Allogeneic Hematopoietic Cell Transplantation Using a/J3+ T-lymphocyte Depleted Grafts from HLA Mismatched Donors

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

Principal Investigator/Department:	Brian Shaffer, MD	Medicine/BMT
Co-Principal Investigator(s)/Department:	Miguel-Angel Perales, MD	Medicine/BMT
	Scott Avecilla, MD, PhD	Laboratory Medicine
	Maria Cancio, MD	Pediatrics
Investigator(s)/Department:	Juliet Barker, MD, MBBS	Medicine/BMT
	David Chung, MD, PhD	Medicine/BMT
	Sergio Giralt, MD	Medicine/BMT
	Heather Landau, MD	Medicine/BMT
	Boglarka Gyurkocza, MD	Medicine/BMT
	Parastoo Dahi, MD	Medicine/BMT
	Esperanza Papadopoulos, MD	Medicine/BMT
	Doris Ponce, MD	Medicine/BMT
	Craig Sauter, MD	Medicine/BMT
	Jonathon Peled, MD, PhD	Medicine/BMT
	Melody Smith, MD	Medicine/BMT
	Christina Cho, MD	Medicine/BMT
	Alan Hanash, MD, PhD	Medicine/BMT
	Scott James, MD, PhD	Medicine/BMT
	Michael Scordo, MD	Medicine/BMT
	Gunjan Shah, MD	Medicine/BMT
	Roni Tamari, MD	Medicine/BMT
	Ioannis Politikos, MD	Medicine/BMT
	Arnab Ghosh, MD	Medicine/Genitourinary One
	Richard Lin, MD	Medicine/BMT
	Kate Markey, MD, PhD	Medicine/BMT
	Katharine C. Hsu, MD, PhD	Medicine/BMT
	Oscar Lahoud, MD	Medicine/ Leukemia
	Cheryl Goss, MD	Laboratory Medicine
	Sean Devlin, PhD	Epidemiology/Biostatistics
	Farid Boulad, MD	Pediatrics
	Kevin Curran, MD	Pediatrics
	Nancy Kernan, MD	Pediatrics
	Andrew Kung, MDm PhD	Pediatrics
	Richard O'Reilly, MD	Pediatrics
	Susan Prockop, MD	Pediatrics
	Andromachi Scaradavou, MD	Pediatrics
	Barbara Spitzer, MD	Pediatrics
	Jaap Jan Boelens, MD PhD	Pediatrics
	Ann Jakubowski, MD	Medicine/BMT
	Shani Irby, NP	Nursing
	Sheila Kenny, NP	Nursing
	Chelsea Mintz, NP	Nursing
	Courtney McElrath, NP	Nursing

	Jason Batalha, NP	Nursing
	Marci Andrejko, NP	Nursing
	Stefanie Kelly, NP	Nursing
	Kathleen Kilroy, NP	Nursing
	Melissa Cortes, NP	Nursing
	Dayna Palumbo, NP	Nursing
	Victoria Szenes, NP	Nursing
	Melissa Varda, NP	Nursing
	Theresa Elko, PA	Medicine
	Natasia Rodriguez, RN	Nursing
	Caroline Carino, RN	Nursing
	Jennifer Bieler, RN	Nursing
	Linh Nguyen, RN	Nursing
	Naomi Cazeau, NP	Nursing
	Christina Kiss, NP	Nursing
	Kathryn Mazzarella, NP	Nursing
	Christianne Supan, NP	Nursing
	Kathleen Cavalier, NP	Nursing
Consenting Professional(s)/Department:	Brian Shaffer, MD	Medicine/BMT
	Miguel-Angel Perales, MD	Medicine/BMT
	Juliet Barker, MD, MBBS	Medicine/BMT
	David Chung, MD, PhD	Medicine/BMT
	Sergio Giralt, MD	Medicine/BMT
	Heather Landau, MD	Medicine/BMT
	Boglarka Gyurkocza, MD	Medicine/BMT
	Parastoo Dahi, MD	Medicine/BMT
	Esperanza Papadopoulos, MD	Medicine/BMT
	Doris Ponce, MD	Medicine/BMT
	Craig Sauter, MD	Medicine/BMT
	Jonathon Peled, MD, PhD	Medicine/BMT
	Melody Smith, MD	Medicine/BMT
	Christina Cho, MD	Medicine/BMT
	Alan Hanash, MD, PhD	Medicine/BMT
	Scott James, MD, PhD	Medicine/BMT
	Michael Scordo, MD	Medicine/BMT
	Gunjan Shah, MD	Medicine/BMT
	Roni Tamari, MD	Medicine/BMT
	Ioannis Politikos, MD	Medicine/BMT
	Arnab Ghosh, MD	Medicine/ Genitourinary One
	Richard Lin, MD	Medicine/BMT
	Kate Markey, MD, PhD	Medicine/BMT
	Katharine C. Hsu, MD, PhD	Medicine/BMT
	Oscar Lahoud, MD	Medicine/Leukemia
	Cheryl Goss, MD	Laboratory Medicine
	Maria Cancio, MD	Pediatrics
	Farid Boulad, MD	Pediatrics
	Kevin Curran, MD	Pediatrics
	Nancy Kernan, MD	Pediatrics

	Andrew Kung, MD, PhD	Pediatrics
	Richard O'Reilly, MD	Pediatrics
	Susan Prockop, MD	Pediatrics
	Andromachi Scaradavou, MD	Pediatrics
	Barbara Spitzer, MD	Pediatrics
	Jaap Jan Boelens, MD PhD	Pediatrics
	Ann Jakubowski, MD	Medicine/BMT

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

OneMSK Sites
Manhattan

Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, New York 10065

Table of Contents

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

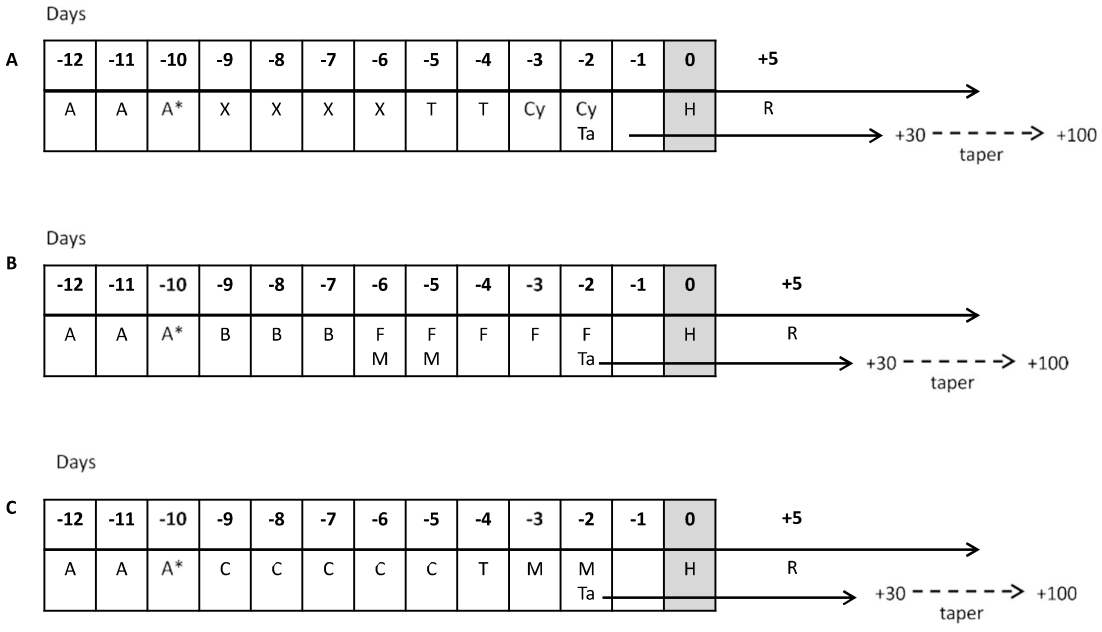
This is a Phase II study evaluating human leukocyte antigen (HLA) mismatched unrelated donor (URD) or related haploidentical donor allogeneic hematopoietic cell transplantation (allo HCT) using TCR-a/W lymphocyte depletion based graft *versus* host disease (GVHD) prophylaxis.

The major barrier to successful outcomes in HLA mismatched allograft recipients is the propensity of recipients to develop severe acute GVHD. This protocol will evaluate the use of unrelated donor hematopoietic progenitor cells depleted of TCR-a/W lymphocytes using immunomagnetic (CliniMACS™) methodology. The primary objective of the study is to estimate the incidence of severe acute GVHD in recipients of this allograft type. Secondary endpoints are to estimate core clinical outcomes in recipients including cumulative incidence of non-relapse mortality, relapse, and death at one year post transplant. Additionally, this study will explore immune reconstitution in all recipients.

Subjects with high risk hematologic malignancies who lack a HLA matched sibling or matched unrelated donor and who otherwise meet the eligibility outlined in section 6.0 may be offered enrollment. Three regimens for cytoreduction will be employed (figure): Regimen A will consist of hyperfractionated total body irradiation, cyclophosphamide, and thiotepa, Regimen B will consist of busulfan, melphalan, and fludarabine, and Regimen C will consist of clofarabine, melphalan, and thiotepa. All regimens are myeloablative in intensity and have demonstrated safety and efficacy in facilitating engraftment of CD34+ selected allografts in MSKCC protocol 10-050. All recipients will additionally receive rabbit antithymocyte globulin and short course tacrolimus after transplantation. Finally, all recipients will receive a single infusion of rituximab shortly after transplantation to prevent development of lymphoproliferative disorders that may result from infusion of CD19+ donor cells. The use of similar cytoreductive regimens to 10-050 participants will facilitate comparison in terms of core clinical outcomes and immune reconstitution to those patients receiving CD34+ selected allografts. Patients will receive standard supportive care as per adult and pediatric BMT service guidelines.

