labeling and washing, the cells are passed through a high-gradient magnetic separation column in the CliniMACS clinical cell selection device. Magnetically labeled CD34+ cells are retained in the magnetized column, and $CD34^{-negative}$ cells flow through as the effluent fraction and discarded. The $CD34^{+positive}$ cells retained in the column are eluted by removing the magnetic field from the column, then washing the cells through the column and collecting them. The final CD34+ cell enriched product is concentrated by centrifugation and tested before final release for administration. Before infusion, the CD34+ cells will be washed in normal saline for intravenous infusion containing 1% human serum albumin, and suspended in a volume of 25-50 ml. for intravenous administration.

Throughout the process, critical control points and associated assays are identified and performed on each cellular therapy product. Aliquots of the same product sample are taken for in-process and final product testing. After each step (apheresis, platelet washing, CD34 labeling and washing, enrichment), QC testing includes; Sterility. Endotoxin, gram stain, Total nucleated cells (TNC), flow cytometry phenotype/analysis of CD45, CD34, and CD3, and viability assessed by 7-AAD is assessed. To ensure sterility, 14-day sterility tests are performed for in-process sterility testing and a gram stain is performed on the final product prior to release.

9.5.2 Transplantation of the T-cell depleted stem cells.

The CD34+T-cell depleted peripheral blood progenitor cells or the SBA-E- fraction of the bone marrow, suspended in a volume of approximately 20-50 ml will be infused intravenously over approximately 15 minutes with monitoring of vital signs.

9.6. *Supportive Care*

a. *Prophylaxis against infections*

Standard of care guidelines will be followed for prophylaxis against post transplant infections by opportunistic organisms, including Pneumocystis jiroveci, fungal organisms, DNA herpesviruses and more specifically CMV.

b. *Prophylaxis against menorrhagia*

All post-pubertal females will receive prophylaxis against menorrhagia according to our standard of care guidelines.

c. *Transfusions*

Following initiation of the pre-transplant cytoreduction, all blood products for transfusion, with the exception of the peripheral blood stem cell, will be irradiated to 3,000 cGy to inactivate lymphocytes capable of initiating lethal GvHD. Blood products are irradiated in the blood bank, using a cesium gamma emitter. Platelets will be administered for clinical evidence of active hemorrhage. Platelets will be administered at the discretion of the treating attending and as per standard of care.

d. *Nutritional support*

Nutritional status will be carefully monitored by the physician, and high-calorie parenteral alimentation will be introduced as needed. Vitamin supplements will be as clinically indicated.

e. CNS leukemia Prophylaxis

Patients with ALL, those with M4/M5, and those transplants with AML in bone marrow relapse may receive intrathecal infusions of cytarabine at monthly intervals, beginning approximately 2 months posttransplant, and thereafter on a monthly basis for a total of 5 doses. Patients with a prior history of CNS leukemia may receive 11 doses of IT cytarabine from approximately 2 until 12 months post-transplant. Intrathecal Cytarabine will be dosed according to age as per institutional guidelines.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

10.1 Post-transplant evaluation

The chart below shows the approximate dates for tests and procedures performed after transplant. Certain tests may be held or repeated at the discretion of the treating physician if deemed in the best clinical interest of the patient.

Activity	Transplant to Discharge	DISCHARGE TO DAY 100	Day 100	6 Months	12 months	24 Months
Blood counts and chemistry (CBC, Comprehensive Metabolic Panel)	CBC: Daily CMP: 2xs a week	CBC/CMP: Weekly , 2x a week or 2-3 weeks	Every 2-4 weeks		X	X
Physical exam for GVHD evaluation	Monitored routinely as per inpatient transplant service guidelines	Weekly	Every 2-4 weeks		X	X
Disease evaluation BMA [1], [2]	30 - 100 days after transplant, if clinically indicated			X	X	X
T-cell chimerism[2]	NA	30 days after transplant	X	X	X	X
Peripheral blood lymphocyte (PBL) phenotyping[3]	NA	NA	X	X	X	X
In vitro response of PBL to standard panel of mitogens [2,4]	NA	NA	NA	X	X	X

1. This should apply to patients with malignant disease in which the BM was previously involved. BM Chimerism should also be assessed whenever possible. BMA may be delayed if patient is clinically unstable.
2. May be done more frequently if clinically indicated. Tests need not be done if patient has a low number of peripheral white cells.
3. PBL phenotyping should be performed until CD34 counts is >200 or if patient is being immunized.
4. Should be performed until normal or reached plateau

Patients will be followed for life by telephone assessments conducted once a year to assess overall and disease free (DFS) survival.

11.0 TOXICITIES/SIDE EFFECTS

Patients recruited to this transplantation trial are individuals who are either referred by physicians or self-referred for peripheral blood stem cell transplantation as a potentially curative treatment for their malignancy or other life threatening disorders. Prior to consideration for transplant, all patients undergo a series of 1-3 hour consultations discussing the risks and potential benefits of an allogeneic stem cell transplantation and the

different procedures which will be a normal part of the transplant course. The risks and potential benefits of the transplant procedure, as well as the participation in any given research, experimental, or therapeutic protocol are also discussed.

11.1 Toxicities/Side effects of conditioning regimens

The risks of short-term treatment with G-CSF are likely negligible. However, administration of GCSF is frequently associated with low grade fever and low back pain which usually resolves within one day following cessation of GCSF treatment. Furthermore, there has now been one recorded patient who developed acute splenomegaly and splenic rupture in response to high dose GCSF. The bone pain may require treatment with analgesics. The risks of a leukapheresis are negligible, involving an occasional vasovagal response to venipuncture and the minimal hemodynamic alterations associated with single unit phlebotomies. To protect against these risks, leukapheresis are conducted in the Blood Bank Donor Room with full medical and nursing supervision and support systems to address adverse events.

11.1.1 Total Body Irradiation

Likely

- Nausea and vomiting - virtually all patients will experience nausea and vomiting after irradiation. This can be diminished somewhat with mild anti-emetics. Strong sedatives or phenothiazine derivatives should be avoided just before radiation treatment, as they frequently cause excessive drowsiness and/or symptomatic orthostasis, which prevent delivery of TBI done in the standing position.

- Myelosuppression is the major dose-limiting toxicity.

- Hyperpigmentation - most patients will get some degree of hyperpigmentation within 2-3 weeks of transplantation.

- Increased risk of infection

- Mucositis - most patients will develop moderate to severe mucositis of the oral and GI tracts, which will be managed with aggressive nursing mouth care, analgesics and prophylactic antifungal and antiviral agents.

- Late effects - there is the possibility of cataract formation. Although mild cataracts may occur in up to 70% of cases with single dose TBI, we have seen cataracts in < 30% of patients treated with hyperfractionated TBI.

- Sterility is extremely common following total body irradiation and administration of alkylating chemotherapy; the risk increases with the number of years since puberty.

Less Likely

- Parotitis - some patients will experience symptomatic parotitis within the first 24 hours post radiation. This resolves spontaneously over several days.

