Biparental HLA Haplotype Disparate T-cell Depleted Transplants for Patients Lacking an HLA-Compatible Donor

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

1. Study Objectives: To evaluate biparental HLA-haploidentical T-cell depleted $CD34^+$ peripheral blood stem cell (PBSC) transplants when applied to the treatment of patients with hematologic malignancies or lethal disorders of hematopoiesis who lack an HLA compatible related or unrelated donor. Primary endpoints will include: 1) incidence, tempo and completeness of engraftment and hematopoietic reconstitution; 2) incidence of graft failure 3) incidence of acute and/or chronic GvHD; 4) incidence and severity of opportunistic infections; 5)incidence of Transplant-related mortality, overall survival and disease-free survival. Secondary, endpoints will include: 1) levels of initial and sustained engraftment of hematopoietic cells and their lineage-specific progenitors from each parental donor; 2) the tolerance or allo responsiveness of T-cells generated *in vivo* from each parental donor against cells of the host or other parent and 3) the capacity of engrafted T-cells from each donor to respond to viral antigens presented by HLA alleles shared by the recipient and each donor or by HLA alleles unique to each donor.

2. Design: Single center pilot study to provide initial data on the safety and efficacy of a biparental HLA haplotype disparate T-cell depleted transplant for patients lacking an HLA-compatible donor.

3. Patient Inclusion Criteria: Patients diagnosed with histologically, proven high risk forms of acute or chronic leukemia, non-Hodgkins, myelodysplastic syndrome or other rare lethal disorders of hematopoiesis and lymphopoiesis for which a T-cell depleted transplant is indicated (e.g. hemophagocytic lymphohistiocytosis, refractory aplastic anemia or congenital cytopenias, non-SCID lethal immune deficiencies such as Wiskott-Aldrich Syndrome, CD40 ligand deficiencies, ALPS). To be eligible, patients must lack an available HLA identical or 9/10 HLA allele matched related or unrelated donor. The patient must have two consenting related donors of whom one shares with the patient the same paternal HLA A, B, C, DR, DQ haplotype and the other shares the same maternal HLA A, B, C, DR, DQ haplotype, who are willing and able to donate GCSF-mobilized PBSC. The patient must also meet the following clinical criteria: creatinine ≤ 1.2 mg/dl or if not, CrCl> 40ml/min/1.73m2, and total bilirubin < 2xnl, ALT < 3xnl, adequate cardiac function i.e., LVEF $\geq$ 50%, adequate pulmonary function-asymptomatic or if not, DLCO > % 50% (corrected for Hgb); Karnofsky performance status $\geq$ 70%; negative pregnancy test (where applicable); signed informed consent of patient and donor.

4. Patient Exclusion Criteria: Pregnancy or lactation, unwillingness to comply with protocol treatment or follow-up uncontrolled infection, HIV or HTLV seropositivity, active leukemia in CNS.

5. Pretreatment Testing: The following tests will be performed within approximately 30 days before beginning treatment: complete history and physical examination, dental evaluation (may be performed outside of approx 30 day window), HLA typing as per institutional guidelines, complete blood count (CBC), PT/PTT/INR, RBC type and screen and RFLP (may also be out of 30 day window) on the two donors and patient, comprehensive panel (including BUN, creatinine, electrolytes, glucose, total protein, albumin, liver function tests - AST, ALT, bilirubin, alkaline phosphatase), urinalysis, serum or urinary HCG in women of childbearing potential, and bone marrow aspiration and/or biopsy, if indicated within approx 1

month or sooner if there is indication that the disease is changing. In addition, chest X-ray and other types of scans (CT scan and PET scan, if needed), electrocardiogram, echocardiogram or a gated pool scan if needed, and pulmonary function test for patients older than 7 years, if indicated. Cytogenetic and molecular evaluations will be performed as clinically indicated. Microbial testing will be obtained as per the Blood Bank standards and BMT Service Guidelines.

All patients with ALL or with M4 or M5 type AML must have a diagnostic LP performed within 1 month prior to enrolling in this study. If there are signs or symptoms of CNS disease, or the hematologist/ oncologist feels it is indicated, an LP will be performed to exclude the presence of CNS disease prior to beginning therapy. Donors will undergo standard screening for PBSC donation. Whenever possible, samples of the patient's hematologic disease will be obtained and prepared for cryopreservation for later in vitro studies.

6. Summary of Treatment Plan: There are two myeloablative preparative regimens that can be used for transplants in this protocol.

Regimen 1 consists of hyperfractionated TBI – 1375 cGy (or 1500 cGy for children with ALL > 1 CR) administered in 11 or 12 doses of 125 cGy each over a total of four days, with three doses on three days and two doses on the last day, fludarabine 25 mg/m2 IV x 5 days, and thiotepa 5mg/kg IV x 2 days (or 10mg/kg x 1 day).

Regimen 2, to be given to patients non-malignant, life-threatening diseases and patients with hematologic malignancies, with extensive prior therapy and comorbidities who are unable to receive TBI, consists of Melphalan 70mg/m^2 IV x 2 days, thiotepa 5mg/kg IV x 2 days (or 10mg/kg x 1 day), and fludarabine 25 mg/m2 IV x 5 days.

Recipients of these biparental HLA haplotype matched related stem cell transplants will receive ATG for three days prior to the transplant as per the BMT Service guidelines. G-CSF mobilized CD34 PBSCs obtained from each HLA- haplotype matched donor will be infused on day 0. Post transplantation G-CSF will be administered only if clinically indicated and should begin on or after d+7. Supportive care will be administered as per the BMT Service guidelines.

Patients will be clinically evaluated daily as inpatients and at each clinic visit for incidence and severity of acute and chronic GVHD and transplant associated morbidity. Sequential evaluation of engraftment and functional reconstitution of hematopoiesis and immunity will be made as per the BMT Service guidelines.

7. Approximate Evaluation during Treatment: CBCs may be obtained daily and chemistries will be obtained at least two times per week until d+30 or until discharge from the hospital, whenever possible. Thereafter, they are performed as clinically indicated. Assessment of disease will be made by bone marrow aspiration (when appropriate), and appropriate cytogenetic and molecular studies. Chimerism will be molecularly assessed to measure the proportion of cell populations derived from each donor and the host. These studies will be performed at approximately 30-100 days post transplant, 6, 12, and 24 months post transplant, and thereafter as clinically indicated. Chimerism will be assessed by

RFLP analysis. Immune competence will be evaluated by peripheral blood lymphocyte (PBL) phenotyping at approximately 1, 3, 6, 9 months, and q3-6 months thereafter until normal. In vitro response of PBL to a standard panel of mitogens and antigens will be performed at approximately 3 and 6 months and then q3-6 months until normal. HLA- restricted T-cell responses to CMV and/or EBV as well as Mixed Leukocyte responses to irradiated PBMCs from the host, each donor and third party allogeneic cells will also be assessed at 3 and 6 months and then q3-6 months until normal.

8. Estimated number of study subjects: 21 patients

9. Selection and evaluation of related donors for transplantation: Any pair of HLA A, B, C, DR, DQ haplotype- matched healthy family members in which one donor inherits the paternal HLA haplotype and the other inherits the maternal HLA haplotype shared by the patient will be considered for PBSC donation. Selection of the donors will be based on genotype matching of HLA-A, B, C, DR, DQ haplotype, to be carried out on the recipient, parents, siblings, or possibly other family members such as aunts, uncles, or first cousins. Each prospective donor must be matched for one or the other HLA haplotype (HLA-A, B, C, DR, DQ) of the patient. Each donor must also provide signed informed consent, to receive a 5-6-day course of G-CSF and to undergo two leukaphereses. In addition, if the PBSC collection is inadequate the donor must consent to undergo a bone marrow harvest under general anesthesia. The donor will undergo a medical evaluation as per the BMT Service Guidelines for a related donor.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary

To obtain a preliminary estimate of the efficacy of HLA-haploidentical biparental T-cell depleted $CD34^+$ peripheral blood stem cell transplants in the treatment of patients with high risk leukemia, lymphoma, MDS or other lethal disorders of hematopoiesis who lack an HLA compatible related or unrelated donor as measured by:

a) incidence of transplant-related mortality, overall survival and disease-free survival at 1 year post transplant.
b) incidence, tempo and complications of engraftment and hematopoietic reconstitutions and conversely, the risk of graft failure
c) incidence and severity of acute and/or chronic GVHD
d) incidence and severity of opportunistic infections developing following engraftment

Secondary

To evaluate recipients of biparental HLA haploidentical T-cell depleted $CD34^+$ peripheral blood stem cell transplants at intervals post transplant as to:

a) levels of engraftment and persistence of hematopoietic cells and their myeloid and lymphoid progressing from each donor post transplant.

b) The tolerance or reactivity of engrafted T cells from each donor detected in the blood at 3, 6, and thereafter every 3-6 months until normal, post transplant against host cells and cells derived from the other parent as measured by standard mixed lymphocyte culture and cell mediated cytolysis assays.
c) The patterns of HLA restriction exhibited by virus antigen-specific T cells detected in the blood at 3, 6, and thereafter every 3-6 months until normal, post transplant, to determine whether and to what degree these T-cells can respond to viral antigens presented by shared HLA alleles in comparison to HLA allels unique to each parental donor.