Donors for study participants will include unrelated donors matched at 6 or 7 loci among HLA-A, -B, -C, and -DRB1 or haploidentical related donors. Donor selection will be based first on donor availability and second on treating physician preference. All donors will undergo a standard GCSF mobilization to obtain a peripheral blood derived graft. The cell product will subsequently undergo TCR-a/W lymphocyte depletion using the Miltenyi CliniMACS device. The target CD34+ cell dose will be >5.0 x 10^6 CD34+ cells per recipient kg body weight. Patients will receive pharmacologic immune suppression with tacrolimus from two days before the allograft infusion until 100 days post allograft infusion.

Protocol Schema: Cytoreductive Regimens



B = Busulfan (adult/ped dose)
F = Fludarabine 25 mg/m2 IV
C = Clofarabine 20-30 mg/m2 IV
X = Hyperfractionated total body irradiation
Cy = Cyclophosphamide 60 mg/kg IV
T = Thiotepa 5 mg/kg IV
*if required by nomogram

M = Melphalan 70 mg/m2 IV
A = Rabbit antithymocyte globulin 2.5 mg/kg IV
H = HPC(A) stem cell allograft
Ta = Tacrolimus dose adjusted
R = Rituximab 200 mg/m2 IV

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary objectives:

- To estimate the incidence of grade 3-4 acute graft *versus* host disease in persons undergoing HLA-mismatched allogeneic hematopoietic cell transplantation with grafts depleted of TCR-a/W cells.

Secondary objectives:

- To estimate overall survival at one year.
- To estimate the cumulative incidence of non-relapse mortality at one year.
- To estimate the cumulative incidence of relapse at one year.
- To determine reconstitution of CD4+, COB+ T-lymphocytes, CD19+ B-lymphocytes.

Translational objectives:

- To determine reconstitution of TCR-a/W T-lymphocytes after TCR-a/W lymphocyte depleted allografts at 30, 100, 180, and 365 days post transplantation.

3.0 BACKGROUND AND RATIONALE

Use of HLA-Mismatched Unrelated Donor Sources in Allogeneic Hematopoietic Cell Transplantation

Allogeneic hematopoietic cell transplantation (allo HCT) is a curative therapy for persons with advanced hematologic malignancies. Successful application of allo HCT depends upon managing the toxicities of conditioning and graft *versus* host disease as well as obtaining a histocompatible donor. Outcomes after allo HCT are influenced by the degree of human leukocyte antigen (HLA) matching between donor and recipient. The most obvious and preferred HLA-matched stem cell donors are siblings that share both HLA haplotypes with the recipient, however; this occurs in only 25-30% of potential candidates. Among those who do not have a matched sibling, the use of a volunteer unrelated donor (URD) matched at the HLA-A, -B, -C, and DRB1 loci confers a similar outcome to transplantation with a HLA-matched sibling donor.[1] Unfortunately, not all recipients will have a suitable HLA-matched donor available. Due to allotypic diversity in various ethnic populations, the likelihood of obtaining a suitable HLA-matched URD varies by the ethnic background of the recipient. For example, among persons who identify primarily as of European descent the likelihood of finding a HLA matched URD is 75%, whereas the probabilities in African Americans, persons from Latin America, and Asia are approximately 16%, 30-40%, and 25-45%, respectively.[2] Therefore, access to a HLA matched donor remains a significant barrier to patients seeking allo HCT, particularly those from racial and ethnic minority backgrounds.

Lack of a HLA matched donor necessitates the use of a HLA mismatched donor for such recipients. If unrelated adult donors that are mismatched at a single HLA loci are included as suitable donors, the likelihood of finding a donor increases to 97%, 76%, 80-90%, and 75-90% for recipients who identify as from European, African, Latin-American, or Asian descent, respectively. Haploidentical related family donors are also feasible allo HCT donors and are available in approximately 80% of patients presenting to Memorial Sloan Kettering Cancer Center for transplantation evaluation (unpublished data).

T-Lymphocyte Depletion to Prevent Graft *Versus* Host Disease after Allogeneic Hematopoietic Cell Transplantation

The primary barrier to successful outcomes after HLA mismatched transplantation is GVHD.[3] Several studies support that the incidence of clinically significant acute GVHDusing HLA mismatched adult unrelated donors is 30-40%. [1, 4] A larger study sponsored by the NMDP and CIBMTR conducted by Lee and colleagues demonstrate more significant findings, where a mismatch at a single HLA class 1 antigen or allele or DRB1 results in an approximately 60% increase in the likelihood of severe acute GVHD and similar increase in the likelihood of treatment related mortality (TRM).[3] The incidence of disease relapse in the HLA mismatch group was less than that of the HLA matched group, indicating that TRM due to GVHD remains the most significant problem in HLA mismatched allograft recipients.

GVHD results from infusion of alloreactive CD3+ T-lymphocytes and other cells within the hematopoietic progenitor cell product.[5] Despite pharmacologic immune suppression, these lymphocyte populations may engraft and cause GVHD. We and others investigated broadly the concept of purifying CD34+ hematopoietic progenitor cells from the donor allograft prior to infusion. This effort has led to several prospective clinical trials at this institution. More recently, CD34+ cell enrichment has been performed via magnetic cell separation techniques using the CliniMACS device (Miltenyi Biotec, Auburn, CA). In this system, the G-CSF mobilized HPC(A) product is introduced into a closed device where the cells are incubated with anti-CD34+ murine monoclonal antibody coated magnetic beads. The cell product is then passed through an electromagnetic field, retaining the CD34+ cells bound to the magnetic beads and allowing other cell populations to wash through the device in the eluent. The enriched CD34+ cell fraction is then infused into the patient. Using this approach we found acceptable CD34+ cell doses (range 0.6-28.8 CD34+ cells/recipient kg) and neutrophil engraftment in 102 patients recently reported.[6] The incidence of acute GVHD in HLA matched transplant recipients was approximately 10%. These findings are similar to those reported in a national study examining this method.[?] Based on the strength of these and other's findings the CliniMACS CD34 Reagent System has been approved by the FDA as a Humanitarian Use Device for the prevention of GVHD in patients with AML in first complete remission undergoing matched sibling donor allo HCT.

In order to address the problem of acute GVHD in HLA-mismatched allo HCT we performed CD34+ selection using the CliniMACS device in 87 individuals undergoing HLA mismatched URD allo HCT (any HLA class 1 or DRB1). Results from these individuals were compared to 169 recipients of HLA matched, CD34+ selected URD allografts. The 2 year survival was 52% (41-63%) in the HLA mismatched group compared to 60% (51-67%) in the HLA matched group

(P = 0.17). The incidence of grade 2-4 and grade 3-4 acute GVHD was 15.6% and 4.7%, respectively in 64 subjects undergoing 7/8 HLA mismatched allografts with complete data. These outcomes are comparable to the incidence of acute GVHD in HLA matched transplant recipients using CliniMACS based CD34+ selection, indicating that the increased death in HLA mismatched transplant recipients results from poor immune reconstitution. Among 64 recipients of HLA mismatched CD34+ selected allografts with complete data, poor graft function contributed to 5 deaths and infection resulted in 6 deaths. Thus, improving immune reconstitution in these individuals is the most likely mechanism to improve outcomes in HLA mismatched, CD34+ selected transplant recipients.

TCR-yl>+ T-Lymphocyte Subsets are Beneficial after Hematopoietic Cell Transplantation

Blood and tissue T-lymphocytes may be subdivided by the T-cell receptor (TCR) isotype. The canonical TCR-a/W lymphocyte population represent approximately 95% of circulating cells, are responsible for the majority of antigen specific T-cell responses, and are implicated in the formation of GVHD after hematopoietic cell transplantation.[8, 9] In contrast, TCR-yiY T-cells are capable of antigen recognition but are more akin to innate immune effector cells such as natural killer cells in that they are activated against conserved peptide epitopes. [10, 11] Similar to other elements of innate immunity, TCR-yo+ cells are activated by immunoglobulin-like, lectin-like, and other natural cytotoxicity receptors.[12] Importantly, this allows for activation of TCR-yo+ lymphocytes outside of the canonical HLA pathway, thereby opening the possibility of anti-tumor potential in the context of an HLA disparate transplant.

In the context of human transplantation, TCR-yo+ T-lymphocytes play an important role in pathogen control early after transplantation. TCR-yo+ T-cells proliferate in response to CMV reactivation and demonstrate similar activity as TCR-a/W cells against CMV in murine models.[13, 14] TCR-yo+ T-cells may also confer protection from adenoviral mediated end organ injury.[15] Perko and colleagues demonstrate that improved reconstitution of TCR-yo+ T-cells after allo HCT was associated with lower incidence of peri-transplant infections and better event-free survival in 102 pediatric transplant recipients.[16] These results would suggest that removal of alloreactive TCR-a/W cells while preserving other beneficial lymphocyte subpopulations may be advantageous in terms of improving post transplant immune function without increasing the incidence of GVHD.