- Diarrhea - most patients develop some diarrhea in the first week post irradiation. This can be treated symptomatically.

- Fever – low grade fever [greater than 38°C] may occur for 24 hr post irradiation. This can be treated symptomatically.

- Erythema - this may occur in patients within 24 hours and resolves in 2-3 days.
- Hypothyroidism has been reported in small numbers of adult patients following TBI plus alkylating chemotherapy, and this will be routinely monitored post transplant with hormonal replacement as indicated.
- There is a possibility that secondary malignancies may develop, particularly due to the combined effects of radiation and an alkylating agent [cyclophosphamide]. The age-adjusted incidence of secondary cancer in transplant patients after radiation and chemotherapy has recently been estimated to be 6.7 times higher than that of first cancers in the general population; and most of these were non-Hodgkin's lymphomas.
- veno-occlusive disease of the liver (VOD)

11.1.2 Thiotepa

Likely

- Myelosuppression is the major dose-limiting toxicity, occurring regularly at doses >405mg/m^2. Other non-fatal toxicities have been observed almost exclusively after administration of thiotepa at doses greater than 1000mg/m^2.
- Increased risk of infection.
- Cutaneous erythema and bronzing is seen in most patients given ≥900mg/m^2. Erythema develops 4-14d after the first dose and may last up to 3wks. Bronzing may persist for months.

Less Likely

- Nausea, vomiting, diarrhea - occasional, rarely severe.

Rare but Serious

- CNS toxicity manifested by mild cognitive dysfunction, disorientation, confusion, irritability, bizarre behavior, is usually not observed at doses <1000mg/m^2.
- Interstitial pneumonia
- Renal failure
- Transient hepatic transaminase elevations are occasionally seen, but rarely severe.

11.1.3 Cyclophosphamide

Likely

- Myelosuppression
- Increased risk of infection
- Edema and weight gain secondary to inappropriate ADH secretion can occur in some patients, but can usually be corrected with medical management
- Gastrointestinal: anorexia, nausea, vomiting, and diarrhea occur commonly, but are reversible.

- Elevated transaminases are usually transient
- Alopecia, amenorrhea, azoospermia would be expected to occur.

Less Likely

- Hemorrhagic cystitis can be severe and in few cases, life threatening.

Rare but Serious

- Cardiotoxicity can be severe and in few cases, life threatening.

11.1.4 Busulfan

Likely

- Myelosuppression
- Fatigue
- Anorexia
- Nausea and Vomiting
- Mucositis
- Diarrhea
- Edema
- Alopecia
- Increased risk of infection
- Sterility

Less Likely

- Cataracts
- Under-activity of the thyroid gland

Rare but Serious

- Seizures
- veno-occlusive disease of the liver (VOD)

11.1.5 Melphalan

Likely

- nausea and vomiting - Diarrhea
- mucositis
- myelosuppression and pancytopenia
- elevated liver function tests
- sterility
- alopecia
- fever

Less Likely

- Syndrome of Inappropriate anti-diuretic hormone (SIADH)
- Interstitial pneumonitis
- Pulmonary fibrosis

Rare but serious

- secondary leukemia
- anaphylaxis
- seizures
- kidney failure
- veno-occlusive disease of the liver (VOD)

11.1.6 Fludarabine

Likely

- Nausea, vomiting, mouth sores, stomach cramps and diarrhea, jaundice, and elevations of liver enzymes.
- Scaling and redness of the skin, which is usually of short duration.
- Transient but significant myelosuppression at the doses called for in this protocol. Fludarabine is also profoundly immunosuppressive. These toxicities place the patient at increased risk for infections for periods of 1-2 months.

Rare but Serious

- Effects on the nervous system are not usually seen at the fludarabine dose used in this protocol, but, when they occur, can include cerebellar dysfunction with loss of balance and trouble walking, blurring of vision or, in extremely rare cases, blindness, and mental agitation or confusion.

11.1.7 Clofarabine

Likely

- Reversible hepatotoxicity is the major dose-limiting toxicity. This is most commonly demonstrated by a transient elevation of transaminases. Hyperbilirubinemia was less common.
- Dermatitis and pruritus
- nausea, vomiting , diarrhea, abdominal pain
- myelosuppression and pancytopenia
- headache
- fever - chills or rigors

Less Likely

- acrodermatitis
- anorexia, weight loss myalgias, arthralgias
- headache, fatigue, dizziness, somnolence, tremor
- shortness of breath
- infections

Rare but serious

- Capillary leak syndrome
- Systemic inflammatory response syndrome

11.1.8 Rabbit Antithymocyte Globulin (Thymoglobulin)

The ATG to be used in this trial is a purified preparation of rabbit gamma globulin containing high concentrations of antibodies against human lymphocytes. The preparation may contain low levels of antibody that cross-react with human platelets, white cells or red cells. The potential side effects of ATG are:

Likely

- Fever, shaking, chills and lowered blood pressure: These are regularly observed, particularly during initial infusions of the rabbit globulin. They probably result from a breakdown of cells binding the antibody.

- Skin rash and itching: A frequent complication which is probably due to minor allergic reactions to rabbit globulin. These symptoms will usually be prevented by or controlled with anti-histamines as well as with concomitant administration of corticosteroids.

- Platelet and white cell count depression: These are frequently observed and are probably caused by the binding of the rabbit antibody to human blood elements. Platelet transfusions will be administered to reduce the chance of bleeding or life threatening hemorrhage.

Less Likely

- Serum sickness: Approximately 30% of patients treated with rabbit globulin will develop a late immune reaction to the globulin resulting in serum sickness 3-10 days after the administration. This may lead to

severe skin rashes, mouth and vaginal sores, pain and swelling of the joints, or kidney damage. Serum sickness is transient and its damage reversible but it may require prolonged treatment with corticosteroids.

Rare, but potentially serious

- Anaphylaxis: A rare but severe allergic reaction which may cause a life threatening drop in blood pressure, wheezing and difficulty breathing and severe hives. This complication can be treated with anti-histamines and steroids.

11.2 Toxicities/Side effects of the transplant and the combined effects of the conditioning regimen and transplant

In addition to tracking patients for survival and relapse, all participants may also be monitored for post-transplant toxicities.

Grade 3-4 toxicities which occur within 30 days following a transplant AND are also possibly, probably, or definitely attributable to the CliniMACS device will be graded and attributed. Additional toxicities will be reviewed and graded/attributed at the discretion of the PI.

Toxicities which are attributable to underlying malignancy and/or expected toxicities from the transplant will not be tracked.

The grading for monitoring transplant related toxicities will be based on the CTCAE 4.0 (57).

11.2.1 Potential Sensitization to Murine Proteins

Mouse protein antibodies are used in the CliniMACS processing procedures. If the recipient has a pre-existing allergy, he or she may be at risk for allergic reactions during infusion of the processed cells, although the residual amount of murine protein in the final product is very low (estimated maximum dose for a 50 kg patient would be 30 μg). To date, no allergic reactions are reported in patients receiving cells processed with the CliniMACS System. Epinephrine and antihistamines will be available at the recipient's bedside during the PBSC infusion.