3.0 BACKGROUND AND RATIONALE

Allogeneic hematopoietic stem cell transplants (HSCT) derived from HLA-matched related donors are now recognized as a potentially curative treatment of choice for patients afflicted with acute leukemia relapsing after initial remission, high risk forms of ALL and AML in 1° CR and MDS and advanced stages of NHL.[1-4] Furthermore, as a result of the improvements in the precision of HLA typing provided by DNA typing and the efficiency of donor identification and recruitment from the NMDP and its integrated network of donor registries in other countries, the results of transplants from matched unrelated donors for these diseases have also markedly improved [5-8], encouraging the application of such transplants in earlier, more responsive stages of disease. However, despite the fact that the NMDP and other cooperating donor registries have accrued over 13 million HLA typed donors willing to provide hematopoietic progenitor cell transplants (HSCT), normal HLA-matched or single HLA allele disparate donors can only be identified for 70% of the patients for whom a transplant is indicated.[9]

To extend the application of HSCT to patients lacking an HLA compatible donor, two major strategies have been explored: 1) transplants of partially matched allogeneic umbilical cord blood cells, and; 2) transplants of HLA-haplotype matched T-cell depleted marrow or peripheral blood stem cells.

Applications of single unrelated allogeneic cord blood transplants (UCBT) in the treatment of children with leukemia and genetic disorders of hematopoiesis have demonstrated the capacity of such transplants to achieve consistent durable engraftment and to reconstitute hematopoiesis and immunity.[10] Indeed, in children, the results of matched or 1 antigen/disparate UCBT are comparable to those achieved with matched unrelated grafts.[11] Published reports also suggest that the incidence and severity of acute and chronic GVHD are less following UCBT than adult marrow or PBSC transplants. Indeed, the severity of GVHD observed following 2 HLA allele disparate UCBT is approximately that seen in recipients of 1 HLA allele disparate marrow grafts.[12] However, the incidence of graft rejection and the incidence of acute and chronic GVHD increases with greater HLA disparate and may be prohibitive when more than 2/6 HLA serologically typed antigens differ between donor and host.[13] Initial results of UCBT in adults with leukemia or aplastic anemia were markedly inferior to those achieved in children, in large part because single UCBT were associated with an unacceptably high incidence of graft rejection or failure.[14, 15] Furthermore, despite the increase in the cumulative number of cord bloods now available

for transplantation, there remains a sizable proportion of individuals for whom a sufficiently partially HLA matched UCB donor cannot be identified.

However, as shown by Barker et al [16] consistent engraftment can be achieved in adults when two comparably matched UCBT units from different donors are administered. The strikingly consistent and more rapid engraftment and reconstitution suggests that the two allogeneic CBTs are mutually costimulatory. However, for as yet unclear reasons, when double cord grafts are administered, only one of the transplants durably engrafts and survives to reconstitute hematopoiesis.[17] As in the pediatric recipients of single UCBTs, the incidence and severity of acute and chronic GVHD are lower than in recipients of comparably matched unrelated marrow or PBSC transplants. However, HLA disparaties exceeding 2/6 serologically defined HLA antigens are associated with a significantly higher incidence of severe acute and chronic GVHD and associated transplant-related mortality.[18]

In order to extend the application of allogeneic HSCT to all patients for whom it is an indicated and potentially curative approach, we and several other transplant centers have explored the use of T-cell depleted HLA haplotype disparate marrow or PBSC transplant. In 1980, our group initially demonstrated the potential of this approach as a treatment for children with severe combined immunodeficiency [19] or leukemia [20] who lacked an HLA compatible donor. In these initial cases, we showed that marrow transplants from HLA half-matched partial donors depleted of T-cells by lectin agglutination and E rosette depletion could engraft and reconstitute hematopoietic as well as T and B cell line function without either acute or chronic GVHD. Of 80 patients with SCID we have transplanted using this approach since that time, 70% survive, durably engrafted, with full T-cell, and in patients pretreated with myeloablative conditioning, B cell function.[21] The overall incidence of acute GVHD in this series is 7%; no patients developed chronic GVHD. Our experience with HLA haplotype matched parental grafts depleted of T-cells by this technique has been replicated in several centers throughout the United States, Europe and Asia, the largest experiences other than ours being at Duke University,[22] Ulm University [23] and UCSF.[24] Unfortunately, centers employing antibody based methods of T-cell depletion have been less successful in preventing lethal GVHD,[25] likely reflecting the fact that these methods only deplete T-cells by 1.5-2 $\log_{10}$ while the lectin agglutination E rosette depletion technique consistently depletes 3-3.5 $\log_{10}$ T-cells. Consistent with this hypothesis are the recent results of trials evaluating transplants of HLA haplotype disparate parental PBSC transplants depleted of T-cells by positive selection for $CD34^+$ progenitor cells by immunoabsorption to anti:CD34 conjugated immunomagnetic beads and selection on the Clinimacs device,[26] this approach achieves a 4.0 x $\log_{10}$ depletion of T-cells.

Initial experience with HLA haploidentical parental SBA^-E^- marrow grafts in the treatment of leukemia again demonstrated their capacity to abrogate GVHD.[20, 27] However, these transplants were associated with an unacceptably high incidence of graft failure. Our transplant program then demonstrated that these graft failures were mediated by residual host T-cells that exhibited cytotoxic activity specifically against donor unique HLA alleles.[28-30] Subsequently, by step-wise modification of preparatory cytoreduction protocols to include additional T-cell targeted immunosuppressive agents, specifically anti-thymocyte globulin and thiotepa, our group and the transplant program at the University of Perugia developed conditioning regimens that have reduced the incidence of graft failure following such T cell

depleted transplants to < 3% in HLA-matched or 1-2 allele disparate patients [31, 32] and < 10% in HLA haplotype disparate patients.[33, 34] These incidences are equivalent to those recorded after unmodified marrow grafts of similar HLA disparity.[35]

Using such effective conditioning regimens, our group has shown that both $SBA^{-}E^{-}$BMT and $CD34^{+}$ E^{-}PBSCT can achieve consistent engraftment and extended disease-free survival rates of 70% in adults transplanted for high risk AML in 1° or 2° CR or high risk ALL in 1° CR when HLA- matched related or unrelated donors are employed.[32, 34] The Perugia group has also recorded comparable outcomes for patients receiving CD34+ T cell depleted PBSC transplants from HLA-matched related donors after the same conditioning.[36] However, at the same time, the Perugia group has been extensively evaluating related HLA-haplotype disparate transplants, initially employing $SBA^{-}E^{-}$ BMT and, more recently, $CD34^{+}$ T cell depleted PBSC transplants fractionated with the Miltenyi Clinimacs device. Their studies have clearly demonstrated the potential of such transplants to achieve a high incidence of durable engraftment (>90%), and extremely low rates of both acute and chronic GVHD.[33] However, extended disease-free survival rates recorded for such patients with AML or ALL transplanted for high risk disease is 1° CR or in 2° CR have been inferior to those achieved with the same conditioning and T-cell depleted grafts from HLA-matched donors. Strikingly, the difference in results between the HLA matched and HLA haplotype disparate transplants do not reflect differences in rates of engraftment, acute or chronic GVHD or relapse. Rather, they are due to a marked increase in mortality due to infections in recipients of HLA haplotype- disparate grafts.[33, 37]

Furthermore, this increase in infection-related mortality is principally due to infections caused by reactivated latent viruses, principally CMV, and to a lesser extent adenovirus. While the risk of reactivation of CMV in seropositive adult patients is approximately 50% in recipients of allogeneic HLA-matched transplants, be they related or unrelated, T-cell depleted or unmodified, clinical infections, which develop between 1-5 months post transplant, can be cleared with current antiviral drugs in all but 3-5% of patients treated.[38] In contrast, the risk of serious CMV infection in HLA haplotype disparate seropositive recipients is higher and the period of risk may extend to a year post transplant. Furthermore, these infections often fail to clear despite prolonged antiviral therapy. As a consequence, the mortality of such infections has ranged from 15-30%.[33]

The basis for this striking disparity in sensitivity to life-threatening CMV infections is still unclear. However, since several studies have demonstrated clearance of CMV infection post HSCT occurs only in individuals who develop CMV-specific cytotoxic T-cells [39, 40] it is reasonable to hypothesize that the failure of certain of these patients to clear CMV infection may reflect a failure to generate effective CMV-specific T-cell immunity early after viral reactivation.