CD34+ selection results in a broad depletion of both TCR-a/W and TCR-yo+ T-lymphocytes as well as B-lymphocytes and NK cells. While this is effective at reducing alloreactive T-lymphocytes, beneficial populations such as NK cells and TCR-yo+ cells are removed as well. More recently, Miltenyi Biotec has made available the TCRa/13 Selection Reagent for selection depletion of TCR-a/W T-cells from hematopoietic progenitor cell products. In a series of 102 depletions, Schumm and colleagues were able to achieve a median log 4.1 (range 3.0-4.7) reduction in TCR-a/W cells using the CliniMACS device and TCRa/13 Selection Reagent protocol.[17] The median absolute number of TCR-a/W cells was 2.8 x 10 (range 0.4-12.8 x 1O. The median number of mononuclear cells was 5.8 x 10 (range 1.2-10.4 x 10 and the median number of CD34+ cells was 358 x 10^6 (range 92-1432 x 10^6). The recovery of

mononuclear cells was 55% (33-77%) and the recovery of CD34+ cells was 73% (43-98%). Viability of the cell product was not affected by TCRa/f3 selection.

Outcomes of TCR-a/W Lymphocyte Depletion in Human Transplantation

TCR-a/W cell depletion is effective at preventing GVHD in the context of HLA mismatched related donor transplantation. Bertaina and colleagues evaluated the use of TCR-a/W cell depletion using the CliniMACS system as the sole GVHD prophylaxis in 23 children undergoing haploidentical related donor transplantation for non-malignant conditions.[18] All patients received pre-transplant anti-thymocyte globulin and rituximab to prevent graft rejection and post-transplant lymphoproliferative disorder (PTLD), respectively. The median number of CD34+ cells/recipient kg infused was 15.8 x 10^6 (range 10.2-40.0 x 10^6) and the median number of TCR-a/WCD3+ cells infused was 4 x 10^4 (range 1.0-9.5 x 10^4). The median time to neutrophil engraftment was 13 days (10-20 days) and platelet engraftment was 10 days (7-40 days). Four patients failed to engraft but responded to second allograft. Three patients developed grade 1-2 acute GVHD of the skin, there was no incidence of severe or visceral acute GVHD or chronic GVHD. The two year survival probability was 91%. Lang and colleagues have additionally treated 24 pediatric patients with malignant diagnoses with a similar protocol of fludarabine, melphalan, and thiotepa based conditioning with anti-thymocyte globulin and TCR-a/W cell depleted G-CSF mobilized haploidentical related donor allograft. (Unpublished results) The outcomes in this series are similar, with the median time to neutrophil engraftment being 10 days. Four patients (16%) developed grade 3+ acute GVHD and 70% of patients are alive albeit with a relatively short median follow up period of 0.5 years.

Bertaina and colleagues expanded the use of TCR-a/W cell depletion based GVHD prophylaxis pediatric patients undergoing matched sibling donor (n = 41) or matched URD (n = 51) allo HCT for acute leukemia.[19] The majority of recipients were treated with total body irradiation based myeloablative conditioning. All treated patients achieved sustained donor cell engraftment. The cumulative incidence of acute GVHD was 41% and 42% for recipients of matched sibling and matched URD, respectively. Visceral involvement was 17% and 16.3%, respectively. The cumulative incidence of extensive chronic GVHD was 8 and 14%, respectively. Seven children died of non-relapse mortality (8%). The three year event-free survival was 66.1% and 65.4% in sibling and unrelated donor recipients, respectively.

Results in recipients of HLA mismatched and HLA matched donor allo HCT using TCR-a/W cell depletion based GVHD prophylaxis are thus favorable when compared to unmodified allografts. The greater degree of acute GVHD noted in the latter Bertaina study suggests that short course pharmacologic immune suppression may be beneficial in preventing acute GVHD in recipients. Tacrolimus levels within the first 4 weeks post allo HCT correlate strongly with the incidence of acute GVHD, suggesting that early pharmacologic immune suppression is the most critical in reducing the incidence of GVHD.[20, 21] In the context of this study we will propose to include a short course (30 days) of tacrolimus based pharmacologic GVHD prophylaxis in order to maximize GVHD prevention without instituting long term pharmacologic immune suppression in study participants.

Use of post-transplantation Rituximab to deplete CD19+ donor B-lymphocytes

The majority of published studies using TCR-a/W cell depletion based GVHD prophylaxis include an additional *ex vivo* depletion of CD19+ B-lymphocytes prior to allograft infusion. The purpose of this additional step is to minimize the propensity for donor derived EBV driven PTLD by reducing the reservoir for EBV from the donor allograft. This additional step may reduce the quantity of infused CD34+ hematopoietic progenitor cells and increases the cost and complexity of the allograft manipulation. More recently, infusion of low-dose, peri-transplant rituximab has demonstrated efficacy to reduce EBV viremia and PTLD without impacting long-term immune reconstitution.[22] Dominietto and colleagues demonstrate that 200 mg/m^2 of rituximab on day +5 post transplant reduced the incidence of EBV viremia from 85% to 56% compared to untreated control patients. These results suggest that peri-transplant rituximab may be administered safely and will reduce the incidence of EBV related disorders. This practice is in use in the ongoing studies of TCR-a/W cell depletion based GVHD prophylaxis for haploidentical related donors conducted by the Italian group.

Summary of Rationale for the Proposed Study

In the current study we propose to conduct HLA mismatched allotransplant using TCR-a/W cell depletion based GVHD prophylaxis. The TCR-a/W cell depletion platform has validated results in HLA matched donors and in related HLA-haploidentical donors with an acceptable rate of acute GVHD and excellent engraftment and long term disease control. This platform represents a logical extension of the CD34+ selection based GVHD prophylaxis for mismatched donors in that it allows for innate effector cells such as NK and TCR-yiY T-cells to participate in pathogen control early after allo HCT. The proposed study addresses a shortcoming in the use of CD34+ selection for HLA mismatched donors in that diminished immune reconstitution is a major contributor to early transplant related mortality.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a phase 2 study evaluating the rate of acute grade 3-4 GVHD following allo-HCT using a/WT-lymphocyte depleted grafts from HLA haploidentical or mismatched unrelated donors. Eligible candidates will include persons with advanced hematologic malignancies who do not have a suitable HLA-matched sibling or unrelated donor available and who meet criteria for myeloablative conditioning. The target accrual will be 38 individuals over 24 months. Three myeloablative conditioning programs are included and are outlined below. All participants will receive rabbit antithymocyte globulin and short course tacrolimus based GVHD prophylaxis. All recipients will receive a TCR-a/W lymphocyte depleted allograft from a HLA mismatched URD. The conditioning regimens are similar to those employed in the MSKCC 10-050 study.

The primary objective of the study will be to determine the incidence of grade 3-4 acute GVHD by day +100 after transplantation. The intervention will be considered unpromising if the rate of GVHD is greater than 40% and promising if the rate is 20% or less. A Simon two-stage optimal design will accrue 11 patients in the first stage. If 5 or more develop grade 3-4 acute GVHD, the study will close due to a lack of efficacy; otherwise an additional 27 will accrue. If at the end of study, at least 27 out of the 38 patients are free of grade 3-4 GVHD, the study will be considered promising. The study also includes sequential safety stopping rules to monitor for non-relapse mortality. Secondary objectives will evaluate other clinical outcomes. Translational endpoints will define immune reconstitution in study participants which will be benchmarked against the estimates from MSKCC 10-050.

4.2 Intervention

Patients will be identified appropriate for this therapy in the weekly Adult or Pediatric BMT Service meeting. Eligible patients will be registered and enrolled prior to the start of conditioning therapy. All patients will receive conditioning treatment with either Regimen A, B, or C outlined below. Selection of the conditioning regimen is outlined in section 9.1. All patients will receive anti-thymocyte globulin based conditioning followed by a G-CSF mobilized, peripheral blood hematopoietic progenitor cell HPC(A) product depleted of TCR-a/ + T-lymphocytes using the CliniMACS system. Participants will receive pharmacologic immune suppression to prevent graft *versus* host disease using tacrolimus for 30 days followed by a taper post transplantation. Standard supportive care will be administered per Adult and Pediatric BMT Service guidelines.

Donors will be evaluated according to standard criteria for unrelated and haploidentical donors. Donors are considered eligible if they are matched at 6 or 7 alleles among HLA-A, -B, -C, and -DRB1 (unrelated donors) or if they are related haploidentical donors. Donors will donate a standard, G-CSF mobilized peripheral blood hematopoietic progenitor cell (HPC(A)) product that will subsequently be transferred to the MSKCC Cell Therapy Laboratory for processing. HPC(A) products will then undergo TCR-aW depletion using the CliniMACS reagents according to the manufacturer's guidelines. The cell dose will be capped to prevent infusion of >1 x 10^5 TCR-aW cells/kg.

Patients will be assessed daily until after hematopoietic recovery and hospital discharge post transplantation. After hospital discharge patients will be assessed for acute GVHD weekly until day +100. Thereafter patients will be assessed for overlap or chronic type GVHD at 3 month intervals or more frequently if clinically indicated. Disease response and GVHD assessments will be performed at +100, +180, +270, and +360 days after transplant.

Translational Endpoints

Immune reconstitution of major lymphocyte subsets will be performed according to service guidelines.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 TCR-a/rl+ lymphocyte depleted stem cells from donor (related or unrelated) peripheral blood stem cells (PBSC)

The investigational product in this study is TCR-a/!3+ lymphocyte depleted stem cells from donor (related or unrelated) peripheral blood stem cells (PBSC), administered intravenously.

In this study, donor apheresis will be divided in two portions: one for the manufacture TCR-a/!3+ lymphocyte depleted stem cells and the other, for the manufacture of CD34+ enriched stem cells (section 5.2). Both products will be manufactured in the Cell Therapy Processing Facility at MSK using the CliniMACS Miltenyi Device. TCR-a/!3+ lymphocyte depleted stem cells will be separated using the investigational reagent kit from Miltenyi and following institutional SOPs. Reference is made to Miltenyi BB-MF 15, 678 for the CliMACS TCR depletion reagent. All testing is performed in a CUA-certified laboratory. Pre-release testing includes identity, viability, purity, sterility (gram stain) endotoxin, potency, and cell count/volume. The final product will contain no more than $1x10^5$ TCR-a/!3+/kg (matched related) and $1x10^4$ TCR-a/!3+/kg (mismatched). Post-release testing consists of sterility (14 day culture, 21CFR610.12).