11.2.2 PBSC Infusion

Symptoms may include changes in heart rate and/or rhythm, changes in blood pressure, fever, chills, sweats, nausea, vomiting, diarrhea, abdominal cramping, hemoglobinuria, acute renal failure, allergic reactions, respiratory dysfunction, or headache.

11.2.3 Infections

Transplantation puts the patient at higher risk for bacterial, viral, or fungal infections, which are potentially life threatening. These risks are potentially higher with TCD transplants. Prophylaxis will be initiated and patients will be closely monitored for signs of infections and will receive early and appropriate treatment.

11.2.4 Microbial Contamination of PBSC

There is a potential that processing the leukapheresis product will inadvertently introduce microorganisms that could cause infection in the recipient after the cells are infused. All precautions to maintain sterility will be taken. Cultures of the leukapheresis product and the selected product will be obtained to monitor for contamination.

11.2.5 Graft Failure / Poor Marrow Function

T cell depletion of donor cells is associated with an increased incidence of graft failure in allogeneic transplant recipients. After allogeneic transplantation, the recipient's marrow function may be poor and leukopenia, anemia, or thrombocytopenia may result from many causes including graft rejection induced by surviving host immune T-cells, or ongoing suppression of engrafted donor blood-forming cells by GVHD, infection or marrow suppression or immunosuppressive drugs and other medications. Graft failure may result in death if not reversed. In patients with immune rejection second transplants can be administered with immunosuppressive therapy, including non-myeloablative conditioning regimens. 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.

11.2.6 Graft-versus-host Disease

Acute or chronic GVHD may develop after allogeneic transplantation that can be disabling and can lead to death. GVHD is thought to be initiated by T cells contained in the PBSC graft. $CD34^+$ selection and $CD3^+$ depletion reduces the number of T cells in the PBSC but GVHD can occur after TCD transplants. Acute and/or chronic GVHD will be treated with immunosuppressive drugs as per the transplant service guidelines.

11.2.7 Veno-occlusive Disease (VOD) of the Liver

VOD is a manifestation of damage to the liver by the conditioning regimen that usually develops within two weeks after allogeneic transplant and is characterized by at least two of the following:

- Hyperbilirubinemia (total bilirubin > 2 mg/dL)
- Hepatomegaly or right upper quadrant pain, or
- Rapid weight gain (> 5% above baseline)

Recipients developing VOD will be monitored closely and will receive appropriate supportive care and careful fluid management. TCD is not expected to affect the risk of VOD.

11.2.8 End Organ Damage

End organ damage of all or any of the major organs, including the brain, may occur as a result of cumulative toxicity from anti-neoplastic therapy, reactions to other drugs, and as a result of destructive processes (e.g., infection, GVHD, etc.) and may have a fatal outcome. Toxicities may occur in any individual patient due to multiple events and cumulative effects that may involve any and all organs, including the brain. Brain damage can result in severe loss of cognitive or neurologic function. Data from previous studies do not suggest that the risk of end organ damage is appreciably affected by TCD or the preparative regimens to be used in this study.

11.2.9 Disease Relapse

Allogeneic transplantation using T cell depleted peripheral blood stem cells has, in some cases, been associated with an increased incidence of leukemic relapse in patients with chronic myelogenous leukemia, compared to recipients who receive unmanipulated donor cells. The risk of relapse has not been increased in patients with acute leukemias. Nevertheless, despite cytoreduction and a transplant, relapse may occur.

11.2.10 Lymphoproliferative Syndrome

Recipients of TCD allogeneic grafts have an increased risk of developing lymphoproliferative syndromes caused by EBV infection (47, 48). This syndrome should be included in the differential diagnosis of recipients with unexplained symptoms such as fever, diarrhea, hepatomegaly or lymphadenopathy. Biopsy evaluation is required to make the diagnosis. EBV PTLD may rapidly progress and can be fatal if not treated. Management of suspected EBV PTLD should be discussed with one of the Protocol P.I.s. EBV PTLD can be treated with rituximab and/or infusion of 10^6 T cells/kg from the donor. Rituximab has been shown to induce regression in 50 - 70% of cases (49). However, Rituximab does not enter the CNS and is not effective in treating CNS disease. Donor lymphocyte infusions may induce regression in > 90% of cases of EBV PTLD and are effective in CNS disease but may cause GVHD (50). EBV-immune cells are experimental, but can also induce regression of EBV PTLD without risk of graft vs host disease (51, 52).

11.2.11 Death

There is an approximately 5-10% risk of treatment related mortality within the first month of transplant due to the risk of severe regimen related toxicity, hemorrhage, opportunistic infection, or other complications. It is not expected that the regimens to be used in this protocol will increase this risk.

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, 100 days, 6 months, 12 months, and 24 months posttransplant or as needed thereafter.

12.2 Graft failure or rejection

Primary non-engraftment is diagnosed when the patient fails to achieve an ANC ≥500/μl at any time in the first 28 days post-transplant. If the patient's leukemia recurs during this interval, the patient is scored as having refractory leukemia. In such a situation, the absence of donor hematopoiesis is not evaluable for graft failure or rejection. If host T-cells capable of specifically inhibiting donor hematopoietic progenitor growth in vitro are concurrently detected during graft failure, a presumptive diagnosis of immune mediated rejection is made (53, 54). If (1) after achievement of an ANC $\geq 500/mm^3$, the ANC declines to $<500/mm^3$ for more than 3 consecutive days in the absence of relapse, or, (2) there is absence of donor cells in the marrow and/or blood as demonstrated by chimerism assay in the absence of relapse, a diagnosis of secondary graft failure is made. If, however, recurrence of host leukemia is detected concurrently, the patient is not evaluable for graft failure or rejection.

Patients with evidence of graft failure without evidence of recurrence of host leukemia may have additional studies drawn to ascertain cause and define relevant histoincompatibilities. These analyses may include (1) Evaluation of bone marrow aspirates and biopsies for residual or recurrent leukemia, when indicated, (2) Culture and/or molecular analyses of marrow and blood for viral pathogens potentially causing graft failure including CMV, HHV6 and parvovirus B 19, (3) Immunophenotypic and genetic analysis of circulating T-cells and NK cells to ascertain their origin and potential function, (4) Analysis of the functional activity of residual circulating lymphocytes to determine whether and to what degree they exhibit cytotoxic or cyto-inhibitory activity against donor host or third party PHA-stimulated blasts or clonogenic hematopoietic progenitor cells. If donor-specific reactivity is identified, attempts will be made to identify targeted specificities (HLA or minor alloantigens) whenever possible.

Patients who suffer graft failure may 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.

12.3. Graft-versus-host disease

Standard BMT-CTN and IBMTR systems clinical criteria as defined by Rowlings, et al (55) will be used to establish and grade acute GvHD.