Recent findings from our laboratory provide a plausible reason why such failures might occur more frequently in a recipient of an HLA haplotype disparate graft. We have been evaluating T-cell responses generated by seropositive donors against a pool of 15-mer peptides spanning the sequence of CMVpp65, the CMV protein that most commonly elicits cytotoxic T- cell responses. These studies have shown that normal seropositive donors maintain responses directed against only 1-2 immunodominant peptide epitopes of CMVpp65 that are presented by no more than 1 or 2 HLA alleles inherited by the donor.[41, 42] Since the

frequencies of clonogenic CMV-reactive CTL precursors in the circulation are 1-5/ 10^4 T-cells, only these immunodominant CMV pp65-specific T-cells would be expected to be transferred to a patient in a T cell depleted transplant that provides 1-10 x 10^4 T-cells/kg recipient weight. In an HLA-matched recipient, these immunodominant T-cells would be able to consistently interact with CMV-infected tissues of the host since all epitope-presenting HLA alleles are also shared by the host. In contrast, in an HLA haplotype- disparate recipient, the transferred CTLp will only be functional if the T-cells transferred recognize immunodominant epitopes presented by HLA alleles shared by the host. If, on the other hand, donor CMV-specific T-cells are restricted by HLA alleles on the haplotype not shared by the recipient, they will not recognize, respond to, or control CMV infected cells of the host.[43] In such patients, effective T-cell immunity will only be provided when new T-cells, generated from hematopoietic progenitors in the graft, develop within the host thymus and there became restricted by HLA alleles of the host. In patients 40-65 years of age, this may not occur until 12 months post transplant.[43, 44]

In fact, we have found that, in 46% of the HLA haplotype matched transplant donors we have tested, CMV T-cells generated from the blood in response to pools of overlapping 15-mers of CMVpp65 are exclusively restricted by HLA alleles not shared by the transplant recipient. Furthermore, we have found that CMV infections occurring in two children who received such transplants were both exceptionally severe and protracted, and resolved only after several months of antiviral drugs. In these patients, CMV-specific T-cells were ultimately generated from transplanted precursors that were educated in and restricted by HLA alleles expressed on the host thymus.[43]

To circumvent these limitations to the effectiveness of HLA haplotype disparate grafts, several approaches have been proposed and explored as adjuncts to T-cell depleted HSCT including: co-administration of large numbers of donor T-cells selectively depleted of alloreactive T-cells capable of initiating GVHD,[45] co-administration of unselected donor T- cells transduced to express a “suicide gene”, such as HSV thymidine kinase, so that they can be eliminated if they induce GVHD,[46] and adoptive transfer of virus-specific or tumor- reactive T-cells sensitized with viral or tumor epitopes presented only by HLA alleles shared by donor and recipient.[47][43] The first approach has been effective in murine models but has had limited success in human trials because T-cells in outbred humans do not respond to different alloantigens all at once. As a result, in vitro treatments used to deplete alloreactive cells remove only a fraction of these cells and do not consistently prevent GVHD. The second approach has recently shown promise in one multicenter trial.[46] However, while GVHD could be abrogated in recipients of suicide vector modified T-cells by treatment with acyclovir; this treatment also eliminates virus-specific T-cells. As a result, long term results were not significantly different from these reported from HLA-haplotype disparate T-cell depleted grafts alone.[33] The third approach is a rational proposal but has thus far not been evaluated because identification of immunogenic viral epitopes that can be presented by HLA alleles other than HLA A0201 and other prevalent HLA alleles is still rudimentary. Furthermore, until recently [41] the logistics of production of T-cells specific for subdominant epitopes has been limiting.

In this protocol, we propose to examine another approach which could facilitate reconstitution of antigen-specific T-cell responses restricted by HLA alleles on either

of the patient's HLA haplotypes and further improve the consistency of durable engraftment and the quality of reconstitution: the concomitant use of two T-cell depleted transplants, one expressing the genotype HLA haplotype of the patient's father; the other, the genotype HLA haplotype of the mother.

The scientific data supporting the use of biparental T-cell depleted hematopoietic progenitor cell transplants was provided initially in the first papers describing the use of allogeneic T-cell depleted transplants as a method for preventing GvHD in histocompatible mice. In 1975, von Boehmer, Nobholz and Sprent [48] demonstrated that co transplantation of CBA/J and DBA/2 marrow and spleen cells depleted of T-cells by treatment with a T-cell specific antibody into CBAXDBA F_1 hybrid mice resulted in durable split chimerism in lymphoid and hematopoietic lineages, with the fraction of cells derived from each donor maintained for over 6 months of observation post transplant. In this study, they also demonstrated that T-cells generated in the host from each parental donor were not reactive against cells of either the F_1 hybrid host or the fully allogeneic second donor. However, they were fully responsive against third party H-2 disparate hosts. In a second study, they further demonstrated that engrafted T-cells from either donor would also cooperate with, and provide effective help to, B cells of the other donor's H-2 type in the generation of antibody responses to sheep red cells.[49]

In our early studies of HLA haplotype disparate parental T-cell depleted SBA^-E^-BMTs in the treatment of children with SCID, we had three patients who did not promptly engraft following a transplant from one parental donor who then received secondary transplants from the other parent. Each of these patients subsequently developed biparental mixed chimerism. Again, as n the murine model, the functional T-cells subsequently generated in vivo from precursors in the graft were mutually tolerant, as evidenced, in vitro, by their failure to respond to either parent's cells or cells from the patient in mixed lymphocyte culture or T-cell mediated cytolysis assays, and, in vivo, by their continued coexistence and the absence of any graft vs. host disease.[50] More recently, we have also shown that transplants of human T-cell depleted CD34+ progenitors isolated from 2 HLA non-identical allogeneic cord blood grafts on the Clinimacs device in immunodeficient NSG mice also potentiate engraftment induce a durable state of dual chimerism suggesting mutual tolerance.[51]

Thus, based on these early studies of T-cell depleted marrow grafts in murine models, our recent studies of the engraftment of $CD34^+$ progenitors from double cord blood transplants and our own early studies of children with SCID who received, sequentially, transplants of SBA^-E^- T-cell depleted marrow from both parents, we hypothesize that biparental transplants will:

1) Provide consistently higher doses of $CD34^+$ progenitor cells and, like double cord blood grafts potentiate engraftment

2) Result in durable mixed chimerism with progenitors from both donors contributing to all hematopoietic and lymphoid lineages

3) Increase the probability that limited but sufficient numbers of immunodominant T-cells will be transferred that can recognize viral epitopes presented by HLA alleles on either haplotype shared by the host

4) Exhibit sustained tolerance of the patient and of each other, as measured clinically by the absence of acute GvHD or other alloimmune or autoimmune disorders, and, in vitro, by absence of reactivity of engrafted cells from either donor against cells of the host or the other transplant donor in mixed leukocyte reactions or assays of cytokine and cytotoxic T-cell responses. As a result of this tolerance, these patients will also be rendered tolerant of adoptively transferred antigen-specific T-cells from either parent.

5) Cooperate in the generation of new, lymphoid progenitor-derived antigen-specific T-cells in the host thymus capable of interacting with antigen presented by HLA alleles shared by each donor and the host, thereby securing for the host the capacity to respond to pathogens with T-cells restricted by HLA alleles on either the maternal or paternal haplotype

6) Allow the engraftment of NK cells from both parental donors thereby enhancing NK-mediated resistance to the host's leukemia [52]

An additional advantage of biparental T-cell depleted transplants to the host in terms of enhanced T-cell mediated resistance to residual leukemia is suggested by the recent studies of Ceciri et al.[53] These investigators observed that among patients with AML who had relapsed following an HLA haplotype disparate transplant, fully 30% of the AMLs exhibited deletion mutations of segments of the host's unique chromosome 6, including the complex of genes encoding the entire host unique HLA haplotype. As a result, the AML blasts were rendered unrecognizable to either alloreactive or tumor-antigen-specific HLA restricted T-cells of the donor. Biparental transplants, by permitting the generation of antigen-specific T-cells restricted by HLA alleles of either parental HLA haplotype should reduce this possibility.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a pilot study to provide initial data on the feasibility, efficacy and safety of biparental HLA haplotype disparate T-cell depleted transplants in the treatment of patients with high risk forms of acute or chronic leukemia, NHL, MDS or other lethal disorders of hematopoiesis who lack an HLA-matched or 9/10 HLA allele compatible donor. The trial allows selection of either of two myeloablative regimens for preparatory cytoreduction that have secured engraftment of HLA non-identical and haplotype disparate T-cell depleted transplants. Thereafter, two GCSF-mobilized PBSC transplants depleted of T-cells by positive selection for CD34+ progenitors on the Clinimacs device will be administered. The paired CD34+ TCD PBSC transplants will be derived from the two parents of the patient, or from first degree relatives, each providing one of the two genotypically identical HLA haplotypes inherited by the patient. Patients will be clinically evaluated for engraftment, incidence and severity of acute and chronic GVHD, transplant related complications, relapse and both overall and disease-free survival. Stopping rules are incorporated to insure against an excess risk of graft failure, severe GVHD or transplant-related mortality. Patients will also be evaluated sequentially for state of chimerism, and for quality and extent of immune reconstitution, particularly as reflected by tolerance or alloreactivity against donor, host or third party alloantigens, and the HLA restrictions of antigen-specific T-cell responses.