In the event that the 14-day sterility culture and/or endotoxin assay yield a positive result, the the physician and patient will be notified immediately as well as the Co-Principal Investigators and Data Coordinating Center. The cell processing laboratory will be notified and will perform a thorough review of all processes and procedures during the manufacturing to determine if any deviations may have occurred leading to the positive culture or endotoxin assay. Further actions will be dependent on this review and will be left to the discretion of the PI and the laboratory personnel. If the sterility culture is positive, the organism will undergo speciation and drug sensitivity testing and the results will be reported to the PI and/or treating physician, patient, study chairpersons, and Data Coordinating Center. The IRB and the FDA will be notified of these results within 15 days.

The final product is stored between 2-6°C until ready for infusion.

5.2 Allogeneic CD34+ selected stem cells

CD34+ enriched stem cells will be obtained using the FDA approved reagents from Miltenyi, according to the instruction manual. The Miltenyi CD34+ Reagent System is an FDA approved Humanitarian Use Device. MSK has extensive experience using the Miltenyi CliniMACS® system under institutional INDs.

CD34+ enriched stem cells are manufactured in the Cell Therapy Processing Facility at MSK, an FDA-registered, NY State Licensed Tissue Bank lab. Operations and quality assurance (QA) activities of the facility are overseen by the MSK Bone Marrow Transplant service QA Committee, by the MSK Blood Bank Transfusion Committee, and by the MSK Clinical Research Compliance Office. All testing is performed in a CUA-certified laboratory. Pre-release testing includes identity, viability, purity, sterility (gram stain) endotoxin, potency,

and cell counUvolume. The final product will contain no more than $1x10^5$ CD3/kg (matched related) and $1x10^4$ CD3/kg (mismatched). Post-release testing consists of sterility (14 day culture, 21CFR610.12).

In the event that the 14-day sterility culture and/or endotoxin assay yield a positive result, the the physician and patient will be notified immediately as well as the Co-Principal Investigators and Data Coordinating Center. The cell processing laboratory will be notified and will perform a thorough review of all processes and procedures during the manufacturing to determine if any deviations may have occurred leading to the positive culture or endotoxin assay. Further actions will be dependent on this review and will be left to the discretion of the PI and the laboratory personnel. If the sterility culture is positive, the organism will undergo speciation and drug sensitivity testing and the results will be reported to the PI and/or treating physician, patient, study chairpersons, and Data Coordinating Center. The IRB and the FDA will be notified of these results within 15 days.

The final product is stored between 2-6°C until ready for infusion.

5.3 Melphalan (Evomela®)

Formulation and stability: A lyophilized powder of 50 mg melphalan.
Solution preparation:

1. Vial/50 mg: Reconstitute with 8.6ml NS to yield a final concentration of 5 mg/ml.
2. Shake vigorously until the solution is clear.
3. Dilute the dose to be administered in 0.9% Sodium Chloride, USP, to a concentration of 0.45 - 5 mg/ml

Storage and stability: Should be stored at room temperature (15-30°C) protected from light. Infusions with concentration of 0.45mg/ml have a 4 hour stability at room temperature
Administration: Intravenous, over 30 minutes.

5.4 Fludarabine (FLUDARA®)

Supplied as: 50mg vial
Reconstitution directions: add 2ml of sterile water for injection to a 50mg vial; yields a final concentration of 25 mg/ml.
Storage and stability:

1. Store vials under refrigeration.
2. Refrigerated: prepare infusion in D5W; stable for 16 days.
3. Room temperature: prepare infusion in D5W; stable for 16 days.

Solution Preparation:

1. Standard iv fluid: D5W.
2. Final infusion concentration range: 1 mg/ml.
3. IV piggyback volume: 50 ml.

Clinical considerations:

1. Hydration: None.
2. Emetic potential: low.
3. Supportive medications: none.

Incompatibilities: acyclovir, amphotericin B, chlorpromazine, daunorubicin, ganciclovir, hydroxyzine, miconazole, prochlorperazine.

5.5 Antithymocyte globulin (Rabbit)

Supplied as: Each package contains two 7 ml vials: Vial 1 contains freeze-dried thymoblobulin formulation active ingredient: Anti-thymocyte globulin 25 mg plus inactive ingredients including glycine 50 mg, sodium chloride 10 mg, and mannitol 50 mg. Vial 2 contains Diluent sterile water for injection, USP 5 ml.

Reconstitution directions and solution preparation: Vial 1 and 2 are combined to form a solution of 5 mg/ml antithymocyte globulin. Transfer the contents of the calculated number of thymoglobulin vials into the bag of infusion solution (saline or 5% dextrose). The recommended volume for the infusion solution is 50 ml of solution per vial 1 of thymoglobulin. Mix the solution by inverting the bag 1-2 times.

Storage and stability: Store un reconstituted vials in refrigerator between 2-8 degrees C. Protect from light. Do not freeze. Do not use after the expiration date indicated on the label. Reconstituted vials should be used within 4 hours. Infusion solutions should be used immediately. Any unused drug after infusion will be discarded.

Clinical considerations: Patients will receive pre-hydration and anaphylaxis prophylaxis to include diphenhydramine and acetaminophen according to institutional guidelines. The infusion solution will be administered through a 0.22-micron filter with flow rate set to deliver the dose over 12 hours. Methylprednisolone 1 mg/kg may be administered concurrently with ATG.

5.6 Busulfan (Busulfex®)

Supplied as: Busulfex is supplied as a clear solution in 10 ml single use vials. Each ampoule of Busulfex contains 60 mg of busulfan dissolved in N,N-dimethyl acetamide 33% wt/wt and polyethylene glycol 400 67% wt/wt.

Reconstitution directions: Busulfex is diluted prior to use with 0.9% sodium chloride. The diluent should be 1OX the volume of Busulfex and the final concentration of busulfan is approximately 0.5 mg/ml

Storage and Stability: Unopened ampules of Busulfex must be stored at 2-8 degrees C. Reconstituted busulfan is administered immediately.

Clinical considerations: Busulfan is emetogenic. Busulfan is administered intravenously over 2 hours. Seizure prophylaxis is co-adminstered according to institutional guidelines. Acetaminophen and metronidazole should not be given 24 hours pre or post busulfan administration.

5.7 Filgrastim (Neupogen®)

Supplied as: 300 mcg/ml; 1 ml vial (300 mcg) and 1.6 ml vial (480 mcg); 300 mcg/0.5 ml pre filled syringe; 480 mcg/0.8 ml pre-filled syringe.

Storage and Stability: Store in a refrigerator (2-8°C). Do not freeze. If inadvertently the filgrastim is exposed to freezing temperatures for up to 24 hours, it may be thawed and refrigerated for use. Avoid shaking. Filgrastim may be allowed to reach room temperature for 24 hours prior to use.

Solution Preparation:

1. For IV infusion, dilute filgrastim in 25-50 ml O5W.
2. The minimum concentration must not be less than 5 mcg/ml.
3. If the final concentration of filgrastim in solution is between 5-15 mcg/ml, albumin 2 mg/ml must be added to the solution prior to addition of the drug.
4. Stability (IV) once diluted in 25-50 ml of O5W, filgrastim is stable for 7 days.
5. Stability (plastic syringe) filgrastim is stable for two weeks in BO 1 ml plastic TB syringes at 2-8°C.

5.8 Tacrolimus (Prograf®)

Supplied as: Capsules (1 and 5 mg) for parenteral administration or as a sterile solution in 1 ml vials containing 5 mg anhydrous tacrolimus per ml, in boxes of 10 vials, for intravenous administration. Tacrolimus compounded suspension can be used.

Storage and Stability: Tacrolimus injection ampules are stored between 5°-25°C. Prograf capsules are stored at room temperature (15°-30°C).

Solution Preparation: Tacrolimus injection must be diluted with 0.9% sodium chloride injection or 5% dextrose injection to a concentration between 0.004 and 0.02 mg/ml prior to use. Diluted infusion solution should be stored in glass or polyethylene containers and should be discarded after 24 hours. The diluted infusion solution should not be stored in a PVC container due to decreased stability and the potential for extraction of phthalates. Parenteral preparations should be inspected visually for particulate matter and discoloration prior to administration.

Clinical Considerations: Due to chemical instability of tacrolimus in alkaline media, injection should not be mixed or co-infused with solutions of pH 9 or greater (e.g. ganciclovir or acyclovir). Prograf capsules should be taken regularly at 12 hour intervals.

5.9 Thiotepa (Thioplex®)

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

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

Storage and Stability:

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

Preparation:

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

Clinical Considerations: Emetogenic potential high.

Hydration: NA

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

5.10 Clofarabine (CLOLAR®)

Supplied as: 20 mg vial (1 mg/ml). The pH range of the solution is 4.0 to 7.0. The solution in clear to yellow in color and should be free from particulate matter.

Storage and Stability: Undiluted vials are stored at room temperature.

Solution Preparation: Clofarabine for injection should be filtered through a sterile 0.22 µm syringe filter and then further diluted with 5% dextrose or 0.9% sodium chloride injection. The resulting mixture may be stored at room temperature but must be used within 24 hours of dilution.

Clinical Considerations: Clofarabine should be administered over 2 hours through a centralized venous catheter. No other medications should be administered through the same line concurrently.