To determine the severity of acute GvHD, data will be collected approximately weekly to characterize the severity of symptoms and signs caused by GvHD and to evaluate possible confounding factors. Real time data collection will include descriptive characteristics of rash and estimated body surface area involved, extent of dermal/epidermal separation, identification of 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 after engraftment, peak diarrhea volume with annotations concerning the presence or absence of urinary mixing and estimates of 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.

Patients will be observed for acute and/or chronic GvHD as long as they have not received donor derived leukocytes infusion (DLI). If at any time, a patient receives DLI, that time will represent the end-time for evaluation of GvHD. Graft-versus-host disease occurring after DLI infusions will be analyzed separately.

Patients with moderate to severe acute GvHD (grade II-IV) will be treated in standard fashion with high-dose I.V. methylprednisolone (2-20mg/kg/day) or in combination with other immunosuppressants as per ongoing trials on GvHD and at the discretion of the treating attending. Patients failing to respond to steroids will be considered for treatment with experimental treatments available at the time of diagnosis of GvHD.

Chronic GvHD will be diagnosed and graded according to the criteria of Sullivan (56) treated with standard or experimental immunosuppressive therapy. Treatment will consist of corticosteroids, cyclosporin A or azathioprine, or combinations of these agents. Other novel treatments could be used if available, i.e. thalidomide and psoralen/ultraviolet A phototherapy (PUVA).

12.4. Transplant related mortality

Transplant-related mortality (TRM) includes fatal complications resulting from the allogenic transplant and/ or treatment regimens such as graft failure, GvHD, hemorrhages, and infections.

12.5. Infections

The occurrence of life-threatening opportunistic infections will be evaluated according to the criteria established by BMT CTN 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 or GVHD before death will be excluded from the above definition, even if an infection was the final cause of death.

12.6. Disease relapse and recurrence

Relapse of MDS, ALL and AML will be defined by an increasing number of leukemic blasts in the marrow over 5%, by the presence of circulating peripheral blasts, or by the presence of blasts in any extramedullary

site. Cytogenetic and/or Molecular analysis of the marrow and/or peripheral blood will also be obtained for the diagnosis of relapse.

Relapse of myeloma and lymphoma will be diagnosed by clinical and radiological findings coupled with biopsy of affected tissue. A sustained risk in the serum concentration of myeloma paraprotein will also be considered evidence of relapse.

Disease recurrence

In non-neoplastic diseases, disease recurrence would be considered if engraftment of donor hemopoietic cells have not been achieved and function or phenotypic abnormalities of hemopoietic or lymphoid lineages affected, occur.

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.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Research participants may be removed from the study if requested by the research participant. Management will depend on where they are in their treatment course. Such research participants will receive appropriate supportive care. Patients may also be removed from the study at any point deemed appropriate by the principle investigator.

Patients may be removed from study, and not followed further, if one or more of the following events occur:

- Significant noncompliance on the part of the patient
- Refusal of the patient to continue treatment or observations
- Progressive disease or relapse
- Decision by the investigator that termination is in the patient's best medical interest
- Unrelated medical illness or complication
- Lost to follow-up

14.0 BIOSTATISTICS

The intent of this phase 2 trial is to determine if the introduction of the CliniMACS system, as a method of T-cell depletion, maintains or reduces the current rates of graft failure, graft versus host disease, and non-relapse mortality in patients with hematologic disease that require a stem cell transplant. The patient population for this study will be divided into three groups based on the cytoreduction regimen administered. Because patients at

later stages of disease may be more susceptible to toxicities induced by the three conditioning regimens, accrual to the three groups will also be stratified according to transplant risk as standard risk or poor risk. Cytoreduction regimens are detailed in section 4.2. Risk strata are detailed in Section 6.1.

Each of the six treatment strata will accrue 30 patients, and to insure patient safety, will be monitored for graft failure, graft versus host disease, and non-relapse mortality. The same stopping boundaries for excessive graft failure, acute GvHD, and the corresponding power calculations will be used for each stratum. The boundary for 100 day non-relapse mortality in the poor risk transplant patients is 20% in each treatment group and 15% for standard risk transplant patients in each treatment group. This is because patients in the poor risk transplant group have been repeatedly shown to have a higher treatment related mortality than patients in earlier stages of ALL, AML, and CML, who constitute the standard risk group. In the event that the stopping boundary is crossed for one treatment stratum, the study will continue accrual in the other treatment groups. The power calculations are computed for each of the three failure endpoints separately.

Table of Stopping boundaries – poor risk group

Failure Endpoint	# of failures needed to stop the arm	Failure rate in the population	Probability boundary is crossed
Graft failure	2 in the first 26 patients	0.02	0.10
	3 within 30 patients	0.15	0.93
Acute GvHD at d100 (Grades 3-4)	3 in the first 16 patients	0.06	0.10
	4 in the first 26 patients	0.23	0.91
	5 within 30 patients		
Non-relapse mortality by d100	3 in the first 11 patients	0.08	0.10
	4 in the first 19 patients		0.88
	5 in the first 27 patients	0.25	
	6 within 30 patients		

Table of Stopping boundaries – standard risk group

Failure Endpoint	# of failures needed to stop the arm	Failure rate in the population	Probability boundary is crossed
Graft failure	2 in the first 26 patients	0.02	0.10
	3 within 30 patients	0.15	0.93
Acute GvHD at d100 (Grades 3-4)	3 in the first 16 patients	0.06	0.10
	4 in the first 26 patients	0.23	0.91
	5 within 30 patients		
	3 in the first 20 patients	0.02	0.10

Non-relapse mortality by d100	4 within 30 patients	0.15	0.93

We have modified the eligibility criteria to include individuals under the age of 70. In addition, patients with mismatched donors will receive an additional dose of ATG.

Given the modification in the patient population, treatment, and the classification of risk used for patient evaluation, we proposed amendment 3 to the protocol to accrue an additional 30 patients to each of the six strata, determined by the two risk groups and the three cytoreductive regimens (section 4.2). The stopping rules below will be used to monitor the additional 30 patients for excessive graft failure, day 100 graft versus host disease, and day 100 non-relapse related mortality.

An analysis of the initial set of patients on this protocol indicated that the day 100 acute graft versus host disease occurred more frequently in patients with a mismatched donor and that the ratio of matched to mismatched donors was approximately 3:1. As a result, for the additional 30 patients, the projected unacceptable failure rate for day 100 acute graft versus host disease has been updated to reflect the differential failure and accrual rates. The updated stopping boundaries for graft failure, day 100 acute graft versus host disease, and day 100 non-relapse related mortality are the same for each cytoreduction regimen but may differ with respect to patient risk.

As a result of the changes (detailed above) to the study design, accrual for the study re started from zero as of the Amendment 3 approval on 5/31/11.

