A Phase II Trial of Alpha Beta T-cell and CD19 B-cell depleted Peripheral Blood Stem Cell Transplantation using the CliniMACS System for patients with Non-Malignant Hematologic Disorders from matched or mismatched, related or unrelated donors

PROTOCOL FACE PAGE FOR
MSK THERAPEUTIC/DIAGNOSTIC PROTOCOL

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

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- **1.0 PROTOCOL SUMMARY AND/OR SCHEMA**

This is a Phase II trial for the use of

- Two specific chemotherapy-only cytoreductive regimens including Melphalan Thiotepa + [Clofarabine or Fludarabine], followed by
- GCSF-mobilized peripheral blood stem cell (PBSC) grafts from HLA-matched or HLA-mismatched, related or unrelated donors
- Using a novel T-cell depletion method with TCR αβ T-cell depletion and CD19 B-cell depletion using the Miltenyi CliniMACS device
- In patients with non-malignant hematologic disorders

The primary objective of the trial is to assess two-year overall survival.

The secondary objectives of the trial are to assess:

1. Neutrophil engraftment following T- and B- cell depleted grafts in patients with non-malignant hematologic disorders using a chemotherapy-only cytoreduction.
2. The rate of grade III-IV acute and chronic GvHD following this novel T- and B- cell depleted approach.
3. Graft composition CD34, T-α/β, T-γδ, B, and NK cells post T-cell and B-cell depletion.
4. The immune reconstitution following α/β T-cell depleted stem cell transplant as measured at approximately 1, 2, 3, 6, and 12 months.
5. The rate and specifics of the immune reconstitution post transplant compared to those achieved in CD34 positively selected grafts as part of protocol 10-050.
6. The CD34+ cell dose using this negative selection method with TCR alpha beta T-cell and CD19 B-cell depletion method and compare to the cell dose achieved using a positive selection method in CD34 positive grafts as part of protocol 10-050.
7. The incidence of post-transplant infectious complications, including CMV, EBV, adenovirus and HHV6 (viremia, organ involvement and infection).
8. The incidence of transplant-related mortality and of disease free survival post transplant.

For this trial, patients will be assigned to receive one of two conditioning regimens, based on their disease, disease severity, organ status and history of red blood cell alloimmunization. Both regimens have demonstrated safety and efficacy in facilitating engraftment of CD34+ selected allografts in MSKCC protocol 10-050.

The two conditioning regimens are:

Regimen A: Melphalan/Thiotepa/Clofarabine
Melphalan 70 mg/m2/day x 2, Thiotepa 7.5 mg/kg/day x 2 and Clofarabine 20-30 mg/m2/day x 5.

Regimen B: Melphalan/Thiotepa/ Fludarabine
Melphalan 70 mg/m2/day x 2, Thiotepa 7.5 mg/kg/day x 2 and Fludarabine 30 mg/m2/day x 5.

Patients will also receive rabbit anti-thymocyte globulin at 2.5 mg/kg/day x 3 doses prior to the start of conditioning. If a severe adverse reaction to rabbit ATG occurs, the patient will receive horse ATG for subsequent doses. If hATG is not tolerated, alemtuzumab will be requested through the Campath Distribution Program.

On day -1, patients will receive rituximab 200mg/m2 for in vivo donor and recipient B-cell depletion to reduce the risk of Epstein-Barr virus (EBV)-related posttransplantation lymphoproliferative disease (PTLD).

Approximately twenty-four to forty-eight hours after completion of cytoreduction, patients will receive an αβ T-cell and CD-19 depleted, GCSF-mobilized PBSC transplant.

Donors for patients enrolled on this protocol will be

HLA matched related	HLA matched unrelated
HLA mismatched related	HLA mismatched unrelated

Stratification will be by donor. The sample size is as follows:

Donor Type 1 (Related or Unrelated ≥ 7/8 HLA-antigen matched): 30 patients
Donor Type 2 (Related donors 4-6/8 HLA-antigen matched): 30 patients

2.1 OBJECTIVES AND SCIENTIFIC AIMS

Primary
The primary objective of the trial is to assess: Two-year overall survival.

Secondary:
The secondary objectives of the trial are to assess:

1. Neutrophil engraftment following T- and B- cell depleted grafts in patients with non-malignant hematologic or immune disorders using a chemotherapy-only cytoreduction.
2. The rate of grade III-IV acute and chronic GvHD following this novel T- and B- cell depleted approach.
3. Graft composition CD34, T-α/β, T-γδ, B, and NK cells post T-cell and B-cell depletion.
4. The immune reconstitution following α/β T-cell depleted stem cell transplant as measured at approximately 1, 2, 3, 6, and 12 months.
5. The rate and specifics of the immune reconstitution post transplant compared to those achieved in CD34 positively selected grafts as part of protocol 10-050.
6. The CD34+ cell dose using this Negative Selection method with TCR alpha beta T-cell and CD19 B-cell depletion method and compare to the cell dose achieved using a Positive Selection method in CD34 positive grafts as part of protocol 10-050.
7. The incidence of post-transplant infectious complications, including CMV, EBV, adenovirus and HHV6 (viremia, organ involvement and infection).
8. The incidence of transplant-related mortality and of disease free survival post transplant.

3.0 BACKGROUND AND RATIONALE

Allogeneic hematopoietic cell transplantation (alloHCT) has long been recognized as a cure for many life threatening non-malignant hematologic and immunologic disorders such as sickle cell disease, thalassemia major, inherited and acquired bone marrow failure syndromes, and immunodeficiency disorders. While HCT offers the only curative option for patients resistant to standard therapies, it may be associated with acute and chronic morbidity and mortality secondary to both the cytoreductive regimens and transplant related complications, primarily GVHD. AlloHCT from donors other than HLA-matched siblings has been performed primarily using unmodified grafts, while T-cell depleted transplants have traditionally required conditioning regimens that include total body irradiation (TBI), especially for recipients of related mismatched or unrelated donors, due to the higher risk of graft rejection in multiply transfused patients. However, this approach leads to a high risk

for GVHD and to the late effects of TBI. In patients with non-malignant hematological disorders, reducing toxicity of cytoreduction using chemotherapy only and reducing the chance of GVHD by using T-cell depletion are fundamental in reducing acute and late complications.

Only 25% of patients who are candidates for allogeneic transplantation have matched related donors. In addition, for patients with genetic disorders, potential matched related donors may not be suitable as they may be affected by the genetic disease. For patients lacking a matched related donor, the likelihood of finding an unrelated donor depends on the frequency of the patient's HLA haplotype as determined by racial and ethnic background.

Based on recent data from our institution, 20% of patients had related stem cell donors, while 80% of patients required transplants from unrelated or mismatched related donors. Alternative donor transplantation, using haploidentical related donors is being increasingly used for many individuals, particularly those belonging to minority groups. However, advances in alternative donor transplantation have been hindered by GVHD and graft rejection, mediated by donor and recipient alloreactive T-cell response, respectively.(1)

The risk of acute GVHD following allogeneic 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 (2-5). Grades II-IV acute GVHD is 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). Higher rates of acute and chronic GVHD are observed following matched unrelated (6) and unmanipulated haploidentical transplants (>30% and >70% respectively) (7, 8).

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 (9-13). Comparative analyses have demonstrated that these techniques vary widely in their capacity to deplete T-cells (1.5-3.5 $\log_{10}$ depletion) (14). 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 (15-18), as well as patient and donor characteristics including underlying disease, age, gender and parity (19-21). Our previous studies in more than 200 patients 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.

Two 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) (9, 22) and the University of Perugia (23). This method is 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 and transplantation of these $CD34^+$ cell-enriched, T-cell depleted cell fractions (24, 25).

In the CliniMACS system (Miltenyi Biotec) system, stem cells (GCSF mobilized PBSC) are introduced into a 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 (26). In a multicenter trial conducted by the BMT Clinical Trials Network under an FDA IND, for which Dr. Richard 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 HCT fractionated by this approach were administered after cytoreduction with our protocol of hyperfractionated TBI, thiotepa and cyclophosphamide in patients with hematologic malignant disorders. 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.