5.11 Rituximab (Rituxan ®)

Supplied as: 100 mg and 500 mg vials (10 mg/ml)

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

Solution Preparation: Rituximab for injection should be diluted in normal saline to a concentration of 1-4 mg/ml.

Clinical Considerations: Anticipate infusion reactions, particularly in individuals who have not previously received rituximab infusion. Infusion schedule will be adjusted according to standard guidelines. Emetogenic potential is low. Patients should receive

acetaminophen and diphenhydramine according to service guidelines 30-60 minutes prior to infusion.

5.12 Cyclophosphamide

Supplied as: 200 mg, 500 mg, 2000 mg vials
Reconstitution directions: add sterile water for injection to yield a final concentration of 20 mg/ml.
Storage and stability:

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

Solution Preparation:

1. Standard iv fluid: D5W.
2. Final concentration range up to: 20mg/ml.
3. IV piggyback volume: for doses < 1200mg/m2, infuse in 25cc D5W; for doses> 1200mg, infuse as straight drug.

Clinical considerations:

1. Hydration: as per MSKCC guidelines for BMT patients receiving> 3000 mg/m2.
2. Emetic potential: high and delayed.
3. Supportive medications: anti-emetics and mesna.

Incompatibilities: do not administer with other drugs.

5.13 Total body irradiation

Hyperfractionated 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 (1,375 or 1,500 cGy) over 4 days (days -9 through -6). If general anesthesia is required, 150 cGy q12h x 8 doses to a total dose of 1,375 cGy may be given. Sequential doses are administered in an anterior/posterior or lateral orientation. Compensators and lung blocks are used to shield the lung so that the lung receives 800 cGy. The blocked areas of the chest will be boosted with high-energy electrons so that the cumulative chest wall dose is approximately 1,500 cGy. This insures that marrow sites int eh ribs are adequately treated.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

Describe the characteristics of the patient/subject population.

6.1 Subject Inclusion Criteria

- Patients with any of the following hematologic malignancies who are considered to be eligible for allogeneic transplantation:

    - Acute lymphoid leukemia (ALL) in first complete remission (CR1) with high risk for relapse including:
        - Detectable minimal residual disease by either multicolor flow cytometry or by genomic assay after initial induction therapy
        - t(9;22) or detected *BCR-ABL1* translocation by genomic methodologies
        - *BCR-ABL*1-Like B-ALL [23] including mutations of IKZF1 or CRLF2
        - Translocations or mutations involving 11q23 **(MLL)** gene.
        - Hypodiploid karyotype
        - Deletion of 9p
        - Loss of 17p or *TP53* mutation
        - T-lymphocyte lineage antigen expression (T-ALL)
        - Prior CNS or other extramedullary involvement
        - WBC count 100,000 cells/μL at diagnosis

    - Acute biphenotypic or bilineal leukemia in CR1

    - Acute myeloid leukemia (AML) in CR1 with
        - Detectable minimal residual disease (MRD) by either multicolor flow cytometry or by genomic assay after initial induction therapy.
        - In the absence of MRD any intermediate or high risk features according to the European LeukemiaNet 2017 guidelines[24], including:
            - Mutated *FLT3-ITD* or *FLT3-TKO*
            - Cytogenetic abnormalities not classified as favorable
            - Cytogenetic abnormalities associated with myelodysplastic syndrome including abnormalities of chromosome 5, 7, or 17p
            - Complex karyotype or monosomal karyotype
            - t(9;11)(p21.1;q23.3); *MLL-KMT2A* or other rearrangements of *KMT2A*
            - t(9;22); *BCR-ABL1*
            - Inversions or translocations of chromosome 3
            - T(6;9)(p23;q34.1); *DEK-NUP214*
            - Somatic mutation of *RUNX1, ASXL1* or *TP53*
        - Extramedullary involvement
        - WBC count 100,000 cells/μL at diagnosis

    - Relapsed acute leukemia with :5 5% blasts in the bone marrow prior to transplantation (i.e. CR2 or greater).

- Myelodysplastic syndrome, myeloproliferative neoplasms, or MDS/MPN overlap syndrome with ≤ 10% blasts and at least one of the following:
  - Revised International Prognostic Scoring System risk score of INT, HIGH, or VERY HIGH at the time of transplant evaluation.
  - Life-threatening cytopenias.
  - Karyotype or genomic changes that indicate high risk for progression to acute myelogenous leukemia, including abnormalities of chromosome 7 or 3, mutations of *TP53,* or complex or monosomal karyotype.
  - Therapy related disease or disease evolving from other malignant processes.

- Chronic myelomonocytic leukemia (CMML) with ≤ 10% blasts prior to transplantation.

- Chronic myeloid leukemia (CML) meeting one of the following criteria:
  - Failed or are intolerant to *BCR-ABL* tyrosine kinase inhibitors.
  - CML with BCR-ABL mutation consistent with poor response to tyrosine kinase inhibition (e.g. T351I mutation).
  - CML with accelerated or blast phase with <10% blasts after therapy.

- Chronic lymphocytic leukemia (CLL) with high risk disease as defined by the EBMT consensus criteria.

- Hodgkin lymphoma meeting both of the following criteria:
  - Responding to therapy prior to enrollment
  - Relapse after autologous bone marrow transplant or are ineligible for autologous bone marrow transplant.

- Non-Hodgkin lymphoma meeting both of the following criteria:
  - Responding to therapy prior to enrollment.
  - Relapse after prior autologous bone marrow transplant or are ineligible for autologous bone marrow transplant.

- Patients aged from birth through 65 years old are eligible.

- Patients must have Karnofsky/Lanksy performance status≥70%.

- Cardiac left ventricular ejection fraction ≥ 50% at rest.

- Serum bilirubin ≤ 2 mg/dl. Patients with Gilbert's disease or ongoing hemolytic anemia are acceptable if the direct bilirubin is ≤ 2 mg/dl.

- AST and ALT ≤ 2.5 x ULN unless thought to be disease related.

- Estimated or measured creatinine clearance > 50 ml/min/1.73 m^2 body surface area.

- Adult patients and pediatric patients capable of performing pulmonary function studies must have hemoglobin adjusted pulmonary DLCO 50% of predicted.

6.2 Subject Exclusion Criteria

- Persons with a HLA matched sibling donor or a 8/8 allele level HLA-matched unrelated donor.

- Female patients who are pregnant or breast-feeding.

- Persons with an infection that is not responding to antimicrobial therapy.

- Persons who are seropositive for HIV.

- Persons with active/detectable central nervous system malignancy.

- Persons who do not meet the age and organ function criteria specified above.

- Presence of psychiatric or neurologic disease, or lack of social support that limits the patient's ability to comply with the treatment protocol including supportive care, follow-up, and research tests.

- Prior allogeneic hematopoietic cell transplantation are ineligible.

- Patients with history of other malignancy within 5 years of study therapy are ineligible with the following exceptions: Low grade prostate cancer (Gleason's :::;6) treated with curative intent, breast ductal carcinoma *in situ* treated with curative intent, or non-melanomatous skin carcinomas.

- Patients with previous checkpoint inhibitor therapy such as PD-1/PDL-1 inhibitors cannot undergo *transplantation* less than 6 weeks from the last checkpoint inhibitor therapy.

6.3 Donor Inclusion and Exclusion Criteria

- Partially HLA-matched unrelated volunteers (allele level matched at 6-7 of 8 HLA loci: -A, -B, -C, and -DRB1) are eligible.

- Related, haploidentical donors are eligible.

- Able to provide informed consent to the donation process

- Meet standard criteria for donor collection as defined by the National Marrow Donor Program Guidelines.

7.0 RECRUITMENT PLAN

Eligible patients are identified via the weekly Adult or Pediatric BMT/Leukemia Patient Review Conference. Patients that do not have a matched sibling or unrelated adult donor option and who do have either an unrelated donor that is matched at least 6/8 loci (HLA-A, -8, -C, ORB1) or a haploidentical related donor and meet the other eligibility criteria will be offered participation in this study.

This protocol will take due notice of NIH/ADAMHA policies concerning inclusion of women and minorities in clinical research populations. We anticipate that this study will preferentially benefit persons of racial or ethnic minority populations as these individuals are less likely to have a HLA matched allo HCT donor. Otherwise, we expect that the study population will be fully representative of the range of patients referred for transplant without exclusion as to age or gender with the exception that pregnant women are excluded from participation in this study.

8.0 PRETREATMENT EVALUATION

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

- Complete history and physical exam
- Dental evaluation
- Complete blood count, PT/PTT, serum chemistries and hepatic panel, and ABO type and screen
- Serum will be tested for CMV serostatus, Hepatitis B (core antibody, surface antigen), Hepatitis C antibodies, EBVantibodies, toxoplasmosis antibodies, HIV antibodies, and HTLV-1/2 antibodies
- Pregnancy test for females of childbearing potential
- Bone marrow aspirate (biopsy if clinically indicated)
- Urinalysis
- Electrocardiogram and echocardiogram
- Spirometry (adults and pediatric patients) and pulmonary diffusion capacity (adults only)
- Donor-specific anti-HLA antibody titers
- Recipient short-tandem repeat (STR) profile for chimerism analysis

9.0 TREATMENT/INTERVENTION PLAN

9.1 Regimen for Cytoreduction

Three cytoreduction regimens are outlined below. Patients with myeloid malignancies such as MOS or AML will be treated with either regimen A or regimen B based on investigator preference. Patients with lymphoid malignancies including ALL will be treated with regimen A or regimen C based on investigator preference. Patients with biphenotypic or mixed phenotypic acute leukemia may be treated with any regimen based on investigator preference. All drugs are administered relative to the date of stem cell infusion (day 0).