4.2 Intervention

Patients with high risk forms of acute leukemia, CML, multiple myeloma, myelodysplastic syndrome (MDS) or other lethal disorders of hematopoiesis who fulfill eligibility requirements, consent to treatment and have two healthy consenting donors, each bearing one of the patient's HLA haplotypes will receive myeloablative conditioning consisting of one of the following regimens:

1) 1375-1500 cGy hyperfractionated total body irradiation (depending on age and stage of disease) with lung shielding, followed by thiotepa (5 mg/kg/day x 2 or 10mg/kg/day x 1) and Fludarabine 25mg/m^2/ day x 5 days.

2) Fludarabine 25 mg/m^2/day x 5, melphalan 70 mg/m^2/day x 2 and thiotepa 5 mg/kg/day x 2 (or 10mg/kg/day x 1)

All patients will also receive antithymocyte globulin (ATG) (thymoglobulin 2.5 mg/Kg/day x 3 or equine ATG 15 mg/kg/day x 3 if thymoglobulin is not tolerated) during pre-transplant conditioning to deplete radiation or chemotherapy resistant host T-cells that could hamper engraftment.

Following preparative cytoreduction, all patients will receive the two GCSF mobilized PBSC transplants depleted of T-cells by positive selection of CD34+ progenitor cells with the CliniMACS system. The targeted dose progenitor cells for each transplant will be > 5 x 10^6 $CD34^+$ cells/kg with the dose of T-cells limited to < 2.0 x 10^4/CD3+ cells/kg. The cell doses of each transplant will be adjusted to provide the same dose of $CD3^+$ T-cells/kg. +/- 0.1X$10^4$$CD3^+$ T-cells/kg.

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

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

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

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

5.1.1. Total Body Irradiation

Hyperfractioned TBI is administered by a linear accelerator at a dose rate of < 20 cGy/minute. Doses of 125 cGy/fraction are administered at a minimum interval of 4 hours between fractions, three times/day for a total of 11 or 12 doses (1375 or 1500 cGy) over 4 days (Day -10, -9, -8, and -7). Sequential doses are administered in an anterior/posterior or lateral orientation. Compensators and lung blocks are used to shield the lung, so that the

lung receives 800 cGy. The blocked areas of the chest will be boosted with high-energy electrons so that the cumulative chest wall dose is approximately 1500 cGy. This insures that marrow sites in the ribs are adequately treated.

If general anesthesia is required for TBI administration (e.g. young children), a dose of 200cGy q12h x 7 doses to a total dose of 1400 cGy may be given.

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

5.1.2. Thiotepa (Thioplex®)

Thiotepa is administered at a dose of 5mg/Kg/day for two days (d -6, d -5) or 10mg/kg for one day (d-5). Thiotepa is infused intravenously over approximately 4 hours.

Formulation: 15 mg vial lyophilized powder; must be diluted prior to infusion.

Reconstitution: Add 1.5 ml of Sterile Water for injection to 15mg vial to yield 10mg/ml. Solutions which are grossly opaque or contain a precipitate, should not be used. In order to eliminate haze, solutions should be filtered through a 0.22-mcron filter prior to administration.

Storage and Stability:

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

Preparation:

1. Standard IV fluid: NSS
2. Final concentration range up to: 5mg/ml.
3. IV piggyback volume: 500 cc.
4. Spike infusion bag with IMED 2200 tubing, primed with non-chemo containing fluid (i.e. NSS).

Clinical Considerations:

Hydration: NA

Emetic Potential: High

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

5.1.3 Fludarabine (FLUDARA®)

Fludarabine is administered intravenously at a dose of 25mg/m^2/day for 5 days (days -6 to -2).

a. Source and Pharmacology: Supplier: Berlex Laboratories, Inc. FLUDARA FOR INJECTION contains fludarabine phosphate, a fluorinated nucleotide analog of the

antiviral agent vidarabine, 9-ß-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination by adenosine deaminase. The chemical name for fludarabine phosphate is 9H-Purin-6-amine, 2-fluoro-9-(5-O-phosphono-ß-D-arabinofuranosyl). Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

b. **Formulation and Stability:** Each vial of sterile lyophilized solid cake contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Reconstitution with 2 mL of Sterile Water for Injection USP results in a solution containing 25 mg/mL of fludarabine phosphate intended for intravenous administration. FLUDARA FOR INJECTION is supplied in a clear glass single dose vial (6 mL capacity) and packaged in a single dose vial carton in a shelf pack of five

c. **Solution Preparation:** FLUDARA should be prepared for parenteral use by aseptically adding Sterile Water for Injection USP. When reconstituted with 2 mL of Sterile Water for Injection, USP, the solid cake should fully dissolve in 15 seconds or less; each mL of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. The pH range for the final product is 7.2-8.2. In clinical studies, the product has been diluted in 100 cc or 125 cc of 5% Dextrose Injection USP or 0.9% Sodium Chloride USP

d. **Storage and Stability:** FLUDARA is supplied as a white, lyophilized solid cake. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Store under refrigeration, between 2°-8° C (36°-46° F).

e. **Administration:** Intravenous

5.1.4 Melphalan (Alkeran®)

Melphalan is administered intravenously at a dose of 70mg/m^2/day for 2 days (days -8 to -7).

a. **Source and Pharmacology:** Supplier: Glaxo Wellcome. A derivative of nitrogen mustard, an analog of mustard gas. It is a polyfunctional alkylating agent that causes miscoding, cross-linkage of DNA, and single-strand breakage of DNA. It also inhibits cellular glycolysis, respiration, and protein synthesis. It is cell cycle-phase non-specific.

b. **Formulation and Stability:** A lyophilized powder of 50 mg melphalan and 20 mg povidone per vial. Also provided is 10 ml of sterile diluent for use in reconstituting the product and a 0.45 micron filter. The special diluent has the following composition: Sodium citrate 0.2 g, Propylene glycol 6.0 ml, Ethanol (95%) 0.5 ml, and sterile water 10 ml.

c. Solution Preparation: Vial/50 mg: Reconstitute by rapidly injecting 10 ml of the supplied diluent into the vial to yield a final concentration of 5 mg/ml. Shake vigorously until the solution is clear. Immediately dilute the dose to be administered in 0.9% Sodium Chloride, USP, to a concentration no greater than 0.45 mg/ml

d. Storage and Stability: The intact packages should be stored at room temperature (15-30°C) protected from light. Shelf-life surveillance of the intact dosage form is ongoing. Constitution with the special diluent as directed results in a solution that retains at least 90% potency for about three hours at 30°C. Storage at 5°C results in precipitation.

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

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

Anti-thymocyte Globulin is administered intravenously at a dose of 2.5mg/Kg/dose on each of 3 days (d -4, -3, -2).

a. **Source and pharmacology:** Supplier: Sangstat, The Transplant Company®. Thymoglobulin® [Anti-thymocyte Globulin (Rabbit)] is a purified, pasteurized, gamma immune globulin, obtained by immunization of rabbits with human thymocytes. This immunosuppressive product contains cytotoxic antibodies directed against antigens expressed on human T-lymphocytes.

b. **Formulation and stability:** Thymoglobulin is a sterile, freeze-dried product for intravenous administration after reconstitution with sterile Water for Injection, USP (WFI). Each package contains two 7 mL vials: Vial 1: Freeze-Dried Thymoglobulin Formulation Active ingredient: Anti-thymocyte Globulin (Rabbit) 25 mg - Inactive ingredients: Glycine (50 mg), mannitol (50 mg), sodium chloride (10 mg); Vial 2: Diluent Sterile Water for Injection, USP 5 mL. The reconstituted preparation contains approximately 5 mg/mL of Thymoglobulin, of which >90% is rabbit gamma immune globulin (IgG). The reconstituted solution has a pH of 7.0± 0.4. Human red blood cells are used in the manufacturing process to deplete cross-reactive antibodies to non-T-cell antigens. The manufacturing process is validated to remove or inactivate potential exogenous viruses. All human red blood cells are from US registered or FDA licensed blood banks. A viral inactivation step (pasteurization, i.e., heat treatment of active ingredient at 60°C/10 hr) is performed for each lot. Each Thymoglobulin lot is released following potency testing (lymphocytotoxicity and E-rosette inhibition assays), and cross-reactive antibody testing (hemagglutination, platelet agglutination, anti-human serum protein antibody, antiglomerular basement membrane antibody, and fibroblast toxicity assays on every 5th lot).

c. **Solution preparation:** Each reconstituted vial contains 25 mg or 5 mg/mL of Thymoglobulin. Transfer the contents of the calculated number of Thymoglobulin vials into the bag of infusion solution (saline or dextrose). Recommended volume: per one vial of Thymoglobulin use 50 mL of infusion solution (total volume usually between 50 to 500 mL). Mix the solution by inverting the bag gently only once or twice.

d. Storage and stability: Store in refrigerator between +2° C to +8° C (36° F to 46° F). Protect from light. Do not freeze. Do not use after the expiration date indicated on the label. Reconstituted vials of Thymoglobulin should be used within 4 hours. Infusion solutions of Thymoglobulin must be used immediately. Any unused drug remaining after infusion must be discarded.