We retrospectively analyzed data on 18 patients affected by non-malignant hematologic disorders lacking an HLA-identical sibling donor who underwent an alternative donor allogeneic HCT at our institution between 2005 and 2013. Of those, 50% had received immunosuppressive treatments, 72% had a history of infections, and 56% were transfusion-dependent at the time of transplant. Cytoreduction included a combination of three of five agents: fludarabine, melphalan, thiotepa, busulfan and cyclophosphamide. Grafts were T-cell depleted. All evaluable patients engrafted; one developed secondary graft rejection. Five patients died of transplant complications. The cumulative incidence of GvHD was 6%. No patient had recurrence of their non-malignant disease. 5-year overall survival was 77%. Age at transplant <6 years was strongly associated with better survival. Based on these results, transplant with chemotherapy-only cytoreductive regimens and T-cell depleted stem cell transplants could be recommended for patients with high risk non-malignant hematologic disorders (NMHD) especially at a younger age. (27) Although extensive T-lymphocyte depletion from the grafts lowers the rate of GVHD, other immune cells, which may be beneficial to engraftment and innate immunity, are also removed from the graft during CD34 selection, thus contribute to risk of graft rejection and a delayed immune reconstitution (28).

In the era of alternative donor transplantation with T-cell depleted grafts, graft rejection is a major obstacle following allogeneic HCT. A major cause of graft failure is the recipient's immune response against donor immuno hematopoietic cells. The risk of rejection or graft failure is higher in HLA-mismatched or unrelated donor grafts, T-cell depleted transplants and major ABO blood group incompatibility (29, 30). In addition, multiply transfused patients with non-malignant hematologic disorders such as sickle cell disease, thalassemia major and severe aplastic anemia, have an increased risk of graft rejection (5-60%), since their memory T-cells recognize major or minor HLA antigens on donor grafts(31, 32).

To tackle the barrier to engraftment, the use of megadose of GCSF-mobilized peripheral blood stem cells was first reported by the group of Perugia with rate of engraftment over 90% and the incidence of both grade II-IV acute and chronic GVHD less than 10% (33). In addition, aggressive inhibition of the host immune system using either myeloablative or immunoablative drugs as a conditioning regimen was used to augment the likelihood of

engraftment. For instance, the addition of hydroxyurea, azathioprine and fludarabine to busulfan/cyclophosphamide based regimen reduced the risk of rejection from 30% to 8% in high risk thalassemia major patients receiving HLA matched related stem cell transplant (34, 35).

Recently, several studies in T-cell depleted grafts have shown graft failure rates ranging from 0 to 17% (36-40) in haploidentical HCT by using fludarabine/clofarabine, thiotepa and melphalan or fludarabine-based regimens. Whereas all patients receiving matched related or unrelated donor stem cells after fludarabine, busulfan plus ATG conditioning achieved primary engraftment (41-43).

Despite downward trends in GVHD achieved by effective reduction of T cells in the graft , improved engraftment using a megadose of stem cells after GCSF mobilization, and aggressive inhibition of the host immune system by immunoablative conditioning, transplant related mortality rate is still high, ranging between 5-37% (44-47) in pediatric studies. This unacceptably high mortality is attributed to delayed immune reconstitution. (24)

However, there is significant evidence of lower rate of mortality secondary to infection in patients achieving faster T- and B-cell lymphocyte counts compared with those did not during the first 6 months or even during the whole first year post SCT (48).

It is known that, γδ T cells (also termed “innate-like” T cells) constitute 1-10% of peripheral blood lymphocytes and have several functions, including response against intracellular and extracellular pathogens. These cells can recognize their targets in an MHC-independent manner through activating receptors NKG2D, TLRs, DNAM-1 and exhibit a pre-activate phenotype allowing rapid cytokine production suchas IFN- γ and TNF-α and strong cytotoxic response upon activation (36). In contrast, αβ T cells are restricted by MHC molecules, which lead to increased risk of GVHD (37). Hence, a new technique of graft manipulation to hasten immune recovery relies on removal of alpha beta T lymphocytes by using a biotinylated anti-TcR αβ antibody followed by an anti-biotin antibody conjugated to magnetic microbeads. This approach preserves γδ+ T cells, natural killer (NK) cells and other cells in the graft. In addition to TCRab depletion, CD19+ B-lymphocyte depletion is employed for prevention of post-transplant EBV-associated lymphoproliferative disease (49). Over the past several years, this approach has been widely and successfully used in hematopoietic stem cell transplantation for both hematological malignant and nonmalignant disorders.

The outcome of the first clinical experience in pediatrics at the Children’s University Hospital Tubingen with TcRαβ/CD19 depleted haploidentical transplantation with melphalan, thiotepa, fludarabine or clofarabine preparative regimens, showed a rapid donor engraftment and rapid T-cell immune reconstitution at day +28 post-transplant without transplant related mortality (50). Similarly, data published by Bertaina et al. shows sustained engraftment of donor hematopoiesis without GVHD occurrence in most patients and 2-year probability of disease-free survival 91.1%.(38) Several series (see appended tables) demonstrated that this method has superior outcomes with accelerated immune recovery in natural killer (NK)-, B- and T-cell subsets when be compared with positive CD34+ selected stem cells transplant. (38, 49-51)

The recovery of CD 56+ NK cells, the first lymphoid cells to emerge following HCT (48), was detectable in the first week post HCT and significantly increased at day +14 when compared to a historical control. In addition, B cell recovery started at day +30 and normalized at day +127. At day +30, TcRαβ/CD19 depleted recipients had significantly higher CD3+ and CD4+ cell numbers than control group (49, 51). For subsets of T-cells, γδ+ T cells expanded faster than TcRαβ lymphocytes in the first 30 days post HCT; however, αβ T-cells were

predominant by day +90 (40). Similarly, *Bertaina et al* have reported the results of haploidentical related HCT in nonmalignant disorders fractionated by this approach administered after cytoreduction with a fludarabine base regimen. In their experience, γδ+ T cells recovered speedily followed by gradual increase of αβ T lymphocytes in a year, with 9.3% incidence of TRM (38).

Herein we propose to evaluate the potential of HCT grafts selectively depleted of αβ+ T-cells and CD19+ B-cells by the CliniMACS system, when administered after each of two cytoreductive regimens to maximize sustained engraftment among recipients at high risk of graft rejection, particularly those with history of multiple transfusions, prevent or abrogate acute and chronic forms of GVHD and to accelerate immune reconstitution to reduce infection related morbidity and decrease treatment related mortality.

Published studies using TCR- α/β depletion include an additional depletion of CD19+ B-lymphocytes prior to allograft infusion. The purpose of this additional step is to reduce the reservoir for EBV from the donor allograft and in turn minimize the risk for donor derived EBV- post transplant lymphoproliferative disorder (PTLD). Rituximab 200 mg/m2 has also been used for in vivo donor and recipient B-cell depletion to reduce as muchas possible the risk of Epstein-Barr virus (EBV)-related posttransplantation lymphoproliferative disease (PTLD) (37). Infusion of low dose peritransplant rituximab has demonstrated efficacy to reduce EBV viremia and PTLD without impacting long term immune reconstitution (52).

At our Center, we have used the two regimens melphalan/thiotepa/clofarabine and melphalan/thiotepa/fludarabine followed by CD34 selected grafts in patients receiving HCT from alternative donors. Two patients rejected their grafts following melphalan thiotepa clofarabine, while 4 patients suffered recovery of host chimerism post transplant. Moreover, several reports of TCR- α/β and CD19 depleted transplants(38, 53) have described a rate of primary graft failure approaching 17%. In our CD34+ protocol and in the alpha beta negative reports, conditioning included thiotepa 10 mg/kg divided in two doses. We have decided to increase the dose of thiotepa for our new protocol from 10 mg/Kg to 15 mg/Kg for increased immunosuppression and myelosuppression and for the improvement of engraftment and chimerism.

The combination of melphalan 70 mg/m2/day x 2, thiotepa 5 mg/kg/day x 2 (or 10 mg/kg/day x 1) and clofarabine 20-30 mg/m2/day x 5 has been successfully used to treat 64 patients at our center with unmodified and T-cell depleted grafts from HLA-matched, mismatched related, matched unrelated and mismatched unrelated donors. Engraftment occurred in 59 of 61 evaluable patients. Grade II-IV acute GvHD occurred in 4/20 (20%) evaluable patients in the TCD group. With a median follow up of 15.4 months for the TCD group, the overall survival and disease free survival rates were 64.1% and 60.7% (54). This cytoreductive regimen has shown promise as radiation sparing conditioning for patients with acute leukemias, including in the relapsed setting (55).