9.1.1 Conditioning Regimen A: TBI/CY/THIO

Day	Treatment
-12	Rabbit ATG dosing per nomogram
-11	Rabbit ATG dosing per nomogram
-10[8]	Rabbit ATG dosing per nomogram
-9	TBI
-8	TBI
-7	TBI
-6	TBI
-5	Thiotepa 5 mg/kg IV#
-4	Thiotepa 5 mg/kg IV#
-3	Cyclophosphamide 60 mg/kg/day IV
-2	Cyclophosphamide 60 mg/kg/day IV
-1	
0	Cell infusion
+5	Rituximab 200 mg/m^2 IV

hiotepa may be given as 10 mg/kg/d x 1 on day -5 only.

a) If day 3 ATG is not required the conditioning program can begin, moving all subsequent treatments to the prior day, at the investigators discretion.

9.1.2 Conditioning Regimen B: BU/MEL/FLU

Day	Treatment
-12	Rabbit ATG dosing per nomogram
-11	Rabbit ATG dosing per nomogram
-10[8]	Rabbit ATG dosing per nomogram
-9	Busulfan 3.2 mg/kg/day IV#
-8	Busulfan IV*
-7	Busulfan IV*
-6	Melphalan 70 mg/m^2 IV Fludarabine 25 mg/m^2 IV
-5	Melphalan 70 mg/m^2 IV Fludarabine 25 mg/m^2 IV
-4	Fludarabine 25 mg/m^2 IV
-3	Fludarabine 25 mg/m^2 IV
-2	Fludarabine 25 mg/m^2 IV
-1	Rest
0	Cell infusion
+5	Rituximab 200 mg/m^2 IV

#Busulfan dosing modifications for children :54 are outlined below.

*Busulfan will be dosed with pharmacokinetic modifications to target an AUC of 4800 min*mmol/L ± 10%/dose according to institutional guidelines.

a) If day 3 ATG is not required the conditioning program can begin, moving all subsequent treatments to the prior day, at the investigators discretion.

9.1.3 Conditioning Regimen C: CLO/MEL/THIO

Day	Treatment
-12	Rabbit ATG dosing per nomogram
-11	Rabbit ATG dosing per nomogram
-1oa	Rabbit ATG dosing per nomogram
-9	Clofarabine 20* mg/m^2 IV
-8	Clofarabine 20* mg/m^2 IV
-7	Clofarabine 20* mg/m^2 IV
-6	Clofarabine 20* mg/m^2 IV
-5	Clofarabine 20* mg/m^2 IV
-4	Thiotepa 10 mg/kg IV
-3	Melphalan 70 mg/m^2
-2	Melphalan 70 mg/m^2
-1	Rest
0	Cell infusion
+5	Rituximab 200 mg/m^2 IV

*In patients <18 years old clofarabine 30 mg/m^2 may be used at the discretion of the principle investigator.
a) If day 3 ATG is not required the conditioning program can begin, moving all subsequent treatments to the prior day, at the investigators discretion.

9.1.4 Clofarabine administration

Clofarabine will be administered via approximately a 2 hour intravenous infusion. Adult patients may receive hydrocortisone 50 mg/m^2 IV (maximum 100 mg) 30-60 minutes prior to clofarabine infusion.

9.1.5 Melphalan administration

Melphalan will be infused once daily intravenously. Adjusted body weight will be used if the patient's actual weight is > 125% of adjusted body surface area. Dosing melphalan in young children is difficult and may require case by case adjustment. Therefore, investigators are permitted to modify the dosing of melphalan in children :s; 3 years based on the discretion of the principal/co-principal investigators.

9.1.6 Fludarabine administration

Fludarabine is administered intravenously over 30 minutes.

9.1.7 Busulfan administration

Intravenous busulfan is administered at a total dose of 3.2 mg/kg/day of adjusted recipient body weight for adults and children 4 years old (only if the child is >125% of ideal body weight). The dose may be administered either as a single dose or divided over four doses administered every six hours. For children <4 years old busulfan will be administered at a total dose of 1.0 mg/kg/dose q6 hours x 12 doses/3 days of actual body weight. Target dosing

through pharmacokinetic assays will be performed as per institutional guidelines. The target AUC = 4800 min*mmol/L ± 10%.

9.1.8 Rituximab administration

Rituximab will be administered according to institutional protocols to minimize infusion reaction.

9.1.9 Thiotepa administration

Thiotepa dosing will be based on adjusted body weight if a patient is >125% of ideal body weight.

9.1.11 Cyclophosphamide administration

Cyclophosphamide dosing will be based on adjusted body weight if a patient is >125% of ideal body weight. Hydration and mesna administration will be according to institutional guidelines.

9.1.12 Rabbit anti-thymocyte globulin dosing

Rabbit ATG dosing will be based on a dynamic nomogram as defined in the PARACHUTE study (Admiraal, Rick et al; BBMT 26;3,S33-34, Appendix A). This dynamic nomogram is based on absolute lymphocyte count at the start of conditioning and can result in either 2 or 3 day ATG administration. If a patient requires 2 day administration the subjequent chemotherapies may be moved forward by one day at the treating physician's discretion.

9.1.13 Adjustment of chemotherapy based on adjusted body weight

Adult patients who are >125% of ideal body weight that require adjustment of agents listed above will receive therapy based on the following formulas:

Ideal body weight (IBW) formula:
Male IBW = 50 kg+ 2.3 kg/inch over 60 inches
Female IBW = 45.5 kg+ 2.3 kg/inch over 60 inches

Adjusted body weight (ABW) formula:
ABW = IBW + 0.4 x (actual weight - IBW)

Patients <18 years old IBW is calculated as follows (IBW in kg):
Less than 152.4 cm:
IBW = (Height in cm^2 x 1.65)/1000 where ht= cm, IBW = kg

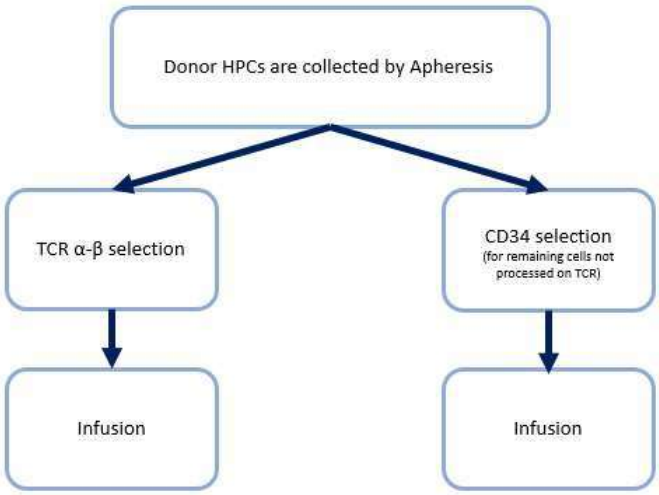
More than 152.4 cm:
IBW (male) = 39.0 + (2.27 x (ht - 60)) where ht= inches
IBW (female) = 42.2 + [2.27 x (ht - 60)]

9.2 Donor mobilization and TCR-a(J+ T-cell Depletion

9.2.1 Mobilization of Donor

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

9.2.2 TCRaJ3+ T-Cell Reduction using the Miltenyi CliniMACS®



Reduction of TCRaJ3 T-cells from the donor HPC(A) product will be performed according to procedures given in the CliniMACS® Users Operating Manual and institutional Standard Operating Procedures (SOP) implemented in the Cell Therapy Laboratory (see Appendix for SOP).

A single TCRaJ3+ T-cell reduction procedure will be performed for adult donors. The target CD34+ cells/kg cell dose for the final product is 5×10^6. An additional CD34+ cell selection (CD34 enrichment) may be performed to achieve the target CD34+ cell dose, if required. If there are less than a total of 2.0×10^6 CD34+ cells/kg recipient body weight in the combined allografts, the laboratory will contact the investigator. If required, CD34+ cell selection on additional HPC(A) will be performed according to procedures given in the CliniMACS® Users Operating Manual and approved institutional SOP. Testing of the product will be conducted in a CUA certified laboratory as described in the CMC section.

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

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

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

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

9.3 Pharmacologic graft *versus* host disease prophylaxis

Tacrolimus will be administered as follows:

- Patients will receive tacrolimus beginning on day -3 and continuing through day +30.
- Tacrolimus will be administered by continuous IV infusion to achieve a level of 5-12 ng/ml plasma concentration.
- When a patient is able to tolerate oral medications tacrolimus may be transitioned to twice (three times daily dosing may be used in pediatric patients <40 kg) to achieve a similar trough level. Tacrolimus will be tapered linearly to off by day +100 after transplantation.
- The tacrolimus taper may be altered or suspended if suspected/confirmed symptoms of GVHD arise. Tacrolimus may be discontinued if patients become intolerant to tacrolimus.

9.3 Supportive Therapy

The following standard therapies will be provided for all patients:

9.3.1 Infection prophylaxis

- Infection prophylaxis will be administered according to institutional guidelines.
- Specific infection prophylaxis will be administered for Pneumocystis *cannu,* Herpesviridae, and fungal infections to all recipients and for Toxoplasmosis *gondii* to seropositive recipients. Seronegative recipients may elect to receive Toxoplasmosis *gondii* prophylaxis if the donor is seropositive at the discretion of the treating physician.

9.3.2 Growth factor support

Patients will receive G-CSF beginning on day +7 at a dose of 5 mcg/kg/dose subcutaneously or intravenously every 12 hours (rounding to nearest vial dose is allowed) until the absolute neutrophil count is 1,000/μL on three consecutive days. Intravenous administration of G-CSF is allowed at the discretion of the treating physician.