An additional treatment regimen, consisting of Melphalan, Fludarabine, and Thiotepa, will be administered to the poor risk patients on this protocol as defined in the Eligibility Criteria section. This regimen was added to the protocol with the approval of Amendment 7 on 12/4/2012. Thirty (30) poor risk patients will receive this fourth conditioning regimen will be monitored for safety according to the stopping boundaries provided in the Table below:

Table of Stopping boundaries for the poor risk group

Failure Endpoint	# of failures needed to stop the arm	Failure rate in the population	Probability boundary is crossed
Graft failure	2 in the first 26 patients	0.02	0.10
	3 within 30 patients	0.15	0.93
Acute GvHD at d100 (Grades 3-4)	3 in the first 16 patients	0.06	0.10
	4 in the first 26 patients 5 within 30 patients	0.23	0.91
Non-relapse mortality by d100	3 in the first 11 patients	0.08	0.10
	4 in the first 19 patients 5 in the first 27 patients 6 within 30 patients	0.25	0.88

Amendment 9 was approved on 5/28/2013. Accrual adjustments for arm B and the updated stopping rules are detailed below:

In addition, recent data at MSKCC suggests that the addition of immune gammaglobulin (IVIG) added to the incubation fluid at a concentration of 1.5 mg IVIG/ml during incubation of the antiCD34 beads with the mobilized PBSC results in a reduction in the rate of grade 2-4 acute graft versus host disease (GVHD). For HLA-matched transplant patients treated on this protocol, 19/98 patients without the addition of IVIG patients have experienced grade 2-4 acute GVHD, whereas there are no reports of acute GVHD in 15 patients treated with immune gammaglobulin exposed products with a minimum follow up of 100 days. We plan to continue the exploration of the IVIG effect in this protocol; however, accrual for cohort B in this study is near completion. As a result, we will expand this cohort and accrue an additional 20 patients. In order to ensure patient safety in this additional cohort, if at any time during accrual 2 patients experience grade 3-4 acute graft versus host disease at day 100 or 2 patients experience graft failure, accrual will stop. The stopping boundaries for the other patient cohorts remain in effect.

As result of the increased accrual proposed in Amendment 15, accrual adjustments for arm A and the updated stopping rules are detailed below:

In order to further evaluate the efficacy of standard risk patients treated on arm A, accrual for this cohort/risk group was increased by an additional 20 patients. For these additional 20 patients, in order to ensure patient safety in this additional cohort, if at any time during accrual 2 patients experience grade 3-4 acute graft versus host disease at day 100 or 2 patients experience graft failure, accrual will stop in Arm A.

Patient accrual for the poor risk and standard risk groups treated with the Arm B regimen is nearly complete. **As a result, after accrual of the additional 20 patients added with amendment 9 is complete, and the stopping boundaries have not been exceeded, we propose to accrue an additional 60 patients to each risk group on this arm with Amendment 15.** These additional patients will provide a more precise estimate of the disease-free and overall survival in this patient population. The stopping rules below will be used to monitor the additional 120 patients for excessive graft failure, graft versus host disease, and non-relapse mortality.

At the conclusion of the study, Kaplan-Meier estimates of overall and disease-free survival will be computed over time. In addition, the probability of graft failure, non-relapse mortality, and acute and chronic graft-versus-host disease will be calculated using the cumulative incidence function. The proportion of patients receiving optimal CD34+, CD3+, and CD3+ T-cell doses will be computed. A logistic regression model and a Cox proportional hazards model will be used to correlate the dose of CD34+ progenitors and CD3+ T cells with engraftment, GvHD, and non-relapse mortality.

As a result of four treatment related deaths within the first nineteen patients in the poor risk group of Arm C have been observed, accrual adjustments for the poor risk group of Arm C are detailed below:

Although the stopping boundary for this risk group has been crossed, accrual will continue. The number of treatment related deaths in this group will continue to be monitored and if the observed proportion exceeds 30% at any time during the study, accrual will be suspended.

Table of stopping boundaries poor risk group

Failure endpoint	# of failures needed to stop accrual within group	Failure rate in the population	Probability boundary is crossed
Graft failure	4 in the first 30 patients 5 in the first 40 patients 6 in the first 50 patients 7 within 60 patients	0.05 0.15	0.09 0.88
Acute GvHD at d100 (Grades 3-4)	5 in the first 20 patients 7 in the first 30 patients 8 in the first 40 patients 9 in the first 50 patients 11 within 60 patients	0.10 0.25	0.10 0.95
Non-relapse mortality at d100	5 in the first 20 patients 7 in the first 30 patients 8 in the first 40 patients 9 in the first 50 patients 11 within 60 patients	0.10 0.25	0.10 0.95

Table of stopping boundaries – standard risk group

Failure endpoint	# of failures needed to stop accrual within group	Failure rate in the population	Probability boundary is crossed
Graft failure	4 in the first 30 patients 5 in the first 40 patients 6 in the first 50 patients 7 within 60 patients	0.05 0.15	0.09 0.88
Acute GvHD at d100 (Grades 3-4)	5 in the first 20 patients 7 in the first 30 patients 8 in the first 40 patients 9 in the first 50 patients 11 within 60 patients	0.10 0.25	0.10 0.95
Non-relapse mortality at d100	4 in the first 30 patients 5 in the first 40 patients 6 in the first 50 patients 7 within 60 patients	0.05 0.15	0.09 0.88

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. 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. The individual signing the Eligibility Checklist is confirming whether or not the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration). **15.2 Randomization**

This research study does not require a randomization.

16.1 DATA MANAGEMENT ISSUES

A Clinical Research Coordinator (CRC) will be assigned to the study and will be responsible for both pediatric and adult accruals. The responsibilities of the CRC and principal investigator include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into The Clinical Research Data Base (CRDB), a secure database. Source documentation will be available to support the computerized patient record.

Dr. Guenther Koehne at Miami Cancer Institute will assist with data analysis. Identifiable data will not leave MSKCC, and only de-identified data will be shared over secured email. Dr. Koehne has cared for some of these patients and has familiarity with this clinical entity in his capacity as a clinician who was previously the Co-Principal Investigator for this study at MSKCC.

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

The risks associated with a T-cell depleted transplant after cytoreduction by any of the four conditioning regimens under study are those associated with the toxicities of the conditioning regimens, as detailed in Section 11.0, as well as the risks of an allogeneic transplant, particularly graft failure, or graft vs. host disease, as also detailed in Section 11.0.

To protect against the toxicities of the cytoreductive regimens, the patient will be transplanted in a single room, HEPA filtered environment. Organ toxicities such as mucositis, enteritis and hepatic dysfunction as well as infectious complications will be treated by standard procedures developed for transplantation to support our patients. Blood and platelet counts will be supported by transfusion. Graft failure might necessitate a second transplant, after additional conditioning. Approaches to the diagnosis and treatment of graft failure that secure consistent engraftment have been developed by the Transplantation Services. Similarly, advanced treatments will be instituted in the event the patient develops graft vs. host disease.

Despite a transplant, the patient's disease may recur. In this case, standard and/or experimental therapies, such as phase I/II drugs, antibodies or cell therapies, will be available to the patient for consideration as treatment options.

Benefits:

A transplant is administered with curative intent. The approaches being evaluated may achieve this goal and may also be effective in preventing acute and chronic graft vs. host disease.