The second combination of melphalan 70 mg/m2/day x 2, thiotepa 5 mg/kg/day x 2 (or 10 mg/kg/day x 1) and fludarabine 30mg/m2/day x 5, was employed as preparative conditioning for seven 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, melphalan and clofarabine alone 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 (3) patients with life threatening nonmalignant acquired and genetic disorders of hematopoiesis and immunity (27).

Summary of Rationale for the Proposed Study:

In the current study we propose to conduct HLA matched or mismatched unrelated and mismatched related donor allotransplant using TCR-α/β$^+$ cell depletion based GVHD prophylaxis. The TCR-α/β$^+$ cell depletion platform has validated results at other centers using HLA matched donors and in related HLA-haploidentical donors with an acceptable rate of acute GVHD and excellent engraftment and long-term disease control (37). This platform represents a logical extension of the CD34+ selection based GVHD prophylaxis for mismatched unrelated donors in that it allows for innate effector cells such as NK and TCR-γδ$^+$ T-cells to participate in pathogen control early after allo HCT. The proposed study addresses a shortcoming in the use of CD34+ selection for HLA mismatched donors in that diminished immune reconstitution is a major contributor to early transplant related mortality. Immune reconstitution in participants of the proposed study will be compared to those undergoing HLA mismatched allotransplant with both unmodified and CD34+ selected grafts.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

o 4.1 Design

This phase 2 trial is designed to assess the efficacy of αβ T-cell and B-cell depleted peripheral blood stem cell transplants for patients with non-malignant hematologic disorders. The study population is divided into two groups defined by donor type: 1) donors who are ≥ 7/8 HLA-antigen matched and 2) related donors who are 4-6/8 HLA-antigen matched. A maximum of 30 patients in each group is planned for accrual onto the study. The primary endpoint of the study is two-year overall survival. It is anticipated that accrual will last three years and each patient will be followed for a minimum of two years. The data analysis and data monitoring will be conducted separately in each donor group.

4.2 Intervention

Patients with non-malignant hematological disorders who fulfill eligibility requirements and consent to treatment, will receive one of two conditioning regimens, based on their disease and clinical parameters including age, prior treatment history, or the presence of comorbidities that could increase risk of graft failure or severe toxicity or in the post transplant period.

The two cytoreduction regimens to be evaluated are:

A. Melphalan/Thiotepa/Clofarabine
   a. Melphalan (70 mg/m2/day x 2)
   b. Thiotepa (7.5 mg/kg/day x 2 or 15 mg/kg/day x1)
   c. Clofarabine (20-30 mg/m2/ day x 5)

B. Melphalan/Thiotepa/Fludarabine

   a. Melphalan (70 mg/m2/day x 2)
   b. Thiotepa (7.5 mg/kg/day x 2 or 15 mg/kg/day x1)
   c. Fludarabine (30 mg/m2/ day x 5)

ATG will be given to all transplant recipients.

ATG dosing will be as follows: antithymocyte globulin (ATG) rabbit ATG 2.5 mg/kg/day x 3 or equine ATG 15 mg/kg/day x 3 (or 40 mg/kg/day x 1 equine ATG if rabbit ATG is not tolerated) during pre-transplant conditioning to deplete host T-cells that could hamper engraftment.

Donors will be evaluated according to standard National Marrow Donor Program criteria. Donors will donate a standard, G-CSF mobilized peripheral blood hematopoietic progenitor cell (HPC (A)) product that will subsequently be transferred to the MSKCC Cell Therapy Laboratory for processing. HPC (A) products will then undergo TCR-αβ+ and CD-19 depletion using the CliniMACS reagents according to the manufacturer's guidelines. Excess starting product(s) not undergoing TCR-αβ+ and CD-19 depletion may be processed for CD34 enrichment when applicable.

Following preparative cytoreduction, all patients will receive HPC (A) depleted of α/β T-cells and CD-19 B-cells. The targeted dose of progenitor cells is $\geq 5 \times 10^6$ $CD34^+$ cells/kg; the targeted dose of relevant T-cells TCR-alpha beta limited to $\leq 10 \times 10^4$ $CD3^+$ cells/kg for matched related donor and $\leq 1.0 \times 10^4$ $CD3^+$ cells/kg for alternative donor; and TCR- gamma delta $>1.0 \times 10^{6.}$

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.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Thiotepa, melphalan, fludarabine and clofarabine are standard cytoreductive agents that will be employed in the two regimens as detailed in the treatment plan. ATG will be administered for further immunoablation.

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 (SWFI) 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-micron filter prior to administration.

Storage and Stability:

1. Store vials in refrigerator and protect from light.
2. Reconstituted solutions are stable for 24 hours under refrigeration and room temperature.
3. Reconstituted solutions further diluted in NS (0.9% sodium chloride) at concentrations of 1mg/mL, 2mg/mL and 5 mg/mL are stable for up to 24 hours at room temperature and 48 hours under refrigeration.
4. Diluted solutions of 0.5 mg/mL in NS should be used immediately after preparation.

Preparation:

1. Standard IV fluid: NS
2. Final concentration range up to: 5mg/mL.

Clinical Considerations:

Hydration: NA

Emetic Potential: High

Incompatibilities: Cisplatin, filgrastim (G-CSF), vinorelbine.

5.1.3 *Melphalan (Evomela®)*

a. **Source and Pharmacology:** Supplier: Spectrum Pharmaceuticals Inc,. 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.

c. **Solution Preparation:** 50 mg/vial: Reconstitute by rapidly injecting 8.6 mL of the supplied diluent into the vial to yield a final concentration of 5 mg/mL. Shake vigorously until the solution is clear.

d. **Storage and Stability:** The intact solution should be stored at room temperature (15-30°C) protected from light. Do not refrigerate reconstituted product. Stability of prepared infusions varies by final concentration. *(*See MSK Pediatric Chemotherapy Guidelines).

Concentration of Solution (mg/mL)	Duration of Stability at Room Temperature (hours)
0.45	4
1	7
2	11
5	25

e. **Administration:** Intravenous, over 30 minutes.

5.1.4 ***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 SWFI 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 SWFI. When reconstituted with 2 mL of SWFI, 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 D5W (5% dextrose in water) or NS.

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). Infusions prepared in D5W or NS are stable for up to 16 days at 25°C.

e. **Administration:** Intravenous, over thirty minutes.

5.1.5 ***Clofarabine (Clolar™)***

a. **Formulation and Stability:**

Clofarabine is formulated at a concentration of 1 mg/mL in sodium chloride (9 mg/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 D5W or 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 simultaneously 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 SWFI. 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 SWFI 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.

5.4 <u>Rituximab (Rituxan ®)</u>

a. Source and pharmacology: Rituximab is a monoclonal antibody directed against the CD20 antigen on the surface of B-lymphocytes. CD20 regulates cell cycle initiation; and, possibly, functions as a calcium channel. Rituximab binds to the antigen on the cell surface, activating complement-dependent B-cell cytotoxicity; and to human Fc receptors, mediating cell killing through an antibody-dependent cellular toxicity. B-cells are believed to play a role in the development and progression of rheumatoid arthritis. Signs and symptoms of RA are reduced by targeting B-cells and the progression of structural damage is delayed.

b. Formulation and stability: Store intact vials at 2°C to 8°C (36°F to 46°F); do not freeze. Do not shake. Protect vials from direct sunlight. Solutions for infusion in NS or D5W are stable at 2°C to 8°C (36°F to 46°F) for 24 hours and at room temperature for an additional 24 hours (although because there is no preservative, the manufacturer recommends storing refrigerated).

c. Solution preparation: Solution, Intravenous [preservative free]: Rituxan: 10 mg/mL (10 mL, 50 mL) [contains polysorbate 80]. Rituximab for injection should be diluted in normal saline to a concentration of 1-4 mg/mL.

d. Storage and stability: Store vials under refrigeration once diluted, protect from sunlight. Diluted rituximab is stable for 24 hours refrigerated and 12 hours at room temperature.