9.3.4 Sinusoidal obstructive syndrome prophylaxis

Patients will receive ursodiol according to pediatric or adult service guidelines.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

10.1 Post transplantation evaluation

Post transplantation evaluations are summarized the table below:

	Day?	Day 14	Day 21	Day28	Day42	Day60	Day 100	Day 180	Day 270	Day 365	Early Term
Window	±3	±3	±3	±7	±3	±3	±7	±14	±14	± 30	± 30
CBC	X	X	X	X	X	X	X	X	X	X	X
Chemistry	X	X	X	X	X	X	X	X	X	X	X
T-Cell and myeloid chimerism (ARC)				X			X	X	X	X	
Bone marrow aspirate and/or biopsy				X				X	X	X	
Restaging Imaging studies (if clinically indicated)				X				X	X	X	
Immune recovery, clinical (Phenotyping, short panel)				X			X	X	X	X	
Research Blood[1]				X			X	X		X	
GVHD Evaluation	X	X	X	X	X	X	X	X		X	X
Toxicity Assessment	X	X	X	X	X	X	X	X	X	X	X

1. For all patients weighing to 10kg, research samples will consist of three 10 ml sodium heparin tubes of blood on the days described above. If a patient is at risk for exceeding maximum allowable blood draw limits (per Seattle Children's Hospital Guideline for Maximum Blood Volumes), clinical laboratory assessments will be prioritized and research samples will be discontinued.

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 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 are also discussed.

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

11.1 Busulfan

COMMON, SOME MAY BE SERIOUS

In 100 people receiving busulfan more than 20 and up to 100 may have:

- Fever
- Headache
- Restlessness
- Hypertension
- Nausea, vomiting, or loss of appetite
- Diarrhea
- Dry or irritated mouth
- Electrolyte abnormalities
- Low blood white cell, platelet, and red cell counts
- Alopecia

OCCASIONAL, SOME MAY BE SERIOUS

In 100 people receiving busulfan from 4 to 20 may have:

- Mouth ulcers
- Abnormal liver function studies
- Rash

RARE, AND SERIOUS

In 100 people receiving busulfan 3 or fewer may have:

- Seizures
- Hepatic sinusoidal obstructive syndrome
- Pericarditis
- Alveolar hemorrhage
- Encephalopathy
- Hemorrhagic cystitis

11.2 Fludarabine

COMMON, SOME MAY BE SERIOUS

In 100 people receiving fludarabine more than 20 and up to 100 may have:

- Decreased blood counts including anemia, low white blood cell count, and low platelet count
- Irritation to the bowel, causing nausea, vomiting, loss of appetite, or diarrhea

OCCASIONAL, SOME MAY BE SERIOUS

In 100 people receiving fludarabine from 4 to 20 may have:

- Abnormal liver function studies (increased ALT or AST)
- Fluid retention/edema
- Rash

RARE, AND SERIOUS

In 100 people receiving fludarabine 3 or fewer may have:

- Neurotoxicity (agitation or confusion, visual disturbances, blindness, loss of hearing, peripheral neuropathy)
- Severe hepatic injury causing hepatic failure
- Hemolytic anemia
- Phlebitis
- Deep venous thrombosis

11.3 Anti-thymocyte globulin

COMMON, SOME MAY BE SERIOUS

In 100 people receiving antithymocyte globulin more than 20 and up to 100 may have:

- Fever
- Chills
- Low white blood cell count
- Low platelet count
- Headache
- Hypertension

OCCASIONAL, SOME MAY BE SERIOUS

In 100 people receiving antithymocyte globulin from 4 to 20 may have:

- Tachycardia
- Allergic reaction

RARE, AND SERIOUS

In 100 people receiving antithymocyte globulin 3 or fewer may have:

- Anaphylaxis infusion reaction (hypotension, allergic symptoms)

11.4 Melphalan

COMMON, SOME MAY BE SERIOUS

In 100 people receiving melphalan more than 20 and up to 100 may have:

- Decreased blood counts including anemia, low white blood cell count, and low platelet count
- Irritation to the bowel, causing nausea, vomiting, loss of appetite, or diarrhea
- Alopecia (loss of hair)
- Fatigue
- Insomnia

OCCASIONAL, SOME MAY BE SERIOUS

In 100 people receiving melphalan from 4 to 20 may have:

- Abnormal liver function studies (increased ALT, AST, or alkaline phosphatase)
- Hyponatremia (low sodium)
- Allergic reaction to infusion (fever, hives, itching)

RARE, AND SERIOUS

In 100 people receiving melphalan 3 or fewer may have:

- Anaphylactic infusion reaction (hypotension, allergic symptoms)
- Interstitial pneumonitis
- Severe hepatic injury or hepatic failure

11.5 Clofarabine

COMMON, SOME MAY BE SERIOUS

In 100 people receiving Clofarabine more than 20 and up to 100 may have:

- Diarrhea
- Nausea or loss of appetite
- Fever or chills
- Fatigue
- Anxiety or restlessness
- Itching
- Rash
- Low blood counts

OCCASIONAL, SOME MAY BE SERIOUS

In 100 people receiving Clofarabine from 4 to 20 may have:

- Increased blood creatinine
- Increased liver function studies (AST, ALT, alkaline phosphatase, bilirubin)
- Infusion reaction

RARE, AND SERIOUS

In 100 people receiving Clofarabine 3 or fewer may have:

- Liver failure due to sinusoidal obstructive syndrome
- Kidney failure
- Pulmonary edema
- Stevens-Johnson syndrome

11.6 Thiotepa

COMMON, SOME MAY BE SERIOUS

In 100 people receiving Thiotepa more than 20 and up to 100 may have:

- Infections
- Nausea
- Headache
- Restlessness
- Body aches
- Loose stool
- Low blood counts

OCCASIONAL, SOME MAY BE SERIOUS

In 100 people receiving Thiotepa from 4 to 20 may have:

- Rash
- Bladder irritation
- Elevation of the liver functions studies (AST, ALT, alkaline phosphatase, bilirubin)
- Minor infusion reaction such as itching or rash
- Fluid retention

RARE, AND SERIOUS

In 100 people receiving Thiotepa 3 or fewer may have:

- Exfoliative skin reaction
- Delirium
- Myocarditis
- Hepatic failure due to sinusoidal obstructive syndrome
- Anaphylactic infusion reactions
- Acute kidney injury

11.7 Rituximab

COMMON, SOME MAY BE SERIOUS

In 100 people receiving Rituximab more than 20 and up to 100 may have:

- Minor infusion reaction such as itching or rash
- Infections
- Low immunoglobulin level
- Nausea
- Headache
- Restlessness
- Body aches
- Loose stool

OCCASIONAL, SOME MAY BE SERIOUS

In 100 people receiving Rituximab from 4 to 20 may have:

- High blood pressure
- Moderate infusion reaction such as fever, chills, shaking, cough, or wheezing
- Low phosphorous level

RARE, AND SERIOUS

In 100 people receiving Rituximab 3 or fewer may have:

- Anaphylactic infusion reaction (hypotension, allergic symptoms)
- Myocardial infarction (heart attack)
- Stroke
- Delayed neutropenia

11.8 Cyclophosphamide

COMMON, SOME MAY BE SERIOUS

In 100 people receiving cyclophosphamide more than 20 and up to 100 may have:

- Decreased blood counts including anemia, low white blood cell count, and low platelet count
- Irritation to the bowel, causing nausea, vomiting, loss of appetite, or diarrhea
- Loss of hair, or alopecia

OCCASIONAL, SOME MAY BE SERIOUS

In 100 people receiving cyclophosphamide from 4 to 20 may have:

- Irritation to the bladder, which may cause discomfort. Rarely, bleeding may occur in the bladder
- Abnormal electrolyte concentration in the blood

RARE, AND SERIOUS

In 100 people receiving cyclophosphamide 3 or fewer may have:

- Irritation to the heart or heart lining, called the pericardium
- Abnormal heart rhythms
- Irritation to the lungs
- Veno-occlusive disease of the liver
- Injury to the kidneys, causing kidney failure
- Allergic reaction to the infusion of cyclophosphamide

11.8 Transplant Related Risks

11.8.1 Blood transfusions

Transfusions may induce allergic reactions. Small, subclinical pulmonary emboli may occur, but these rarely if ever require any intervention. Standard pre-medications for blood products may be used before administration of the marrow graft. Fluid overload can be managed with diuretics. Allergic reactions of variable severity can be prevented or mitigated by premedication with antipyretics, antihistamines, and opiates. These products may also serve as vectors of serious infection (e.g., CMV, hepatitis, AIDS). To circumvent this, prospective blood and marrow donors will be screened per AABB and FACT guidelines. CMV antibody (-) blood products will be used in CMV(-) individuals, whenever possible, regardless of the antibody status of the marrow donor. All blood products are irradiated (3000r, ^{137}Cs) to circumvent the risk of GVHD caused by contaminating lymphocytes in the transfused fractions.

11.8.2 Hematopoietic progenitor cell infusion

Possible side effects include: changes in blood pressure, fever, headache, shortness of breath, chills, sweats, nausea/vomiting, bad taste in the mouth. Pre-medications are given to reduce these side effects. Reactions will be treated as per standard MSKCC guidelines.

11.8.3 Graft-versus-host-disease (GVHD)

GVHD occurs when the donor immune system attempts to reject normal tissues and is described below. Approximately 20-40% of persons may develop acute GVHD and 5-20% may develop chronic GVHD. A biopsy may be necessary to make the diagnosis of GVHD.

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

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

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

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

11.8.4 Serious bleeding

Serious bleeding may result from low platelet counts and/or injury to tissues from treatment. This can happen in spite of platelet transfusions. Bleeding is rarely lethal.