The results of this study will also define risks and benefits of T-cell depleted grafts fractionated on the CliniMACS device, when administered after each of the cytoreduction regimen proposed for study. This may greatly accelerate further development of transplantation approaches employing this approach to T-cell depletion.

Consent Process: Participation in this study is voluntary. All patients will be required to sign a statement of informed consent which must conform to MSKCC IRB guidelines.

Alternatives: Enrollment in this study is voluntary. Alternative treatment options will be presented to the patient prior to taking part in this study. Alternative treatment options may include getting a transplant from a volunteer unrelated donor, if one is available; getting treatment for the cancer with either chemotherapy or a transplant without being on a study; taking part in another study; or getting no treatment.

Costs: 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. Research tests will be done at no cost to the patient.

Confidentiality: Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential.

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).

The consent indicates that individualized de identified information collected for the purposes of this study may be shared with other qualified researchers. Only researchers who have received approval from MSK will be allowed to access this information which will not include protected health information, such as the participant's name, except for dates. It is also stated in the Research Authorization that their research data may be shared with other qualified researchers.

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 participant 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 starts investigational treatment/intervention. SAE reporting is required for 30-days after the participant's last investigational treatment/intervention. Any event

that occur after the 30-day period that is unexpected and at least possibly related to protocol treatment must be reported.
Please note: Any SAE that occurs prior to the start of investigational treatment/intervention and is related to a screening test or procedure (i.e., a screening biopsy) must be reported.
All SAEs must be submitted in PIMS. If an SAE requires submission to the HRPP office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be submitted within 5 calendar days of the event. All other SAEs must be submitted within 30 calendar days of the event.
The report should contain the following information:

• The date the adverse event occurred
• The adverse event
• The grade of the event
• Relationship of the adverse event to the treatment(s)
• If the AE was expected
• Detailed text that includes the following
  o An explanation of how the AE was handled
  o A description of the participant's condition
  o Indication if the participant 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

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

17.2.1

This protocol has an IND, therefore, the SAE will also be reported to the FDA through the IND Office and the report will include the FDA assigned IND number and name.

17.2.2 Definition of SAE

An SAE is an undesirable experience that meets one of the following criteria:

- Is fatal or life-threatening
- Is disabling
- Results in a congenital anomaly or occurrence of malignancy
- Important medical event that jeopardizes the participant AND requires medical or surgical intervention to prevent one of the outcomes above
  Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

Attribution:

- Unrelated: The AE *is clearly NOT related* to the device
- Unlikely: The AE *is doubtfully related* to the device
- Possible: The AE *may be related* to the device
- Probable: The AE *is likely related* to the device
- Definite: The AE *is clearly related* to the device

Expected and Unexpected Event:

- Expected: Any experience *previously reported* (in nature, severity, or incidence) in the current Investigator's Brochure for the CliniMACS device or general investigational plan
- Unexpected: Any experience *not previously reported* (in nature, severity, or incidence) in the current Investigator's Brochure for the CliniMACS device or general investigational plan

REPORTABLE EVENTS:

- Grades 1-2: Adverse Event Reporting NOT required.
- Grades 3-4: Possible, Probable or Definitely attributed to the CliniMACS device, (as detailed as 11.2.1), will be reported *.
- Grades 5: Regardless of Attribution will be reported*.

*Reportable events are those which occur within 30 days of the last dose of treatment on protocol. Events beyond 30 days will be reported at the discretion of the PI.

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.

19.0 REFERENCES

1. Cornelissen, J. J., W. L. van Putten, et al. (2007). "Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom?" Blood 109(9): 3658-3666.

2. Koreth, J., R. Schlenk, et al. (2009). "Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials." JAMA 301(22): 2349-2361.
3. Brochstein, J. A., N. A. Kernan, et al. (1987). "Allogeneic bone marrow transplantation after hyperfractionated total-body irradiation and cyclophosphamide in children with acute leukemia." N Engl J Med 317(26): 1618-1624.
4. Barrett, A. J., M. M. Horowitz, et al. (1994). "Bone marrow transplants from HLA-identical siblings as compared with chemotherapy for children with acute lymphoblastic leukemia in a second remission." N Engl J Med 331(19): 1253-1258.
5. Anderson, J. E., F. R. Appelbaum, et al. (1993). "Allogeneic bone marrow transplantation for 93 patients with myelodysplastic syndrome." Blood 82(2): 677-681.
6. Castro-Malaspina, H., R. E. Harris, et al. (2002). "Unrelated donor marrow transplantation for myelodysplastic syndromes: outcome analysis in 510 transplants facilitated by the National Marrow Donor Program." Blood 99(6): 1943-1951.
7. Woessmann W, Peters C, Lenhard M, Burkhardt B, Sykora KW, Dilloo D, Kremens B, Lang P, Führer M, Kühne T, Parwaresch R, Ebell W, Reiter A. Allogeneic haematopoietic stem cell transplantation in relapsed or refractory anaplastic large cell lymphoma of children and adolescents--a Berlin-Frankfurt-Münster group report. Br J Haematol. 2006 Apr;133(2):176-82.
8. Majolino I, Pearce R, Taghipour G, Goldstone AH. Peripheral-blood stem-cell transplantation versus autologous bone marrow transplantation in Hodgkin's and non-Hodgkin's lymphomas: a new matched-pair analysis of the European Group for Blood and Marrow Transplantation Registry Data. Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol. 1997 Feb;15(2):509-17.
9. Bensinger WI. Role of autologous and allogeneic stem cell transplantation in myeloma. Leukemia. 2009 Mar;23(3):442-8. Epub 2009 Jan 29.
10. Blume, K. G., S. J. Forman, et al. (1987). "Allogeneic bone marrow transplantation for acute lymphoblastic leukemia during first complete remission." Transplantation 43(3): 389-392.
11. Chao, N. J., S. J. Forman, et al. (1991). "Allogeneic bone marrow transplantation for high-risk acute lymphoblastic leukemia during first complete remission." Blood 78(8): 1923-1927.
12. Suciu S, Mandelli F, de Witte T, Zittoun R, Gallo E, Labar B, De Rosa G, Belhabri A, Giustolisi R, Delarue R, Liso V, Mirto S, Leone G, Bourhis JH, Fioritoni G, Jehn U, Amadori S, Fazi P, Hagemeijer A, Willemze R; EORTC and GIMEMA Leukemia Groups. Allogeneic compared with autologous stem cell transplantation in the treatment of patients younger than 46 years with acute myeloid leukemia (AML) in first complete remission (CR1): an intention-to-treat analysis of the EORTC/GIMEMAAML-10 trial. Blood. 2003 Aug 15;102(4):1232-40. Epub 2003 Apr 24.
13. Nesbit, M. E., Jr., J. D. Buckley, et al. (1994). "Chemotherapy for induction of remission of childhood acute myeloid leukemia followed by marrow transplantation or multiagent chemotherapy: a report from the Childrens Cancer Group." J Clin Oncol 12(1): 127-135.
14. Amadori, S., A. M. Testi, et al. (1993). "Prospective comparative study of bone marrow transplantation and postremission chemotherapy for childhood acute myelogenous leukemia. The Associazione Italiana Ematologia ed Oncologia Pediatrica Cooperative Group." J Clin Oncol 11(6): 1046-1054.
15. Woods, W. G., S. Neudorf, et al. (2001). "A comparison of allogeneic bone marrow transplantation, autologous bone marrow transplantation, and aggressive chemotherapy in children with acute myeloid leukemia in remission." Blood 97(1): 56-62.