e. Administration: Withdraw necessary amount of rituximab and dilute to a final concentration of 1 to 4 mg/mL with NS or D5W. Gently invert the bag to mix the solution. Do not shake. Do not mix or dilute with other medications. Compatible in polyvinyl chloride (PVC) and polyethylene bags. Anticipate infusion reactions, particularly in individuals who have not previously received rituximab infusion. Infusion schedule will be adjusted according to standard guidelines. Emetogenic potential is low. Patients should receive acetaminophen 10-15 mg/kg/dose PO (max 650 mg/dose) and diphenhydramine 0.5-1 mg/kg/dose PO or IV (max 50 mg/dose) 30-60 minutes prior to infusion.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

6.2.1 Lethal disorders of Hematopoiesis correctable by transplant for which α/β T-cell and CD-19 depleted allogeneic hematopoietic stem cell transplantation is indicated including:

a) Hemoglobinopathies:

- Sickle cell disease (HbSS, HbSC, $HbSB^0$ thalassemia, $HbSB^+$, HbSD, HbSE) with at least one of the following criteria (Walters *et al*):
  1. Cerebrovascular accident lasting longer than 24 hours
  2. Impaired neuropsychological function with abnormal brain MRI/MRA
  3. Recurrent hospitalizations (>2 episodes/year over several years) or exchange transfusions for acute chest syndrome
  4. Recurrent priapism
  5. Stage I or II sickle chronic lung disease
  6. Sickle cell nephropathy (moderate to severe proteinuria or glomerular filtration rate 30-50% of predicted normal value for age)
  7. Bilateral proliferative retinopathy with major visual impairment in at least one eye
  8. Osteonecrosis of multiple joints
  9. Red cell alloimmunization during chronic transfusion therapy

- Thalassemia major with at least one of the following criteria:
  1. Age <16 years
  2. Available HLA-identical sibling
  3. Red blood cell transfusion dependency
  4. Lucarelli class 1 or 2 risk status (i.e. with only 0-2 of the following factors: hepatomegaly, portal fibrosis, or poor response to chelation therapy)

5. Recurrence of disease after previous stem cell transplant

b) Bone Marrow Failure Syndromes:

- Aplastic anemia refractory to immunosuppressive therapy
- Diamond Blackfan Anemia refractory to conventional therapy
- Shwachman-Diamond Syndrome
- Severe Congenital Neutropenia
- Congenital Amegakaryocytic Thrombocytopenia
- Thrombocytopenia Absent Radii syndrome
- Other marrow failure disorders not otherwise specified

c) Autoimmune cytopenias refractory to all conventional treatments

- Autoimmune hemolytic anemia
- Immune thrombocytopenia
- Evan's syndrome
- Pure red cell aplasia

d) Histiocytic disorders:

- Hemophagocytic lymphohistiocytosis
- High risk, recurrent or refractory Langerhans cell histiocytosis
- Secondary HLH

6.2.2 Subject Inclusion

- Recipient's age birth to < *70 years* old
- 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 mg/dl 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 for. 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.

Pulmonary: asymptomatic or if symptomatic, DLCO ≥ 50% of predicted (corrected for hemoglobin).

Renal: serum creatinine ≤1.5x normal for age. If serum creatinine is outside the normal range, then CrCl > 70 mL/min/1.73m2 (calculated or estimated) or GFR (mL/min/1.72m2) >30% of predicted normal for age.

Normal GFR in Children and Young Adults

Age	Mean GFR +- SD (mL/min/1.73 m^2)
1 week	40.6 + / - 14.8
2-8 weeks	65.8 + / - 24.8
>8 weeks	95.7 + / - 21.7
2-12 years	133.0 + / - 27.0
13-21 years (males)	140.0 + / - 30.0
13-21 years (females)	126.0 + / - 22.0

Abbreviations: GFR, glomerular filtration rate; SD, standard deviation

Greater than 2 years old: Normal GFR is 100 mL/ min.

Infants: GFR must be corrected for body surface area

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

6.1.3. Donor Inclusion Criteria

- Each donor must meet criteria outlined by institutional guidelines and be medically eligible to donate according to NMDP (or equivalent donor search organization) criteria including testing for antibodies to Human T-Lymphotrophic Virus Types I & II (Anti-HTLV-I/II) and screening for West Nile Virus, Creutzfeldt–Jakob disease and Zika.
- Pediatric donors should weigh ≥ 25.0 kg, have adequate peripheral venous catheter access for leukapheresis or must agree to placement of a central catheter.
- Donor should be healthy and agree to receive G-CSF followed by donation of peripheral blood stem cells.
- Donors must agree to anesthesia and marrow donation (in cases of inadequate PBSC collection).
- Related or unrelated donors who are 7/8 or 8/8 HLA-antigen matched for haplotypes A, B, C, DRB1 OR
- Related donors who are 4-6/8 HLA-antigen matched.

6.1.4 Donor Exclusion Criteria

Donors who are seropositive for HIV-I/II or HTLV-I/II and female patients who are pregnant or breastfeeding will not be eligible for this study.

6.2 Subject Exclusion Criteria

- Inherited DNA repair deficiency:

  Fanconi Anemia

  Dyskeratosis Congenita

These are presently undergoing transplantation based on a multi-center protocol

- Inherited metabolic disorders:

  Hurler Syndrome

  Sly syndrome (MPSVIII)

  α-Mannosidosis

  X- ALD

  Osteopetrosis
- Stage III-IV sickle chronic lung disease
- Patients with Thalassemia Major with Pesaro risk score >II
- Female patients who are pregnant or breast-feeding
- Active viral, bacterial or fungal infection
- Patient seropositive for HIV-I/II; HTLV-I/II
- Karnofsky (adult)/Lansky (pediatric) < 70%

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. We anticipate that this study will preferentially benefit persons of racial or ethnic minority populations as these individuals are less likely to have a HLA matched allo HCT donor. Otherwise, we expect that the study population will be fully representative of the range of patients referred for transplant without exclusion as to age or gender with the exception that pregnant women are excluded from participation in this study.

8.0 PRETREATMENT EVALUATION

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
- Complete Blood Count (CBC), coagulation studies (PT/aPTT/INR), Blood Type (ABO) and screen, serum chemistries including BUN, creatinine, electrolytes, glucose, total protein, albumin, liver function tests (AST, ALT, bilirubin, alkaline phosphatase)
- Pregnancy test for women of childbearing potential
- Infectious disease markers will be tested per FDA, AABB, NY State, and FACT standards.
- Urinalysis
- Electrocardiogram, echocardiogram
- 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)

- Donor specific class 1 and class 2 anti-HLA antibody titers for mismatched donors
- Pre-transplant research sample

When indicated
- Dental evaluation
- Disease Evaluation: Hemoglobin electrophoresis, bone marrow aspirate and/or biopsy
- Assessment of iron overload (ferritin, liver and/or heart MRI)
- Pulmonary function test for patients older than 7 years
- Chest X-ray and/or other types of scans (MRI, CT scan and PET scan)
- Transcranial Doppler Evaluation (TCD)
- Neuropsychological testing

9.1 TREATMENT/INTERVENTION PLAN

9.2 Selection of Cytoreduction Regimen

Patients eligible for this protocol include individuals with non-malignant disorders of hematopoiesis or immunity who fulfill eligibility requirements and consent to treatment. Stratification of patients to one of the two disease-targeted cytoreductive regimens will be based on the patient's disease as well as clinical parameters that could increase risk of graft failure or severe toxicity in the post transplant period. Examples of disease indications for each of the two cytoreductive regimen are summarized below. The basis for selection of a specific regimen can be briefly described.

Regimen A, 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 hematologic malignancies. (54, 55) This regimen has been successfully used for some non-malignant disorders in MSKCC 10-050 Arm C.

Regimen B, 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. This regimen has been successfully used in MSKCC 10-050 Arm D.

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.

9.3.3 Regimen A: Clofarabine, Thiotepa, Melphalan

9.3.3a Clofarabine

Clofarabine will be administered via approximately a 2-hour intravenous infusion at a dose of 20-30 mg/m2/day for 5 doses. Doses of 30 mg/m2/day are better tolerated by pediatric patients than adults. Patients ≤ 18 years of age deemed suitable may receive clofarabine at 30mg/m2/day with PI approval.

The timing of conditioning may be lengthened by 1-2 days based if deemed clinically necessary because of elevation of liver enzymes, and approved by the PI.

9.3.3b Thiotepa

Thiotepa will be administered via approximately a 4-hour intravenous infusion at a dose of 7.5 mg/kg/day over approximately 4 hr daily x 2 days (Day-5 and Day -4). Thiotepa may also be administered at 15 mg/kg/day for 1 dose (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 70 mg/m2/day for 2 doses. 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.