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

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

The CliniMACS System (Miltenyi Biotec, Auburn, CA) including the CliniMACS^{plus} Instrument, a CliniMACS Tubing Set, the CliniMACS CD34 Reagent and the CliniMACS PBS/EDTA Buffer is intended for the selection and enrichment of human CD34 positive hematopoietic progenitor cells from a leukapheresis product.

The CD34 antigen is a cell membrane glycoprotein expressed by early hematopoietic stem and progenitor cells. The CD34 positive cell separation process may be useful in several areas of clinical stem cell transplantation, including purging of tumor cells, T-cell depletion, *ex vivo* cell expansion and gene therapy. When re-infused after myeloablative chemotherapy, CD34 positive peripheral blood progenitor cells have been shown to reconstitute all hematologic lineages and exhibit both short and long term repopulating capacities.

The ClinMACS System uses selective CD34 monoclonal antibodies conjugated to super-paramagnetic particles. The CD34 positive target cells are selected in an automated continuous flow separation system.

The CD34 positive cells are specifically labeled for selection by incubation with the CliniMACS CD34 Reagent. After unbound reagent is washed from the suspension, the cells are ready for the automated separation process. The CliniMACS System passes the antibody-labeled suspension, the cells are ready for the automated separation process. The CliniMACS System passes the antibody-labeled suspension through a column in which strong magnetic gradients are generated. The Selection Column retains the magnetically labeled CD34 positive cells, while unwanted cells flow through the Selection Column and are collected in the Negative Fraction Bag. The system performs several washing steps, disposing most of the liquid into the Buffer Waste Bag. The Separated CD34 positive cells are released from the column by removing the column from the magnetic field and cluting the cells into the Cell Collection Bag.

The components of the CliniMACS System include:

5.3.1 The CliniMACS Instrument

The CliniMACS Instrument is a bench-top instrument consisting of a supporting structure to hold the column/tubing assembly and various bags, a series of valves through which the tubing set is fitted, a magnet between the poles of which the separation column is placed, a peristaltic pump through which a section of tubing is placed, software to control the instrument and user

interface and a computer touchpad with a display window. The instrument is operated at ambient temperature and it is intended to be multi-use item.

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

5.3.2. CliniMACS Tubing Set

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

The tubing element consists of a series of tubes, connectors, spikes, Luer locks, and collection bags. The tubing of the tubing element is comprised of materials that have been qualified for use in this application by testing to ISO 10993. The principal constituents are polyvinyl chloride (PVC) and silicone. The connectors are made of various polymers (e.g., ABS and PVC) suitable for use in a blood contact environment. They are solvent bonded to the PVC tubing. The silicone pump tubing is softened with petroleum ether for manufacturing and mechanically fixed to connectors. The cell wash bags are composed of PVC.
The CliniMACS Tubing Set is packaged in a thermoformed tray and heat sealed with a Tyvek® lid. The CliniMACS Tubing Set is sterilized by ethylene oxide gas in a validated sterilization cycle and supplied as a single-use component for the CliniMACS Instrument.

5.3.2. CliniMACS CD34 Reagent

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

CliniMACS CD34 Reagent is supplied sterile and pyrogen-free in glass vials containing 7.5 mL and is intended for single use and in vitro use only.

5.4. The CliniMACS PBS/EDTA Buffer

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

Table 1 Formulations of the CliniMACS PBS/EDTA Buffer

Ingredient	Compendial	Amount
NaCl	Ph. Eur.	8.0 g/L
KCl	Ph. Eur.	0.19 g/L
Na2HPO4 anhy.	Ph. Eur.	1.15 g/L
KH2PO4	Ph. Eur.	0.19 g/L
Na2EDTA	Ph. Eur.	0.37 g/L
Water for Injection	Ph. Eur.	ad 1L

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

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

Malignant conditions for which CD34+ selected, T-cell depleted allogeneic hematopoietic stem cell transplantation is indicated such as:

1) AML in 1st remission - for patients whose AML does not have 'good risk' cytogenetic features (i.e. t 8;21, t15;17, inv 16).
2) Secondary AML in 1st remission
3) AML in 1st relapse or $\geq$ 2nd remission
4) ALL/LL in 1st remission clinical or molecular features indicating a high risk for relapse; or ALL $\geq$ 2nd remission
5) CML failing to respond to or not tolerating Imatinib, dasatinib, or nilotinib in first chronic phase of disease; or CML in accelerated phase or second chronic phase.
6) Non-Hodgkins lymphoma with chemoresponsive disease in any of the following categories:
    a) intermediate or high grade lymphomas who have failed to achieve a first CR or have relapsed following a 1st remission who are not candidates for autologous transplants.
    b) any NHL in remission which is considered not curable with chemotherapy alone and not eligible/appropriate for autologous transplant.

7) Myelodysplastic syndrome (MDS): RA/RCMD with high risk cytogenetic features or transfusion dependence, RAEB-1 and RAEB-2 and Acute myelogenous leukemia (AML) evolved from MDS, who are not eligible for transplantation under protocol IRB 08-008.
8) Chronic myelomonocytic leukemia: CMML-1 and CMML-2.
9) Other rare lethal disorders of Hematopoiesis and Lymphopoiesis for which a T-cell depleted transplant is indicated (e.g. hemophagocytic lymphohistiocytosis; refractory aplastic anemia or conjugated cytopenias; non-SCID lethal genetic immunodeficiencies such as Wiskott Aldrich Syndrome, CD40 ligand deficiency, ALPS).

Accrual will include up to 21 patients

The following inclusion criteria are also required:

- Patients may be of either gender and of any racial or ethnic background.
- Patients must have a Karnofsky (adult) or Lansky (pediatric) Performance Status ≥ 70%.
- Patients must have adequate organ function measured by:

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

  Hepatic: < 3x ULN ALT and < 2.0x ULN total serum bilirubin, unless there is congenital benign hyperbilirubinemia.

  Renal: serum creatinine ≤1.2 mg/dl or if serum creatinine is outside the normal range, then CrCl > 40 ml/min (measured or calculated/estimated)

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

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

6.2 Subject Exclusion Criteria

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

6.3 Donor Inclusion Criteria

- Each donor must meet criteria outlined by institutional policies.
- Donor must have adequate peripheral venous catheter access for leukapheresis or must agree to placement of a central catheter.

6.4 Donor Exclusion Criteria

- Evidence of active infection (including urinary tract infection, or upper respiratory tract infection), viral hepatitis exposure (on screening), unless only HB_S Ab+ and HBV DNA negative, or serologic evidence of exposure or infection with HIV-I/II or HTLV-I/II
- If donors do not meet institutional guidelines, exclusion will be considered.

7.0 RECRUITMENT PLAN

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

8.0 PRETREATMENT EVALUATION

8.1. Pretreatment evaluation of the patient

Each patient will receive an extensive medical evaluation within approximately 30 days prior to starting preparatory cytoreduction. This evaluation may include the below tests:

- Complete physical exam and medical history
- Dental evaluation (may be completed outside of 30 day window)
- HLA typing as per institutional guideline
- CBC
- PT/PTT/INR
- Blood Type and screen
- Serum chemistries including BUN, creatinine, electrolytes, glucose, total protein, albumin, liver function tests (AST, ALT, bilirubin, alkaline phosphatase).
- Infectious disease markers will be performed as per each department's guidelines or at the discretion of the treating attending.
- Pregnancy test for women of childbearing age
- Bone marrow aspirate and/or biopsy
- Urinalysis
- Electrocardiogram, echocardiogram or a gated pool scan if needed
- Pulmonary function test for patients older than 7 years if indicated
- Chest X-ray and other types of scans (CT scan and PET scan, if needed)
- Samples of bone marrow and/or peripheral blood cells will be obtained to define donor/host genetic differences and to determine engraftment of donor cells (may be completed outside of 30 day window)
- Patients with ALL or with M4 or M5 type AML will have a diagnostic LP performed

Whenever possible, in addition to these baseline tests, a 25ml aliquot of blood will be drawn within 1 month prior to transplant which will be used 1) to establish a patient derived EBV transfused B cell line as an antigen presenting cell 2) to analyze the patient's HLA restricted T-cell responses to EBV and CMV and 3) to provide irradiated PBMC to test responses of engrafted T-cells against the patient's alloantigens.

8.2. Pretreatment evaluation of the two donors

Whenever possible, in addition to these baseline tests, donors will be asked to consent to the appropriate cellular therapy trial (i.e.: MSKCC IRB # 95-024, 07-055, 11-130, or 12-086) so that we may procure the following: a 25ml aliquot of blood will be drawn within 1 month prior to

transplant which will be used to 1) establish a donor-derived EBV transfused B cell line as an antigen presenting cell of donor origin, 2) to analyze the donor's HLA-restricted T-cell responses to EBV and CMV and 3) to serve as a donor-type target for mixed lymphocyte reactions testing whether the engrafted donor T-cells are tolerant or alloreactive in nixed leukocyte culture post transplant.