16. Smith AR, Baker KS, Defor TE, Verneris MR, Wagner JE, Macmillan ML. Hematopoietic cell transplantation for children with acute lymphoblastic leukemia in second complete remission: similar outcomes in recipients of unrelated marrow and umbilical cord blood versus marrow from HLA matched sibling donors. Biol Blood Marrow Transplant. 2009 Sep;15(9):1086-93.
17. Moore J, Nivison-Smith I, Goh K, Ma D, Bradstock K, Szer J, Durrant S, Schwarer A, Bardy P, Herrmann R, Dodds A. Equivalent survival for sibling and unrelated donor allogeneic stem cell

transplantation for acute myelogenous leukemia. Biol Blood Marrow Transplant. 2007 May;13(5):601-7. Epub 2007 Mar 23.

18. Chao, N. J., G. M. Schmidt, et al. (1993). "Cyclosporine, methotrexate, and prednisone compared with cyclosporine and prednisone for prophylaxis of acute graft-versus-host disease." N Engl J Med 329(17): 1225-1230.
19. Nash, R. A., J. H. Antin, et al. (2000). "Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors." Blood 96(6): 2062-2068.
20. Ratanatharathorn, V., R. A. Nash, et al. (1998). "Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation." Blood 92(7): 2303-2314.
21. Gratwohl, A., R. Brand, et al. (2002). "Graft-versus-host disease and outcome in HLA-identical sibling transplantations for chronic myeloid leukemia." Blood 100(12): 3877-3886.
22. Martin, P. J., G. Schoch, et al. (1990). "A retrospective analysis of therapy for acute graft-versus-host disease: initial treatment." Blood 76(8): 1464-1472.
23. Sasazuki T, Juji T, Morishima Y, Kinukawa N, Kashiwabara H, Inoko H, Yoshida T, Kimura A, Akaza T, Kamikawaji N, Kodera Y, Takaku F. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. Japan Marrow Donor Program. N Engl J Med. 1998 Oct 22;339(17):1177-85.
24. Petersdorf EW, Kollman C, Hurley CK, Dupont B, Nademanee A, Begovich AB, Weisdorf D, McGlave P. Effect of HLA class II gene disparity on clinical outcome in unrelated donor hematopoietic cell transplantation for chronic myeloid leukemia: the US National Marrow Donor Program Experience. Blood. 2001 Nov 15;98(10):2922-9.
25. Warren EH, Fujii N, Akatsuka Y, Chaney CN, Mito JK, Loeb KR, et al. Therapy of relapsed leukemia after allogeneic hematopoietic cell transplant with T cells specific for minor histocompatibility antigens. Blood. 2010.
26. Reisner Y, Kapoor N, Kirkpatrick D, Pollack MS, Dupont B, Good RA, O'Reilly RJ. Transplantation for acute leukaemia with HLA-A and B nonidentical parental marrow cells fractionated with soybean agglutinin and sheep red blood cells. Lancet. 1981 Aug 15;2(8242):327-31.
27. Wagner, J. E., G. W. Santos, et al. (1990). "Bone marrow graft engineering by counterflow centrifugal elutriation: results of a phase I-II clinical trial." Blood 75(6): 1370-1377.
28. Waldmann, H., A. Polliak, et al. (1984). "Elimination of graft-versus-host disease by in-vitro depletion of alloreactive lymphocytes with a monoclonal rat anti-human lymphocyte antibody (CAMPATH-1)." Lancet 2(8401): 483-486.
29. Soiffer, R. J., D. Fairclough, et al. (1997). "CD6-depleted allogeneic bone marrow transplantation for acute leukemia in first complete remission." Blood 89(8): 3039-3047.
30. Wagner, J. E., J. S. Thompson, et al. (2005). "Effect of graft-versus-host disease prophylaxis on 3-year disease-free survival in recipients of unrelated donor bone marrow (T-cell Depletion Trial): a multi-centre, randomised phase II-III trial." Lancet 366(9487): 733-741.
31. Kernan, N. A., N. H. Collins, et al. (1986). "Clonable T lymphocytes in T cell-depleted bone marrow transplants correlate with development of graft-v-host disease." Blood 68(3): 770-773.
32. Lowenberg, B., G. Wagemaker, et al. (1986). "Graft-versus-host disease following transplantation of 'one log' versus 'two log' T-lymphocyte-depleted bone marrow from HLA-identical donors." Bone Marrow Transplant 1(2): 133-140.
33. Beatty PG. Marrow transplantation using volunteer unrelated donors in a comparison of mismatched family donor transplants: a Seattle perspective. Bone Marrow Transplant. 1994;14 Suppl 4:S39-41.
34. Couban, S., D. R. Simpson, et al. (2002). "A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies." Blood 100(5): 1525-1531.
35. Gale, R. P., M. M. Bortin, et al. (1987). "Risk factors for acute graft-versus-host disease." Br J Haematol 67(4): 397-406.