Dosing adjustments for patients ≤ 3 years will be made on case by case basis.

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

Rabbit ATG (Thymoglobulin®) will be administered in the pre-transplant period for all research participants at 2.5 mg/kg/day x 3 days. If the patient has a history of allergy or intolerance to rabbit ATG, equine antithymocyte globulin at a dose of 15 mg/kg x 3 or 40 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 3 days with the ATG administration and will be discontinued thereafter. Additional medications to prevent or treat reactions will be administered as indicated according to institutional guidelines. If the 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.

9.3.3e Rituximab

Rituximab will be administered based on actual weight according to institutional protocols to minimize infusion reaction.

Approximate Schema for Regimen A

EXAMPLE OF ROAD MAP OF PREPARATION FOR TRANSPLANT

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
				-12 **Rabbit ATG** 2.5 mg/Kg/day IV over 12 hrs	**-11** **Rabbit ATG** 2.5 mg/Kg/day IV over 12 hrs	**-10** **Rabbit ATG** 2.5 mg/Kg/day IV over 12 hrs
-9 **Clofarabine** 20-30 mg/m2/dose IV over 2 hours	**-8** **Clofarabine** 20-30 mg/m2/dose IV over 2 hours	**-7** **Clofarabine** 20-30 mg/m2/dose IV over 2 hours	**-6** **Clofarabine** 20-30 mg/m2/dose IV over 2 hours	**-5** **Clofarabine** 20-30 mg/m2/dose IV over 2 hours	**-4** **Thiotepa** 7.5 mg/Kg/dose IV over 4 hours	**-3** **Melphalan** 70 mg/m2/dose IV over 30 min **Thiotepa** 7.5 mg/Kg/dose IV over 4 hours
-2 **Melphalan** 70 mg/m2/dose IV over 30 min **Start Tacrolimus**	**-1** **Rituximab** 200 mg/m^2	**0** **TCR αβ - CD19- Stem cell transplant**	+1	+2	+3	+4

(Haplo)						
+5	**+6**	**+7** Start **G-CSF**				

9.3.4 Regimen B: Fludarabine, Thiotepa, Melphalan

9.3.4a Fludarabine

Fludarabine will be administered via approximately a 30-minute infusion at a dose of 30 mg/m2/day for 5 days. 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 70 mg/m2/day for 2 doses. 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 7.5 mg/kg/day IV over approximately 4 hr daily x 2 days. Thiotepa may also be administered at 15 mg/kg/day for 1 dose 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 3 days. If the patient has a history of allergy or intolerance to rabbit ATG, equine antithymocyte globulin at a dose of 15 mg/kg x 3 or 40 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 3 days with the ATG administration and will be discontinued thereafter. 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.

9.3.4e Rituximab

Rituximab will be administered based on actual weight according to institutional protocols to minimize infusion reaction.

Approximate Schema for Regimen B

EXAMPLE OF ROAD MAP OF PREPARATION FOR TRANSPLANT

MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY	SUNDAY
-11 **Rabbit ATG** 2.5 mg/Kg/d	-10 **Rabbit ATG** 2.5 mg/Kg/d	-9 **Rabbit ATG** 2.5 mg/Kg/d	-8 **Melphalan** 70 mg/m2/day IV X 1	-7 **Melphalan** 70 mg/m2/day IV X 1	-6 **Fludarabine** 30 mg/m2/day IV X 1 **Thiotepa** 7.5 mg/kg/day IV X 1	-5 **Fludarabine** 30 mg/m2/day IV X 1 **Thiotepa** 7.5 mg/kg/day IV X 1
-4 **Fludarabine** 30mg/m2/day IV X 1	-3 **Fludarabine** 30 mg/m2/day IV X 1	-2 **Fludarabine** 30 mg/m2/day IV X 1 **Start Tacrolimus (Haplo)**	-1 **Rituximab** 200 mg/m^2	0 **TCR αβ - CD19- Stem cell transplant**		
				+7 Start **G-CSF**		

9.4 Prophylaxis against acute graft-versus-host disease

Tacrolimus or Mycophenolate mofetil (MMF) will be used as GVHD prophylaxis for haploidentical grafts. The choice of agent will be based on clinical status.
Prophylaxis will be started on day -2, tapered from day +30 to +60 and discontinued.

1. Tacrolimus
   Tacrolimus will be started day –2 at an approximate dose of 0.03 mg/kg/24 hours as a continuous infusion. The dose of tacrolimus will be adjusted to achieve a level between 5 and 15 ng/mL. Once oral medications are tolerated, patients may be converted to oral tacrolimus at 3-4 times the current IV dose divided every 8 to 12 hours.

   In case of major toxicity, such as CNS symptomatology, tacrolimus may be discontinued earlier and substituted with other immunosuppressive medications as per clinical care guidelines.

   The immunosuppressive regimens on this protocol may conform to current practice where additional agents may be introduced in addition to Tacrolimus.

2. Mycophenolate mofetil (MMF)

Prophylaxis Dosing Starting on Day -2:	
Recipient > 12 years and < 50 kg	15 mg/kg q 8 hours
Recipient > 12 years and ≥ 50 kg	15 mg/kg q 8 hours (Min 1 g q 8 hours. Max 1.5 g q 8 hours)
Recipient ≤ 12 years	30 mg/kg IV q 8 hours (Max dose 1.5g q 8 hours)

In preparation for discharge, switch to oral route (CellCept or generic mycophenolate mofetil and same dosing as IV) and round to tablet size.

- No dose adjustments for renal or liver disease are needed routinely unless severe organ dysfunction. Measurements of serum blood levels of MMF should be done routinely as per current MSKCC guidelines to guide assessment of potential MMF toxicity in the event of unexpected myelosuppression. If patient ≥ +28 days and slow engraftment, consideration can be made to reduce dosing to q12 after discussion with PI or co-PI. See MSKCC guidelines for further information about MMF levels.

For patients with active GvHD, tacrolimus or MMF may be continued for longer periods of time according to clinical care guidelines.

9.5. *Stem Cell Transplantation*

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. Mobilization with Plerixafor will be considered in selected cases.

Donor bone marrow cells: Harvesting, Isolation and T-cell depletion

In cases of failed peripheral stem cell donor mobilization, bone marrow harvesting will be performed. Under general anesthesia, and after appropriate surgical preparation, bone marrow will be harvested by repeated aspiration from the iliac crests bilaterally into heparinized syringes, and pooled in heparinized saline, per FACT approved institutional guidelines. After filtration to remove boney spicules, and marrow harvest, collected in a sealed sterile transfusion bag, is transported to the laboratory for processing and selection of CD34+ cells on the CliniMACS device.

Processing prior to selection of CD34+ progenitor cells on the CliniMACS device, consists of initial measurements of total volume and cell count, acquisition of samples required for immunocytometry and cultures, and thereafter, depletion of red cells by hetastarch sedimentation, collection of the leukocyte rich plasma and washing, according to the laboratory's standard operating procedures.

Following this processing, the cells are resuspended in CliniMACS PBS/EDTA buffer supplemented with sterile human intravenous immune globulin at a concentration of 1.5 mg IVIG/mL, and thereafter incubated with the antiCD34 antibody coated paramagnetic

beads, and then separated on the CliniMACS device, as detailed for selection of CD34+ progenitors from GCSF-mobilized PBSCT.

9.5.2 Isolation of CD34+ TCR α/β T-cell and CD-19 Depleted hematopoietic progenitor cells with the CliniMACS TM System, Miltenyi Biotec.