9.0 TREATMENT/INTERVENTION PLAN

9.1 Admission for Transplantation

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

9.2 Cytoreduction Regimen in Preparation for Transplantation:

Patients will be assigned to one of two cytoreductive regimens depending on their disease and treatment history.

Regimen 1 will be the regimen of choice for those patients with hematologic malignancies who are able to tolerate TBI and who are without significant treatment associated comorbidity.

Regimen 2 will be the regimen of choice for patients with non-malignant, lethal disorders of hematopoiesis and immunity, and those patients with hematologic malignancies who cannot receive TBI due to extensive prior treatment and comorbidity.

9.2.1a Regimen 1 - Hyperfractioned Total Body Irradiation, Thiotepa and Fludarabine.

Hyperfractionated TBI is administered at a dose rate of < 20 cGy/minute. Doses of 125 cGy/fraction are administered at a minimum interval of 4 hours between fractions, three times/day for a total of 11 or 12 doses (1375 or 1500 cGy) over 4 days (Day -10, -9, -8, and -7).

1500 cGy will be administered to children $\leq$ 18 years of age with ALL > 1^{o}CR, since the higher dose has greater anti-leukemic activity and is well tolerated. Sequential doses are administered in an anterior/posterior or lateral orientation. Lung blocks will be used to reduce the lung dose to 800 cGy. The blocked chest wall areas will be boosted with high-energy electrons so that the cumulative chest wall dose is approximately 1500 cGy, so as to insure that marrow sites in the ribs are adequately treated.

If general anesthesia is required for TBI administration (e.g. young children), a dose of 200cGy q12h x 7 doses to a total dose of 1400 cGy may be given.

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

9.2.1b Thiotepa

Thiotepa: 5mg/kg/day IV over approximately 4 hr qd x 2 days (Day-6 and Day -5) or 10mg/kg for one day (Day -5).

In select cases in which the G-CSF mobilized PBSC must be harvested a day later than requested due to a scheduling issue with the donor or Stem Cell Processing Laboratory, there may be a day of rest between the last day of radiation and the first day of thiotepa (Donor can only donate on Friday). Alternatively, if the marrow must be handled a day earlier than requested, Thiotepa may be given in a single dose of 10mg/Kg on day -6. 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.

9.2.1c Fludarabine

Fludarabine will be administered at a dose of 25 mg/M^2 IV over approx. 1/2hr qd x 5 days (d -6 through d -2). The first dose will begin approximately 8 hours prior to the thiotepa. On d-5 fludarabine will be administered first followed by Thiotepa 8-10 hours later. On days -4, -3, -2, Fludarabine will also be administered approximately 2 hours prior to ATG. Fludarabine may be adjusted in the case of renal toxicity, as per MSKCC standard of care guidelines.

9.2.1d Rabbit antithymocyte globulin (thymoglobulin®)

Rabbit ATG will be given to all transplant recipients. Rabbit antithymocyte globulin (Genzyme) will be administered on days -4, -3, and -2 at a dose of 2.5 mg/kg (actual body weight/day). If the patient has a history of allergy or intolerance to rabbit ATG, equine antithymocyte globulin at a dose of 15 mg/kg x 3 doses may be used. If severe reaction is encountered after the 1st dose of ATG, the second and third doses can be delayed until day +5 and day +7. Methylprednisolone 1 mg/kg will be given as premedication. Additional medications to prevent or treat reactions will be administered as indicated according to institutional guidelines. ATG dosing will be adjusted if the patient is > 125% of ideal body weight and will be calculated based on adjusted ideal body weight, as per MSKCC standard of care guidelines.

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

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

9.2.1e Approximate Schema of Conditioning Regimen 1

APPROXIMATE SCHEMA OF CYTOREDUCTION AND PREPARATION FOR ALLOGENEIC PBSCT

-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0
TBI	TBI	TBI	TBI							
				THIO	THIO					

				FLU	FLU	FLU	FLU	FLU		
						rATG	rATG	rATG		$CD34^+$ PBSC Transplant
Donor Mobiliza-tion					X	X	X	X	X Begin Leu-kapheresis	X Continue Leu-kapheresis

This schedule may be modified and doses may be adjusted if clinically appropriate.

9.2.2a Regimen 2 – Fludarabine, Thiotepa, and Melphalan.

9.2.2a Fludarabine

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

9.2.2b Melphalan

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

9.2.2c Thiotepa

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

9.2.2d Rabbit antithymocyte globulin (thymoglobulin®)

Rabbit ATG will be given to all transplant recipients. Rabbit antithymocyte globulin (Genzyme) will be administered on days -4, -3, and -2 at a dose of 2.5 mg/kg (actual body weight/day). If the patient has a history of allergy or intolerance to rabbit ATG, equine antithymocyte globulin at a dose of 15 mg/kg x 3 doses may be used. If severe reaction is encountered after the 1st dose of ATG, the second and third doses can be delayed until day +5 and day +7. Methylprednisolone 1 mg/kg will be given as premedication. Additional medications to prevent or treat reactions will be administered as indicated according to institutional guidelines. ATG dosing will be adjusted if the patient is > 125% of ideal body weight and will be calculated based on adjusted ideal body weight, as per MSKCC standard of care guidelines.

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

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

9.3.4e Approximate Schema of Conditioning Regimen 2

APPROXIMATE SCHEMA OF CYTOREDUCTION AND PREPARATION FOR ALLOGENEIC PBSCT

-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0
		MEL	MEL							
				THIO	THIO					
				FLU	FLU	FLU	FLU	FLU		
						rATG	rATG	rATG		$CD34^{+}$ PBSC Transplant
Donor Mobiliza-tion					X	X	X	X	X Begin Leu-kapheresis	X Continue Leu-kapheresis

This schedule may be modified and doses may be adjusted if clinically appropriate.

9.3 Prophylaxis against acute graft-versus-host disease

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

9.4. *Stem Cell Transplantation*

9.4.1. PBSCT

Donor peripheral blood progenitor cells: stimulation, harvesting, isolation and T-cell depletion.

For both related donors, GCSF will be administered according to MSKCC standard of care.

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

The apheresis product is collected from two blood-related, genotypically HLA haplotype matched donors. Aliquots of the apheresis product are collected and then tested and screened as per blood banking guidelines. The apheresis product is then prepped for the CliniMACS Cell Selection System. The mechanism of action of the CliniMACS Cell Selection System is based on magnetic-activated cell sorting, which can select or remove specific cell types depending on the cell-specific immunomagnetic label used. The apheresis product is first co-incubated with the CliniMacs CD34 reagent (antibody-coated paramagnetic particles). After magnetic labeling and washing, the cells are passed through a high-gradient magnetic separation column in the CliniMACS clinical cell selection device. Magnetically labeled CD34+ cells are retained in the magnetized column, and $CD34^{-negative}$ cells flow through as

the effluent fraction and discarded. The $CD34^{+positive}$ cells retained in the column are eluted by removing the magnetic field from the column, then washing the cells through the column and collecting them. The final CD34+ cell enriched product is concentrated by centrifugation and tested before final release for administration. The SOPs from the MSKCC Cytotherapy Lab Manual are appended.

Before infusion, the CD34+ cells will be washed in normal saline for intravenous infusion containing 1% human serum albumin, and suspended in a volume of 25-50 ml. for intravenous administration.

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

9.4.2 Transplantation of the T-cell depleted stem cells.

For the first patient, the CD34+T-cell depleted peripheral blood progenitor cell graft expressing the paternal HLA haplotype, suspended in a volume of approximately 20-30 ml will be infused intravenously over 10-15 minutes with monitoring of vital signs. The second $CD34^{+}$ T-cell depleted graft expressing the maternal HLA haplotype will be infused approximately 30 minutes thereafter. For subsequent patients, the order of transplants will be alternated as to the HLA haplotype of the first donor (i.e. maternal or paternal HLA sharing graft first). Each patient will be premedicated as for blood product transfusions.

9.5. *Supportive Care*

a. *Prophylaxis against infections*

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

b. *Prophylaxis against menorrhagia*

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

c. *Transfusions*

Following initiation of the pre-transplant cytoreduction, all blood products for transfusion, with the exception of the peripheral blood stem cell, will be irradiated to 3,000 cGy to inactivate lymphocytes capable of initiating lethal GvHD. Blood products are irradiated in the blood bank, using a cesium gamma emitter.