36. Patel B, Kirkland KE, Szydlo R, Pearce RM, Clark RE, Craddock C, Liakopoulou E, Fielding AK, Mackinnon S, Olavarria E, Potter MN, Russell NH, Shaw BE, Cook G, Goldstone AH, Marks DI. Favorable outcomes with alemtuzumab-conditioned unrelated donor stem cell transplantation in adults with high-risk Philadelphia chromosome-negative acute lymphoblastic leukemia in first complete remission. Haematologica. 2009 Oct;94(10):1399-406. Epub 2009 Jul 31.
37. Hahn T, McCarthy PL Jr, Zhang MJ, Wang D, Arora M, Frangoul H, Gale RP, Hale GA, Horan J, Isola L, Maziarz RT, van Rood JJ, Gupta V, Halter J, Reddy V, Tiberghien P, Litzow M, Anasetti C, Pavletic S, Ringdén O. Risk factors for acute graft-versus-host disease after human leukocyte antigen-identical sibling transplants for adults with leukemia. J Clin Oncol. 2008 Dec 10;26(35):5728-34. Epub 2008 Nov 3.
38. Loren AW, Bunin GR, Boudreau C, Champlin RE, Cnaan A, Horowitz MM, Loberiza FR, Porter DL. Impact of donor and recipient sex and parity on outcomes of HLA-identical sibling allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2006 Jul;12(7):758-69.
39. Flowers ME, Pepe MS, Longton G, Doney KC, Monroe D, Witherspoon RP, Sullivan KM, Storb R. Previous donor pregnancy as a risk factor for acute graft-versus-host disease in patients with aplastic anaemia treated by allogeneic marrow transplantation. Br J Haematol. 1990 Apr;74(4):492-6.
40. Papadopoulos, E. B., M. H. Carabasi, et al. (1998). "T-cell-depleted allogeneic bone marrow transplantation as postremission therapy for acute myelogenous leukemia: freedom from relapse in the absence of graft-versus-host disease." Blood 91(3): 1083-1090.
41. Aversa, F., A. Terenzi, et al. (1999). "Improved outcome with T-cell-depleted bone marrow transplantation for acute leukemia." J Clin Oncol 17(5): 1545-1550.
42. Aversa, F., A. Terenzi, et al. (2005). "Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse." J Clin Oncol 23(15): 3447-3454.
43. Handgretinger, R., T. Klingebiel, et al. (2001). "Megadose transplantation of purified peripheral blood CD34(+) progenitor cells from HLA-mismatched parental donors in children." Bone Marrow Transplant 27(8): 777-783.
44. Jakubowski, A. A., T. N. Small, et al. (2007). "T cell depleted stem-cell transplantation for adults with hematologic malignancies: sustained engraftment of HLA-matched related donor grafts without the use of antithymocyte globulin." Blood 110(13): 4552-4559.
45. Urbano-Ispizua, A., C. Rozman, et al. (2002). "Risk factors for acute graft-versus-host disease in patients undergoing transplantation with CD34+ selected blood cells from HLA-identical siblings." Blood 100(2): 724-727.
46. Devine S, Soiffer R, Pasquini M, Carter S, Hari P, DeVore S, Stein A, Lazarus H, Linker C, Stadtmauer E, Keever-Taylor C, O'Reilly RJ. HLA-identical sibling matched, CD34^{+} selected, T cell depleted peripheral blood stem cells following myeloablative conditioning for first or second remission acute myeloid leukemia (AML): Results of blood and marrow transplant clinical trials network (BMT CTN) Protocol 0303. Blood, 114 (22),273.
47. Lucas KG, Small TN, Heller G, Dupont B, O'Reilly RJ. The development of cellular immunity to Epstein-Barr virus after allogeneic bone marrow transplantation. Blood. 1996 Mar 15;87(6):2594-603.
48. van Esser JW, van der Holt B, Meijer E, Niesters HG, Trenschel R, Thijsen SF, van Loon AM, Frassoni F, Bacigalupo A, Schaefer UW, Osterhaus AD, Gratama JW, Löwenberg B, Verdonck LF, Cornelissen JJ. Epstein-Barr virus (EBV) reactivation is a frequent event after allogeneic stem cell transplantation (SCT) and quantitatively predicts EBV-lymphoproliferative disease following T-cell--depleted SCT. Blood. 2001 Aug 15;98(4):972-8.
49. van Esser JW, Niesters HG, van der Holt B, Meijer E, Osterhaus AD, Gratama JW, Verdonck LF, Löwenberg B, Cornelissen JJ. Prevention of Epstein-Barr virus-lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation. Blood. 2002 Jun 15;99(12):4364-9.
50. Papadopoulos, E. B., M. Ladanyi, et al. (1994). "Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation." N Engl J Med 330(17): 1185-1191.

51. O'Reilly, R. J., T. N. Small, et al. (1998). "Adoptive immunotherapy for Epstein-Barr virus-associated lymphoproliferative disorders complicating marrow allografts." Springer Semin Immunopathol 20(3-4): 455-491.
52. Rooney, C. M., C. A. Smith, et al. (1995). "Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation." Lancet 345(8941): 9-13.
53. Kernan, N. A., C. Bordignon, et al. (1989). "Graft failure after T-cell-depleted human leukocyte antigen identical marrow transplants for leukemia: I. Analysis of risk factors and results of secondary transplants." Blood 74(6): 2227-2236.
54. Bordignon, C., C. A. Keever, et al. (1989). "Graft failure after T-cell-depleted human leukocyte antigen identical marrow transplants for leukemia: II. In vitro analyses of host effector mechanisms." Blood 74(6): 2237-2243.
55. Rowlings, P. A., D. Przepiorka, et al. (1997). "IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade." Br J Haematol 97(4): 855-864.
56. Sullivan KM, Witherspoon RP, Storb R, Weiden P, Flournoy N, Dahlberg S, et al. Prednisone and azathioprine compared with prednisone and placebo for treatment of chronic graft-v-host disease: prognostic influence of prolonged thrombocytopenia after allogeneic marrow transplantation. Blood. 1988;72:546-554.
57. Cancer therapy and evaluation program cancer terminology criteria for adverse events. Version 4. NCI, NIH, 2003.
58. Small, T. N., D. Avigan, et al. (1997). "Immune reconstitution following T-cell depleted bone marrow transplantation: effect of age and posttransplant graft rejection prophylaxis." Biol Blood Marrow Transplant 3(2): 65-75.
59. Small, T. N., E. B. Papadopoulos, et al. (1999). "Comparison of immune reconstitution after unrelated and related T-cell-depleted bone marrow transplantation: effect of patient age and donor leukocyte infusions." Blood 93(2): 467-480.
60. Keever, C. A., T. N. Small, et al. (1989). "Immune reconstitution following bone marrow transplantation: comparison of recipients of T-cell depleted marrow with recipients of conventional marrow grafts." Blood 73(5): 1340-1350.
61. Venstrom J, Gooley T, Spellman S, et al. Donor activating KIR3DS1 is associated with decreased acute GvHD in unrelated allogeneic hematopoietic stem cell transplantation. Blood. 2010;115.
62. Venstrom J, Pittari G, Chewning J, et al. Donor KIR2DS1 and KIR3DS1 are associated with improved outcomes following unrelated allogeneic stem cell transplantation for acute myeloid leukemia. Biol Blood Marr Transpl. 2011;[Abstract].
63. Cooley S, Weisdorf D, Guethlein L, et al. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. Blood. 2010;116:2411-2419.
64. Giglio F, Venstrom J, Gallagher M, et al. KIR3DL1/S1 and HLA-B alleles combine to influence unrelated hematopoietic stem cell transplantation (HSCT) outcomes Biol Blood Marr Transpl. 2012;[Abstract].
65. Cook MA, Milligan DW, Fegan CD, et al. The impact of donor *KIR* and patient *HLA-C* genotypes on outcome following HLA-identical sibling hematopoietic stem cell transplantation for myeloid leukemia. Blood. 2004;103:1521-1526.
66. Giebel S, Locatelli F, Wojnar J, et al. Homozygosity for human leucocyte antigen-C ligands of KIR2DL1 is associated with increased risk of relapse after human leucocyte antigen-C-matched unrelated donor haematopoietic stem cell transplantation. Br J Haematol. 2005;131:483-486.
67. Gagne K, Busson M, Bignon J, et al. Donor KIR3DL1/3DS1 gene and recipient Bw4 KIR ligand as prognostic markers for outcome in unrelated hematopoietic stem cell transplantation. Biol Blood Marr Transpl. 2009;15:1366-1375.

20.0 APPENDICES

There are no appendices with this protocol.