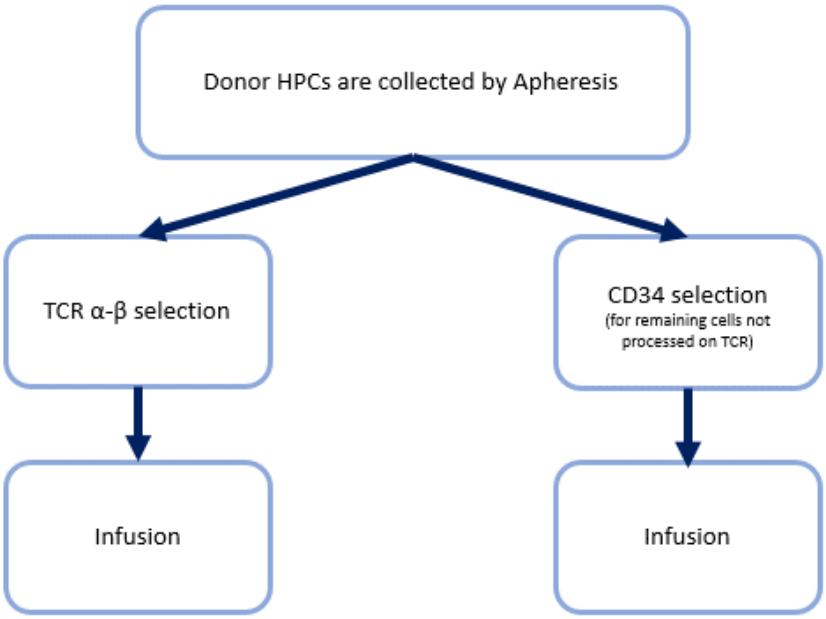
a. Mobilization of Donor

Allogeneic donors will be mobilized per institutional standards.

b. Donor Stem Cell Apheresis

Donor stem cell apheresis, HPC(A) will be performed per institutional standards. The donor shall not undergo more than 2 days of collection unless extenuating circumstances permit for an additional day (total 3 days).

c. TCRαβ+ T-Cell Reduction using the Miltenyi CliniMACS®



Reduction of TCRαβ T-cells and CD19+ B-cells from the donor HPC(A) product will be performed according to procedures given in the CliniMACS® Users Operating Manual and institutional Standard Operating Procedures (SOP) implemented in the Cell Therapy Laboratory (see Appendix for SOP).

The total nucleated cell count, CD34+ cell count, TCRαβ+ CD3+ T-cell count, and CD19+ cell count will be analyzed pre- and post-selection and recorded.

Day 1: TCRαβ + CD19 reduction and Day 2: CD34 selection

A single TCRαβ+ T-cell and CD19+ B cell reduction procedure will be performed for donors to achieve a target of CD34+ cells/kg cell dose for the final product ≥ 5 x 10^6 and CD3+ TCRαβ+ cell count ≤ 1 x 10^4 cells/kg recipient total body weight for mismatched donors. An additional CD34+ cell selection may be performed to achieve the target CD34+

cell dose, if required. If there are less than a total of 2 x 10^6 CD34+ cells/kg recipient body weight in the combined allografts, the laboratory will contact the investigator. If required, CD34+ cell selection on additional HPC(A) will be performed according to procedures given in the CliniMACS® Users Operating Manual and approved institutional SOP. Testing of the product will be conducted in a CLIA certified laboratory as described in the CMC section. If needed, the order of cell selection may be altered in order to accommodate resource limitations.

The TCRαβ graft is depleted of potentially allo-reactive T cells (TCR a-b positive cells). Because all other cells are retained (CD34, TCR gamma-delta, NK cells etc) the size of the graft is similar to an unmodified PBPC graft, and therefore it is usually dispensed in a bag.

The CD34 selected graft only retains the CD34 cell fraction, which is relatively rare and therefore the volume of the product is much smaller and as such the product is dispensed in a syringe.

If both products are available simultaneously to infuse, the recommendation is to first infuse the CD34 selected graft due to its small volume and nearly absent reaction rate. Once the CD34 selected graft has been infused, then the bag of the TCRαβdepleted graft may be administered as per SOP for HPCs in the bag format. There is no necessity for a delay between infusions.

Pre-medications and fluids should be administer per standard BMT practice guidelines for CD34 selected products.

d. **Analysis of Allograft**

Samples will be taken from each leukapheresis product after collection and before processing and on each fraction of cells after processing on a single CliniMACS® tubing set. Pre- and post-selected target cells (CD34+ and CD3+) will be characterized as follows:

- Gram stain (performed locally) post selection
- Total nucleated cell (TNC) count pre- and post-selection
- Endotoxin testing pre-release (performed locally)
- Flow cytometric analysis for CD34+ cells and for CD3+ cell pre- and post-selection performed locally using validated SOP
- Flow cytometric analysis of monocytes, B-cells, NK cells in the cell-selected products
- Viability testing (7-AAD method) pre- and post-selection

Criteria for release of final graft product for infusion.

- Viability ≥ 70% after selection
- Negative Gram stain
- Cumulative CD34+ cell content ≥ 2.0 x 10^6 CD34+ cells/kg
- Cumulative T cell content ≤ 10 x 10^4 TCRαβ+CD3+ cells/kg; related matched donor

- Cumulative T cell content ≤ 1x 10^4 TCRαβ+CD3+ cells/kg; alternative, mismatched donor

9.5.3 Transplantation of the T-cell depleted stem cells.

The CD34+T-cell depleted peripheral blood progenitor cells will be 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 herpes viruses 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.

10.1 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. Additional testing may be performed as is clinically indicated. Scheduled evaluations on day +30 and +100 may occur ±7 days from

the schedule time point. Long term follow up testing may be done ±21 days from the scheduled time point.

Post-transplant GVHD assessments will be performed as per institutional standards.

Activity	**Transplant to Discharge**	**DISCHARGE TO DAY 100**	**Day 100**	**6 Months**	**12 months**	**24 Months**
Blood counts and chemistry (CBC, CMP)	CBC: Daily CMP: 2x a week	CBC/CMP: Weekly, 2x a week, or every 2-3 weeks	Every 2-4 weeks		X	X
Lineage Specific chimerism including T-cells[1]	NA	Day +30 Day +60	X	X	X	X
Peripheral blood lymphocyte (PBL) phenotyping[2]	NA	Day +30 Day +60	X	X	X	X
In vitro response of PBL to standard panel of mitogens [2,3]	NA	NA	X	X	X	X
Viral PCR: CMV, EBV, ADV[4]	Twice weekly[4] As clinically indicated	Twice weekly[4] As clinically indicated	Weekly[4] As clinically indicated	Every 2 weeks[4] If clinically indicated	Every two weeks[4] If clinically indicated	
Research bloods	Once	Day +30 Day +60	X	X	X	X

1. May be done more frequently if clinically indicated
2. PBL phenotyping should be performed until CD34 counts is >200 or if patient is being immunized
3. Should be performed until normal or reached plateau
4. CMV testing if patient or donor CMV positive only. Testing more or less frequently as clinically indicated

For patients weighing ≥10 kg, research samples will consist of three 10 mL solution heparin tubes of blood on the days described above. If a patient is at risk for exceeding maximum allowable blood draw limits (per Seattle Children's Hospital Guideline for Maximum Blood Volumes), clinically laboratory assessments will be prioritized over research samples.

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 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.

In addition to tracking patients for survival and graft failure, all participants will also be monitored for post-transplant toxicities. Toxicities will be graded according to NCI CTCAE version 5.0 at the time points outlined in Section 10.1. All grade 3-4 adverse events will be captured and assessed for attribution to protocol treatment. Additional toxicities will be reviewed and graded/attributed at the discretion of the PI. Toxicities which are attributable to underlying disease and/or grade 1-2 expected toxicities from the transplant will not be tracked.