Platelets will be administered for clinical evidence of active hemorrhage. Platelets will be administered at the discretion of the treating attending and as per standard of care.

d. *Nutritional support*

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

10.0 EVALUATION DURING TREATMENT/INTERVENTION

10.1 Post-transplant evaluation

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

Activity	Transplant to Discharge	Discharge to Day 100	6 Months	Long term follow-up
Blood counts and chemistry (CBC, Comprehensive Metabolic Panel)	CBC: Daily CMP: 2x a week	CBC/CMP: Weekly , 2x a week, or every 2-3 weeks	6 months after transplant	9, 12, & 24 months after transplant; thereafter as clinically indicated
Physical exam for GVHD evaluation	Monitored routinely as per inpatient transplant service guidelines	About weekly until day 100	6 months after transplant	9, 12, & 24 months after transplant; thereafter as clinically indicated
Disease evaluation, BMA [1,2]	30 - 100 days after transplant, if clinically indicated		6 months after transplant	12 & 24 months after transplant; thereafter as clinically indicated
NK cell & T cell chimerism[2]	30 - 100 days after transplant, if clinically indicated		6 months after transplant	12 & 24 months after transplant; thereafter as clinically indicated
Peripheral blood lymphocyte (PBL) phenotyping	30 days after transplant	3 months after transplant	6 months after transplant	9 months after transplant; thereafter every 3 months if clinically indicated
In vitro response of PBL to standard panel of mitogens [2]	N/A	3 months after transplant	6 months after transplant	About every 3-6 months until normal or plateau
In vitro mixed leukocyte responses of PBL to irradiated PBMC of each[3]	N/A	3 months after transplant	6 months after transplant	9 months & 12 months after transplant; thereafter as clinically indicated
In vitro assessment of engrafted T-cell responses to EBV and CMV and their HLA restrictions[3]	N/A	3 months after transplant	6 months after transplant	9 months & 12 months after transplant; thereafter as clinically indicated

1. This should apply to patients with malignant disease in which the BM was previously involved. BM Chimerism should also be assessed whenever possible. BMA may be delayed if clinically indicated
2. May be done more frequently if clinically indicated. Tests need not be done if patient has a low number of peripheral white cells.
3. 4-6 Green top tubes

11.0 TOXICITIES/SIDE EFFECTS

Patients recruited to this transplantation trial are individuals who are either referred by physicians or self-referred for marrow transplantation as a potentially curative treatment for their malignancy. 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.

On this study, we will be capturing and tracking Grade 3-5 toxicities which occur within 30 days post-HSCT and are potentially attributable to treatment on study. Toxicities which are attributable to underlying malignancy and/or were present prior to initiation of therapy will not be tracked. Please see section 17.2 for Serious Adverse Event reporting.

11.1 Toxicities/Side effects of conditioning regimens

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

11.1.1 Total Body Irradiation

Likely

- Nausea and vomiting - virtually all patients will experience nausea and vomiting after irradiation. This can be diminished somewhat with mild anti-emetics. Strong sedatives or phenothiazine derivatives should be avoided just before radiation treatment, as they frequently cause excessive drowsiness and/or symptomatic orthostasis, which prevent delivery of TBI done in the standing position.
- Myelosuppression is the major dose-limiting toxicity.
- Hyperpigmentation - most patients will get some degree of hyperpigmentation within 2-3 weeks of transplantation.
- Increased risk of infection

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

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

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

Less Likely

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

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

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

- Erythema - this may occur in patients within 24 hours and resolves in 2-3 days.

- Hypothyroidism has been reported in small numbers of adult patients following TBI plus alkylating chemotherapy, and this will be routinely monitored post transplant with hormonal replacement as indicated.

- There is a possibility that secondary malignancies may develop, particularly due to the combined effects of radiation and an alkylating agent [cyclophosphamide]. The age-adjusted incidence of secondary cancer in transplant patients after radiation and chemotherapy has recently been estimated to be 6.7 times higher than that of first cancers in the general population; and most of these were non-Hodgkin's lymphomas.

11.1.2 Thiotepa

Likely

- Myelosuppression is the major dose-limiting toxicity, occurring regularly at doses >405mg/m^2. Other non-fatal toxicities have been observed almost exclusively after administration of thiotepa at doses greater than 1000mg/m^2.

- Increased risk of infection.

- Cutaneous erythema and bronzing is seen in most patients given ≥900mg/m^2. Erythema develops 4-14d after the first dose and may last up to 3wks. Bronzing may persist for months.

Less Likely

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

Rare but Serious

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

11.1.3 Fludarabine

Likely

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

Rare but Serious

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

11.1.4 Melphalan

Likely

- nausea and vomiting - Diarrhea
- mucositis
- myelosuppression and pancytopenia, with increased risk of infection
- elevated liver function tests
- sterility
- alopecia
- fever
- Transfusions of platelets and red blood cells

Less Likely

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

Rare but serious

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

11.1.8 Rabbit Antithymocyte Globulin (Thymoglobulin)

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

Likely

- Fever, shaking, chills and lowered blood pressure: These are regularly observed, particularly during initial infusions of the rabbit globulin. They probably result from a breakdown of cells binding the antibody.
- Skin rash and itching: A frequent complication which is probably due to minor allergic reactions to rabbit globulin. These symptoms will usually be prevented by or controlled with anti-histamines as well as with concomitant administration of corticosteroids.
- Platelet and white cell count depression: These are frequently observed and are probably caused by the binding of the rabbit antibody to human blood elements. Platelet transfusions will be administered to reduce the chance of bleeding or life threatening hemorrhage.

Less Likely

- Serum sickness: Approximately 30% of patients treated with rabbit globulin will develop a late immune reaction to the globulin resulting in serum sickness 3-10 days after the administration. This may lead to severe skin rashes, mouth and vaginal sores, pain and swelling of the joints, or kidney damage. Serum sickness is transient and its damage reversible but it may require prolonged treatment with corticosteroids.

Rare, but potentially serious

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

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

11.2.1 Potential Sensitization to Murine Proteins

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

11.2.2 PBSC Infusion

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

11.2.3 Infections

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

11.2.4 Microbial Contamination of PBSC

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

11.2.5 Graft Failure / Poor Marrow Function

T cell depletion of donor cells is associated with an increased incidence of graft failure in allogeneic transplant recipients. After allogeneic transplantation, the recipient's marrow function may be poor and leukopenia, anemia, or thrombocytopenia may result from many causes including graft rejection induced by surviving host immune T-cells, or ongoing suppression of engrafted donor blood-forming cells by GVHD, certain types of infection (e.g. CMV. HHV6), immunosuppressant 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.

It is possible that administration of two T-cell depleted transplants each bearing one of the two parental HLA haplotypes might lead to alloreactions between grafts that could increase the risk of graft failure, although our own extensive experience with allogeneic cord blood transplants that are each partially HLA matched with the patient suggests that engraftment is improved. Our own and published experience in mouse models as well as our experience in children with SCID who received two T-cell depleted transplants also suggest that overall engraftment is not impaired.

It is certainly possible that only one of the two parental grafts will durably engraft and induce hematopoietic and lymphoid chimerism and functional reconstitution. This is usually the case following double cord blood transplants. However, this dominance has been ascribed to either a higher cell dose and viability of one cord blood or the activity of T-cells in the dominant cord. In murine models, and limited clinical experience, both T-cell depleted grafts usually contribute to sustained chimerism. However, even if, in these patients, one T-cell depleted graft proves to be dominant, the biparental grafts, like the cord blood grafts, may induce more rapid engraftment and reconstitution and potentially a lower risk of disease relapse. The level of tolerance for alloantigens unique to the host and to the second parental donor, as well as the pattern of HLA restriction exhibited by virus-specific T-cells both early and late post transplant remain to be determined, but the potential for in vivo selection of functional donor T-cells restricted by shared HLA alleles could be enhanced by the biparental grafts even if only one ultimately becomes dominant.

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. Biparental TCD transplants could reduce or possibly increase this risk. Acute and/or chronic GVHD will be treated with immunosuppressive drugs as per the transplant service guidelines.

11.2.7 Veno-occlusive Disease (VOD) of the Liver

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

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

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

11.2.8 End Organ Damage

End organ damage of all or any of the major organs, including the brain, may occur as a result of cumulative toxicity from anti-neoplastic therapy, reactions to other drugs, and as a result of destructive processes (e.g., infection, GVHD, etc.) and may have a fatal outcome. Toxicities may occur in any individual patient due to multiple events and cumulative effects that may involve any and all organs, including the brain. Brain damage can result in severe loss of

cognitive or neurologic function. Data from previous studies do not suggest that the risk of end organ damage is appreciably affected by TCD or the preparative regimens to be used in this study.

11.2.9 Disease Relapse

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

11.2.10 Lymphoproliferative Syndrome

Recipients of TCD allogeneic grafts have an increased risk of developing lymphoproliferative syndromes caused by EBV infection.[54, 55] 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 EBV-specific T-cells/kg from the donor. Rituximab has been shown to induce durable regression in 50 - 70% of cases.[56] However, Rituximab does not enter the CNS and is not effective in treating CNS disease. EBV-immune T-cells are experimental, but can also induce regression of EBV PTLD without risk of graft vs host disease.[57-59]

11.2.11 Death

Following HLA matched or compatible transplants there is an approximately 5-10% risk of treatment related mortality within the first 100 days of transplant due to the risk of severe regimen related toxicity, hemorrhage, opportunistic infection, or other complications. Based on reported experience, the risk of transplant-related mortality following single HLA-haplotype matched transplants is higher (up to 25%) because of the higher risk of graft failures, GVHD and particularly severe viral infections.[33]

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, 9 months (if clinically indicated), and 12 months, 18 months (if clinically indicated), and 24 months posttransplant or as needed thereafter.