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

11.1.2 Thiotepa

COMMON, SOME MAY BE SERIOUS In 100 people receiving Thiotepa, more than 20 and up to 100 may have:
• Nausea, vomiting • Pain • Loss of appetite • Tiredness • Hair loss • Rash

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Thiotepa, from 4 to 20 may have:
• Infection, especially when white blood cell count is low • Anemia which may require transfusion • Bruising, bleeding • Damage to the bone marrow (irreversible) which may cause infection, bleeding, may require transfusions • Absence of menstrual period • Loss or absence of sperm • Blurred vision • Blood in urine • Swelling of feet or lower legs

RARE, AND SERIOUS In 100 people receiving Thiotepa, 3 or fewer may have:
• Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Cancer of bone marrow caused by chemotherapy

11.1.5 Melphalan

COMMON, SOME MAY BE SERIOUS In 100 people receiving Melphalan, more than 20 and up to 100 may have:
• Sores in mouth which may cause difficulty swallowing

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Melphalan, from 4 to 20 may have:
• Anemia, kidney problems which may cause tiredness, bruising, swelling, or may require dialysis or require blood transfusions • Infection, especially when white blood cell count is low

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Melphalan, from 4 to 20 may have:
• Bruising, bleeding • Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Hepatitis which may cause yellow eyes and skin, tiredness • A new cancer resulting from treatment of a prior cancer • Cancer of bone marrow caused by chemotherapy • Kidney damage which may cause swelling, may require dialysis • Scarring of the lungs which may cause shortness of breath

RARE, AND SERIOUS In 100 people receiving Melphalan, 3 or fewer may have:
• Heart stops beating

11.1.6 Fludarabine

COMMON, SOME MAY BE SERIOUS In 100 people receiving Fludarabine, more than 20 and up to 100 may have:
• Infection, especially when white blood cell count is low • Vomiting, loss of appetite • Tiredness, fever • Pain • Bruising, bleeding • Cough • Increased risk of unusual infections lasting more than 6 months

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Fludarabine, from 4 to 20 may have:
• Anemia, kidney problems which may cause tiredness, bruising, or swelling • Nausea, chills • Feeling of "pins and needles" in arms and legs • Damage to organs (brain, lungs, others) which may cause tiredness, changes in thinking or shortness of breath

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Fludarabine, from 4 to 20 may have:
• Confusion

RARE, AND SERIOUS In 100 people receiving Fludarabine, 3 or fewer may have:
• Kidney damage which may require dialysis

11.1.7 Clofarabine

COMMON, SOME MAY BE SERIOUS In 100 people receiving Clofarabine, more than 20 and up to 100 may have:
• Anemia which may cause tiredness, or may require blood transfusions • Abnormal heartbeat • High blood pressure • Diarrhea, nausea, vomiting, chills • Tiredness • Fever • Bruising, bleeding • Infection, especially when white blood cell count is low • Loss of appetite • Pain • Headache ~~• Worry~~ • Nose bleed • Itching, rash • Low blood pressure which may cause feeling faint • Damage to the liver

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Clofarabine, from 4 to 20 may have:
• Severe blood Infection • Kidney damage which may cause swelling, may require dialysis • Stroke which may cause paralysis, weakness • Severe skin rash with blisters and can involve inside of mouth and other parts of the body

OCCASIONAL, SOME MAY BE SERIOUS
In 100 people receiving Clofarabine, from 4 to 20 may have:
• Fluid in the organs which may cause low blood pressure, shortness of breath, swelling of ankles • Flushing

RARE, AND SERIOUS
In 100 people receiving Clofarabine, 3 or fewer may have:
• Damage to the bone marrow (irreversible) which may cause infection, bleeding, may require blood transfusions

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:

COMMON, SOME MAY BE SERIOUS
In 100 people receiving ATG more than 20 and up to 100 may have:
• Skin rash and itching • Fever and chills • Lowered blood pressure • Decreased ability to form blood clots; this may lead to difficulty in stopping bleeding, easy bruising and blood in urine or stool. • Serum sickness- causes severe skin rashes, mouth and vaginal sore, pain and swelling in the joints or kidney damage. Serum sickness is treatable with antihistamines and steroids • Increased chance of infection

RARE, AND SERIOUS
In 100 people receiving ATG 3 or fewer may have:
• Severe, possibly fatal, allergic reaction

11.1.9 Rituximab

COMMON, SOME MAY BE SERIOUS In 100 people receiving Rituximab, more than 20 and up to 100 may have:
• Nausea • Chills, fever • Reaction during or following infusion of the drug • Infection, especially when white blood cell count is low • Anemia which may require blood transfusions • Numbness and tingling of the arms and legs • Tiredness

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving Rituximab, from 4 to 20 may have:
• Bruising, bleeding • Abnormal heartbeat • Heart attack or heart failure which may cause shortness of breath, swelling of ankles, and tiredness • Sores in eye • A tear or a hole in the bowels that may require surgery • Diarrhea, vomiting • Pain • Swelling of the body • Hepatitis, or liver damage which may cause yellow eyes and skin • Dizziness, headache • Kidney damage which may require dialysis • Cough • Scarring of the lungs • Stuffy nose • Blockage of internal organs which may cause shortness of breath, wheezing, vomiting • Increased sweating • Itching, rash, blisters on the skin • Severe skin rash with blisters and peeling which can involve mouth and other parts of the body • Low blood pressure which may cause feeling faint

RARE, AND SERIOUS
In 100 people receiving Rituximab, 3 or fewer may have:
• Damage to the brain caused by a virus which may result in tiredness, weakness, changes in thinking, and disability. This is called progressive multifocal leukoencephalopathy (PML). • Heart stops beating

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 graft failure, 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 disease and/or grade 1-2 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**.** Low B and T-cell count for an additional 3-12 months after transplantation increases the risk for certain opportunistic infections including pneumocystis *jiroveci* pneumonia, cytomegalovirus, and others. Medications are given to reduce the chance of infections. Patients will receive treatment if they do get an infection and most infections can be treated

successfully with antibiotics. Patients will stay in the hospital longer or be readmitted if found to have an infection. Patients are watched closely for bleeding and given platelet transfusions to prevent serious bleeding, but minor bleeding may occur.

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.

Acute GVHD usually occurs in the first 3 months or as immunesuppressive medications are tapered and may cause: skin rashes, nausea, vomiting, diarrhea, hepatitis, increased risk of infection, ulceration of the surfaces of the oral cavity, esophagus, and intestines, and suppressed or delayed recovery of the hematopoietic and immune system.

Chronic GVHD can occur any time after the first 3 months and is manifested to varying degrees by scleroderma-like changes of the skin, cirrhosis of the liver, sclerosis of lacrimal and salivary ducts, chronic inflammation and scarring of the gastrointestinal tract with consequent malabsorption and diarrhea, chronic bronchitis, and suppression of the immune system. This can be treated with standard or protocol-based experimental immunosuppression, but may be refractory.

Severe GVHD: Rarely, GVHD can be severe or deadly. Severe acute GVHD could involve a severe skin rash like a burn, severe vomiting and/or diarrhea, liver failure and infections or bleeding. Severe acute GVHD will be treated with intense immunosuppressive therapy according to standard clinical practice or other experimental protocol. Severe chronic GVHD could involve similar symptoms but may produce other symptoms such as severe skin changes, severe dry eyes and weight loss.

Steroids, as treatment for GVHD: inability to sleep, high blood sugar, puffiness of the face, changes in the skin, high blood pressure, increased risk of infection, weight gain, reduced growth in children, thinning of the bones.

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 Lymphoproliferative Syndrome

Recipients of TCD allogeneic grafts have an increased risk of developing lymphoproliferative syndromes caused by EBV infection (56, 57). 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 (58). 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 (59). EBV-immune cells are experimental, but can also induce regression of EBV PTLD without risk of graft vs host disease (60, 61).

11.2.11 Serious Bleeding

Serious bleeding may result from low platelet counts and /or injury to tissues from treatment. This can happen despite platelet transfusions. Bleeding is rarely fatal.

11.2.12 Risk of secondary cancer

Secondary cancers may occur after chemotherapy. The risk of developing a secondary cancer of the skin, cervix, etc., which has been seen in other transplant studies is less than 5%.

11.2.13 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 post-transplant or as needed thereafter.

12.2 Graft failure or rejection

Primary non-engraftment is diagnosed when the patient fails to achieve an ANC $\geq$ 500/μl at any time in the first 28 days post-transplant. 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 (62, 63). 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.

Patients with evidence of graft failure may have additional studies drawn to ascertain cause and define relevant histo incompatibilities. These analyses may include (1) Evaluation of bone marrow aspirates and biopsies, when indicated, (2) Culture and/or molecular analyses of marrow and blood for viral pathogens potentially causing graft failure including CMV, HHV6 and parvovirus B19, (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 (64) 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 (2-20 mg/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 NIH Consensus development project on criteria for clinical trials in chronic GVHD (65) 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, hemorrhage, 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. Cellular Immunological reconstitution

Immune recovery after transplantation with a αβ T-cell/CD-19 lymphocyte depleted graft will be evaluated by phenotypic and functional studies. B cell, T-cell subsets and NK cell analysis will be performed at baseline, weekly until day 100 or discharge, and monthly thereafter. Phenotypic analysis will be done by flow cytometry; analysis will be performed on mononuclear cells labeled

with mAb panels to allow identification of the main T cell subsets. Functional studies using T cell subsets shortly after collection and after in vitro expansion.