12.2 Graft failure or rejection

Primary non-engraftment is diagnosed when the patient fails to achieve an ANC ≥500/μl at any time in the first 28 days post-transplant. If the patient's leukemia recurs during this interval, the patient is scored as having refractory leukemia. In such a situation, the absence of donor hematopoiesis is not evaluable for graft failure or rejection. If host T-cells capable of specifically inhibiting donor hematopoietic progenitor growth in vitro are concurrently detected during graft failure, a presumptive diagnosis of immune mediated rejection is made.[60, 61] If (1) after achievement of an ANC ≥500/mm^3, the ANC declines to <500/mm^3 for more than 3 consecutive days in the absence of relapse, or, (2) there is absence of donor cells in the marrow and/or blood as demonstrated by chimerism assay in the absence of relapse, a diagnosis of secondary graft failure is made. If, however, recurrence of host leukemia is detected concurrently, the patient is not evaluable for graft failure or rejection.

Patients with evidence of graft failure without evidence of recurrence of host leukemia will have additional studies drawn to ascertain cause and define relevant histoincompatibilities. These analyses may include (1) Evaluation of bone marrow aspirates and biopsies for residual or recurrent leukemia, when indicated, (2) Culture and/or molecular analyses of marrow and blood for viral pathogens potentially causing graft failure including CMV, HHV6 and parvovirus B 19, (3) Immunophenotypic and genetic analysis of circulating T-cells and NK cells to ascertain their origin and potential function, (4) Analysis of the functional activity of residual circulating lymphocytes to determine whether and to what degree they exhibit cytotoxic or cyto-inhibitory activity against one of the donors, the 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 will be considered for a secondary transplant from one of the initial donors. The need for additional immunosuppression or treatment for viral infection prior to the secondary transplant will be determined by the results obtained from chimerism and viral studies.

12.3. Graft-versus-host disease

Standard BMT-CTN and IBMTR systems clinical criteria as defined by Rowlings, et al [62] 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 infusions (DLI). If at any time, a patient receives DLI, that time will represent the end-time for evaluation of GvHD. Graft-versus-host disease occurring after DLI infusions will be analyzed separately.

Patients with moderate to severe acute GvHD (grade II-IV) will be treated in standard fashion with high-dose I.V. methylprednisolone (2-20mg/kg/day) or in combination with other immunosuppressants as per ongoing trials on GvHD. 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 Sullivan [64] and treated with standard or experimental immunosuppressive therapy.

12.4. Regimen-related and transplant-related mortality

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

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

Toxicities such as alopecia, mucositis, dehydration, anxiety, infection, anorexia, nausea, vomiting, diarrhea, fever, rash, fatigue, sleep disturbance, anemia, leukopenia, thrombocytopenia, abnormal serum chemistries, impaired renal function and hepatic function occur in almost all patients who receive a myeloablative preparative regimen for transplant and are considered expected adverse events. These toxicities will be routinely monitored as part of a clinical assessment and reported as adverse events at the PIs discretion.

The grading for monitoring transplant related toxicities will be based on the NCI/CTEP common toxicity criteria 4.0. Adverse events/toxicities that will be tracked and collected are Serious Adverse Events that are defined in section 17.

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 before death will be excluded from the above definition, even if an infection was the final cause of death.

12.6. Disease relapse

Relapse of MDS, AML and AML will be defined by an increasing number of blasts in the marrow over 5%, by the presence of circulating peripheral blasts, or by the presence of blasts in any extramedullary site. Cytogenetic analysis of the marrow and/or peripheral blood will also be obtained for the diagnosis of relapse.

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

12.7. Disease-free Survival

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

12.8 Overall Survival

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

12.9 $CD34^+$ and $CD3^+$ Cell Doses

Total $CD34^+$ and $CD3^+$ cell doses will be calculated based on results of flow cytometric analysis.

13.0 CRITERIA FOR REMOVAL FROM STUDY

If at any time the research participant is found to be ineligible for the protocol as designated in the section on Criteria for research participant eligibility (e.g. a change in diagnosis), the research participant will be removed from the study. Also 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.

14.0 BIOSTATISTICS

A pilot study is developed to provide initial data on the efficacy and safety of a biparental HLA haplotype disparate T-cell depleted transplant for patients lacking an HLA-compatible donor. The patient population for this study consists of patients transplanted for leukemia, NHL, and MDS, as well as lethal, non malignant diseases of hematopoiesis and immunity without an HLA identical or 9/10 HLA allele matched related or unrelated donor. A maximum of 21 patients will be accrued to the study and the accrual period is expected to last 3 years with an additional two years of follow up after the accrual has been completed.

At the conclusion of the study, Kaplan-Meier estimates of overall and disease-free survival will be computed. Estimated probabilities for the time to treatment related mortality, the time to infectious complication, and the time to graft versus host disease will be computed using the cumulative incidence function.

Immunologic reconstitution will be summarized by averaging the recorded results across patients at each time point. Measures of tolerance and HLA restriction will be assessed using cytotoxicity assays. Graphic descriptions of the trajectories over time will be produced using kernel smoothing.

In order to reduce patient risk, the study design includes early termination in the event of excessive graft failure, graft versus host disease, or early treatment related mortality during the accrual period. The stopping rules are provided in the table below and consider only the marginal failure probabilities.

Failure type	# of failures needed to stop the study	Failure rate in the population	Probability boundary is crossed
Graft failure	3 in the first 13 patients 4 within 21 patients	0.03	0.09
		0.20	0.90
Treatment Related Mortality by day 100	3 in the first 12 patients 4 in the first 20 patients 5 within 21 patients	0.08	0.10
		0.30	0.91
Acute Graft Versus Host Disease Grades 3-4	3 in the first 12 patients 4 in the first 20 patients 5 within 21 patients	0.08	0.10
		0.30	0.91
Chronic Graft Versus Host Disease	3 in the first 12 patients 4 in the first 20 patients 5 within 21 patients	0.08	0.10
		0.30	0.91

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

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

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

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

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic

Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.2 Randomization

This research study does not require a randomization.

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study and will be responsible for both pediatric and adult accruals. The responsibilities of the RSA and 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.

16.1 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.

16.2 Data and Safety Monitoring

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

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.

17.0 PROTECTION OF HUMAN SUBJECTS

The risks associated with a T-cell depleted transplant after cytoreduction by the 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 biparental T-cell depleted grafts fractionated on the CliniMACS device, when administered after the cytoreduction regimen proposed for study. This may greatly accelerate further development of transplantation approaches for those who currently cannot be transplanted because of a lack of a suitably histocompatible donor.

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

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

Costs: The patient's health plan/insurance company will need to pay for all of the costs of treatment in this study. The patient will be responsible for the costs of standard medical care, all hospitalizations and any transplant complications. Pre-authorization for the transplant will be cleared with the health plan/insurance company prior to admission. Patients will not be paid for taking part in this study. Research tests will be done at no cost to the patient.

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

17.1 Privacy

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

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report should contain the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

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

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

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

17.2.1

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

17.2.2 Definition of SAEs

A serious adverse event (SAE) is defined as one of the following:

• Is fatal or life-threatening
• Results in persistent or significant disability/incapacity
• Constitutes a congenital anomaly/birth defect
• Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above
• Requires inpatient hospitalization or prolongation of existing hospitalization,
• Note that hospitalizations for the following reasons should not be reported as serious adverse events:
    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
    - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
    - Social reasons and respite care in the absence of any deterioration in the patient's general condition
    - hospitalizations or prolongation of hospitalizations, due to complications of the patient's previous therapy and/or underlying malignancy

Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event

Attribution:

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

Expected and Unexpected Event:

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

17.2.3 Reporting Requirements

An adverse event is defined as the appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s), or medical condition(s) that occur after patient/guardian signed ICF has been obtained. Serious Adverse Events (SAEs) are any adverse events occurring while on treatment and within 30 days of the transplant that result in any of the following outcomes (Note: Events beyond 30 days will be reported at the discretion of the PI):

UNEXPECTED EVENT:

- Grades 1-2: Adverse Event Reporting NOT required.
- Grades 3-4: Possible, Probable or Definite attribution to the drug and/or device will be reported.
- Grade 5: Regardless of Attribution will be reported.

EXPECTED EVENT

- Grades 1-3: Adverse Event Reporting NOT required.
- Grade 4: Possible, Probable or Definite attribution to the drug and/or device will be reported.
- Grade 5: Regardless of Attribution will be reported.

SAEs will be graded according to NCI CTCAE 4.0.

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. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

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

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

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20.0 APPENDICES

N/A