12.7 Disease recurrence

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.

13.1 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
- Graft failure
- 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

This phase 2 trial is designed to assess the efficacy of αβ T-cell and B-cell depleted peripheral blood stem cell transplants for patients with non-malignant hematologic or immune disorders. The study population is divided into two groups defined by donor type: 1) donors who are ≥ 7/8 HLA-antigen matched and 2) related donors who are 4-6/8 HLA-antigen matched. A maximum of 30 patients in each group is planned for accrual onto the study. The primary endpoint of the study is two-year overall survival. It is anticipated that accrual will last three years and each patient will be followed for a minimum of two years if he/she has not failed. The data analysis and data monitoring will be conducted separately in each donor group. It is anticipated that accrual will last three years and each

patient will be followed for a minimum of two years if he/she has not failed. Patients lost to follow-up prior to the two-year endpoint will be counted as a failure for the purpose of the primary endpoint evaluation. The data analysis and data monitoring will be conducted separately in each donor group.

For patients with donor type 1, a two-year overall survival (OS) rate greater than 0.75 would be considered a success. A single stage design that differentiates between two-year OS population rates of 0.75 and 0.92 will be used to assess treatment efficacy. At the conclusion of the study, if at least 26/30 patients survived for at least two years, then the treatment will be declared a success. The probability of declaring the treatment a success is 0.10 when the two-year OS rate in the population is 0.75 and increases to 0.91 when the two-year OS rate is 0.92.

For patients with donor type 2, a two-year OS rate greater than 0.65 would be considered a success. A single stage design that differentiates between two-year OS population rates of 0.65 and 0.87 will be used to assess treatment efficacy. At the conclusion of the study, if at least 24/30 patients survived for at least two years, then the treatment will be declared a success. The probability of declaring the treatment a success is 0.06 when the two-year OS rate in the population is 0.65 and increases to 0.91 when the two-year OS rate is 0.87.

In order to reduce patient risk, the study design includes early termination in the event of excessive graft failure, acute graft versus host disease, or infection-related mortality during the accrual period. The stopping rules for excessive failure and the corresponding power calculations are derived separately for the two donor groups and are given in the tables below. The boundaries are based on a maximum accrual of 30 patients. In the event that the stopping boundary is crossed for one donor group, the study will continue accrual in the other donor group. The calculations in the table are based on marginal binomial probabilities.

Donor type 1

Failure type	# of failures needed to stop the study	Failure rate in the population	Probability boundary is crossed
Graft failure	2 in the first 26 patients 3 within 30 patients	0.02	0.10
		0.15	0.93
Acute Graft Versus Host Disease (Grades 3-4)	2 in the first 10 patients 3 in the first 20 patients 4 within 30 patients	0.04	0.09
		0.20	0.91
Infection-related Mortality	2 in the first 26 patients 3 within 30 patients	0.02	0.10
		0.15	0.93

Donor type 2

Failure type	# of failures needed to stop the study	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 Graft Versus Host Disease (Grades 3-4)	3 in the first 10 patients 4 in the first 20 patients 5 within 30 patients	0.07	0.08
		0.25	0.92
Infection-related Mortality	2 in the first 10 patients 3 in the first 20 patients 4 within 30 patients	0.04	0.09
		0.20	0.91

At the conclusion of the study, Kaplan-Meier estimates of overall and disease-free survival will be computed. The cumulative incidence function will be used to evaluate the cause-specific probability of graft failure, acute and chronic GvHD, infectious complications, and transplant-related mortality. At the outset of transplant procedure, the immune reconstitution parameters will be recorded and summarized over all patients. Over time post-transplant, nonparametric curve smoothing methods will be used to estimate the population trajectory of the immunologic recovery parameters. Linear mixed models will be employed to compare the recovery rates to patients who received CD34 positively selected grafts as part of protocol 10-050. The Wilcoxon rank sum test will be used to compare the CD34+ cell doses for patients transplanted on this protocol to the cell doses of patients with non-malignant disorders who received CD34 positively selected grafts.

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

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

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

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming 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).

15.3 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.

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 Clinical Research Administration. 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 two 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 with 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

MSK'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 subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

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

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

If an SAE requires submission to the IRB office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a SAE report be submitted electronically to the SAE Office as follows:

All SAEs must be submitted in PIMS. If an SAE requires submission to the Human Research Protection Program (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
    - An explanation of how the AE was handled
    - A description of the participant's condition
    - 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

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

18.0 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; additionally, patients will be offered an option of supportive care).
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

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20.0 APPENDICES

Table I: Alpha beta T-Cell Depleted stem cell transplants for Non-Malignant Disorders (+/- malignant)

	CD19 depleted	Donor	N	ATG	Conditioning	Intensity	GvHD PPx	Rituximab	Engraftment/Graft failure	GVHD a/c	Infection	TRM	OS
Karakukcu M EBMT Abs 2016 **Both** (NMHD13) Ped	No	Haplo	34	ATG	Flu/Mel/Thio	RIC	MMF if **αβ >2.5x10^4/kg** (50% of all)	Yes 11% D-1	Engraft (D+12) 3% graft failure	20% (II skin) 12-15% (III-IV GI/liver)	80% CMV react 15% of CMV react had EBV react	14.7% (GvHD, sepsis, relapsed)	64.7%
Yadav SP EBMT Abs 2016 NMHD (FA, WAS, Thal in Ped/adult)	Yes	Haplo	4 (+2 req 2nd graft)	ATG-T(2/4)	TBI/Flu/Cy +/- thio – failed x 2 pts. Campath/Flu/Cy Campath/Flu/Treo/Thio *(doses not specified)*	RIC	No	No	2/4 failed	50% (II) No chronic	75% CMV react, 25% TMA 25% JC enceph 25% BK encep & emorrhagic cystiti	25% (BK)	75%
Mainardi C Letter to editor BMT 2016 (Hurler)	Yes	MUD	1	ATG (? Dose)	Flu/Treo/Mel	RIC	MMF <2M	No	Engraft on D+14	" mild GI"	CMV reactivation	0	
Lang P BMT 2015 **Both** (5 NMHD) Ped	Yes	Haplo	41	ATG-**F** 15 mg/kg (1-4-5-5 on D-12 to -9) **or** OKT-3 (pre 2012)	Flu 40 mg/m^2/day or Clo 50 mg/m^2/day (D-8 to -5) Mel 70 mg/m^2/day (D-3 to -2) Thio 10 mg/kg D-4	MAC/RIC	MMF until D+30	No	88% Engraft (+D10) Rej 12%	76% (0-I) 10%(II) 15%(III or IV) Chronic: limited	- (focuses on immune reconstitution)	48% died (17/41 died of relapse)	51.2% (relapsed)

				*peak level of ATG						18%; extensive 9%			
Airoldi I Blood 2015 **Both** (12NMHD) Ped	Yes	Haplo	27	**r**-ATG (D-5 to -3)	Treo/Flu +/-Thio Treo/Thio/Mel TBI/Cy/Flu Bu/Thio/Flu Bu/Cy/Mel	MA	No	Yes D-1	-	-	CMV reac 55%	1 from Adeno	81% (all) **100%** in NMHD
Tumino M Letter to BMT 2014 (SAA in ped)	Yes	Haplo	1	ATG-T	Thio 10 mg/kg, Cy 200 mg/kg, TLI 7.5Gy	MA	CSA if **αβ** **>2.5x10^4**	No	Engraft D+14	No	CMV reactivation Invasive spergillosis (lung	No	
Kharya G Allergy and Clinical Immunol 2014 (WAS)	Yes	Haplo	1	ATG-**F**(15mg/Kg)	Bu (AUC 80 mg/L h), Flu 160/m^2, Thio 15mg/kg	RIC	CSA+MMF	No	Engraft D+15	-	CMV reacti	No	100%
Bertaina Blood 2014 NMHD Ped	Yes	Haplo (KIR-B preferred)	23	ATG-**F** 4 mg/kg/day (D-5 to -3)	Bu/Thio/Flu Treo/Thio/Flu Treo/Flu (SCID) Flu/Cy+/-TBI (single dose at 200 cGy) (SAA or FA)	RIC	No	Yes D-1 200 mg/m^2	82% engraft (D+13) 20% graft failure	13% (I-II) no cGvHD	CMV+Adeno=38%	9.3%	91.3%