11.8.5 Infections

Patients will be at increased risk of infections due to pancytopenia induced by the transplant. Low T-cell count for an additional 9-12 months after transplantation increases the risk for certain opportunistic infections such as pneumocystis *jiroveci* pneumonia, cytomegalovirus, and others. Medications are given to reduce the chance of infections. Patients will receive treatment if they do get an infection and most infections can be treated successfully with antibiotics. Patients will stay in the hospital longer or be readmitted iffound to have an infection. Patients are watched closely for bleeding and given platelet transfusions to prevent serious bleeding, but minor bleeding may occur.

11.8.6 Risk of a secondary cancer

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

11.8.7 Graft Failure/rejection

Stem cell grafts may fail to grow or may start to grow and then be rejected by the patient's immune system.

11.8.8 Impaired fertility

Conditioning chemotherapy has a high probability of resulting in impaired fertility of the recipient. Fertility counseling will be offered to appropriate patients pre-transplantation.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

12.1 Graft *versus* Host Disease

- Acute and chronic GVHD are defined by standard criteria.[25, 26]

12.2 Disease response criteria

- Disease will be staged using standard response criteria.

12.3 Engraftment

- Engraftment of neutrophils is defined as sustained 500 neutrophils/μL blood after conditioning chemotherapy.
- Primary engraftment failure is defined as failure to achieve neutrophil engraftment by day 30 after transplantation. The patient may pursue alternative transplantation options in such instances.
- Secondary engraftment failure is defined as <500/μL circulating neutrophils at any time after primary engraftment that is not attributed to disease recurrence or drug therapy.
- Engraftment of platelets is defined as sustained 50,000/μL after conditioning chemotherapy.

12.4 Donor chimerism

- The percentage of donor contribution to CD3+ lymphopoiesis and myeloid hematopoiesis (chimerism) will be determined using short tandem repeat (STR) based analysis of the blood at the time points outlined in section 10.1.
-

12.5 Survival

- Relapse incidence: The duration of time between transplantation and progression of primary malignancy or relapse of primary malignancy. Death in the absence of relapse or progression is considered a competing risk.
- Overall survival: The duration of time between transplantation and death due to any cause.
- Non-relapse mortality: The duration of time between transplantation death from any cause in the absence of disease progression or relapse. Relapse and progression are considered competing risks.

13.0 CRITERIA FOR REMOVAL FROM STUDY

The patient may be withdrawn from the study for the following reasons:

- If at any time the patient is found to be ineligible for the protocol as designated in the section on patient/subject eligibility.
- Subjects with relapsed or progressive disease may pursue other therapies and thus will be monitored for survival but not further toxicity.
- Patients may remove themselves from the study.
- The PI may remove patients from the study
    - Patinet noncompliance that makes continued study therapy dangerous for the patient.

- New information with respect to the patients health status that makes it unsafe for the patient to continue with study therapy.
- Adverse events that make ongoing study therapy unsafe for the patient.

In all instances of study removal, supportive care will continue as is appropriate for the patient.

14.0 BIOSTATISTICS

Primary objectives:
This is a phase II study to evaluate the rate of acute grade 3-4 GVHD following allo-HCT using a/WT-lymphocyte depleted grafts from haploidentical or mismatched unrelated donors. The intervention will be considered unpromising if the rate of GVHD is greater than 40% and promising if the rate is 20% or less. The unpromising rate of 40% or higher is established based on the rate of anticipated GVHD in patients receiving HLA mismatched allografts.[1, 3]

Based on these rates, a Simon two-stage optimal design will accrue 11 patients in the first stage. If six or fewer are free of grade 3-4 GVHD, the study will close due to a lack of efficacy; otherwise an additional 27 will accrue. If at the end of study, at least 27 out of the 38 patients are free of grade 3-4 GVHD, the study will be considered promising. The type I and type II errors are both set at 0.10. Accrual for this study will not be held at the interim analysis if it is uncertain whether seven or more patients are free of GVHD. The study will potentially close after the 11th patient completes the GVHD evaluation window.

Patients who die or relapse without developing acute GVHD will be included in the analysis. They will be considered as not developing acute GVHD for the primary endpoint in order for the primary endpoint to align with published cumulative incidence estimates of acute GVHD. Estimates of patient survival and relapse are separately estimated in the secondary objectives.

The study also includes sequential safety stopping rules to monitor for non-relapse mortality. In the event one of the stopping rules is crossed, accrual on the study will be suspended and all safety data will be reviewed by the PI. These rules are provided in the table below.

Failure Type	# of failures needed to stop the study	Failure rate in the population	Probability boundary is crossed
Non-relapse Mortality	4 in the first 14 patients 5 in the first 21 patients	0.1	0.1
	6 in the first 28 patients 7 in the first 34 patients 8 at any point	0.35	0.99

Secondary objectives:

- Overall survival will be estimated at one year following transplantation using Kaplan-Meier methodology.

- Cumulative incidence functions will be used to estimate non-relapse mortality at one year following transplantation. Relapse is considered a competing risk for this analysis.
- Cumulative incidence functions will be used to estimate relapse incidence at one year following transplantation. Death in the absence of relapse is considered a competing risk for this analysis.
- Summary statistics will be used to describe the reconstitution of CD4+, CD8+ T-lymphocytes, CD19+ B-lymphocytes at 30, 100, 180, and 365 days post transplantation.

Translational objectives:

- Summary statistics will be used to describe the reconstitution of TCR-a/W T-lymphocytes after TCR-a/W lymphocyte depleted allografts at 30, 100, 180, and 365 days post transplantation.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming whether or not the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA 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 Database (CRDB). Source documentation will be available to support the computerized patient record.

16.1 Quality Assurance

Monthly 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. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: https://one.mskcc.org/sites/pub/clinresearch/Documents/MSKCC Data and Safety Monitoring Plans.pdf

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

Risks: From the studies that have been done so far it appears that adding G-PBSC to standard induction chemotherapy can safely be performed in elderly patients with AML and that these patients may benefit from the treatment. However, given this is a new treatment approach, it is possible that there are side effects that have not yet been seen.

Benefits: This protocol may benefit patients by shortening the duration of chemotherapy-induced aplasia, reducing the related severe complications rate (infections, hemorrhagic events etc). This protocol may result in higher anti-leukemic activity compared to standard

chemotherapy, achieving higher CR rate and translating in higher OS. The information from this study will help future leukemia patients.

Possible toxicities/side effects: Toxicities and side effects of the agents used are listed in section 11 and reporting of serious adverse events is found in section 17.2.

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: Alternative treatment options will be presented to the patient prior to taking part in this study. Alternative treatment options may include getting treatment with standard chemotherapy; taking part in another study; or getting no treatment.

Costs/Incentives: No incentives will be offered to patients/subjects for participation in the study. Participation is voluntary. The patient/subject will be responsible for the costs of standard medical care, including the conventional agents cytarabine and filgrastim, antibiotics, blood and platelet transfusions, radiographic studies, laboratory tests, and all hospitalizations, even for complications of treatment. 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).

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

17.2 Serious Adverse Event (SAE) Reporting

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

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect

- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

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

SAE reporting is required as soon as the participant starts investigational treatment/intervention. SAE reporting is required for 30-days after the participant's last investigational treatment/intervention. Any event that occur after the 30-day period that is unexpected and at least possibly related to protocol treatment must be reported.

Please note: Any SAE that occurs prior to the start of investigational treatment/intervention and is related to a screening test or procedure (i.e., a screening biopsy) must be reported.

All SAEs must be submitted in PIMS. If an SAE requires submission to the HRPP office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be submitted within 5 calendar days of the event. All other SAEs must be submitted within 30 calendar days of the event.

The report should contain the following information:

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

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

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.

19.0 REFERENCES

1. Saber, W., et al., *Outcomes after matched unrelated donor versus identical sibling hematopoietic cell transplantation in adults with acute myelogenous leukemia.* Blood, 2012. **119(17):** p. 3908-3916.
2. Gragert, L., et al., *HLA Match Likelihoods for Hematopoietic Stem-Cell Grafts in the U.S. Registry.* New England Journal of Medicine, 2014. **371(4):** p. 339-348.
3. Lee, S.J., et al., *High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation.* Blood, 2007. **110(**13): p. 4576-4583.
4. Kroger, N., et al., *HLA-mismatched unrelated donors* as *an alternative graft source for al/ogeneic stem cell transplantation after antithymocyte globulin-containing conditioning regimen.* Biol Blood Marrow Transplant, 2009. **15(4):** p. 454-62.
5. Ball, L.M. and R.M. Egeler, *Acute GvHD: pathogenesis and classification.* Bone Marrow Transplant, 2008. **41**(S2): p. S58-S64.
6. Tamari, R., et al., *CD34-Selected Hematopoietic Stem Cell Transplants Conditioned with Myeloablative Regimens and Antithymocyte Globulin for Advanced Myelodysplastic Syndrome: Limited Graft-versus-Host Disease without Increased Relapse.* Biol Blood Marrow Transplant, 2015. **21(12):** p. 2106-14.
7. Pasquini, M.C., et al., *Comparative outcomes of donor graft CD34+ selection and immune suppressive therapy as graft-versus-host disease prophylaxis for patients with acute myeloid leukemia in complete remission undergoing HLA-matched sibling allogeneic hematopoietic cell transplantation.* J Clin Oncol, 2012. **30(26):** p. 3194-201.
8. Brooks, E.G., et al., *Human T-ce/1 receptor (TCR) alpha/beta+ CD4-CD8- T cells express oligoclonal TCRs, share junctional motifs across TCR V beta-gene families, and phenotypically resemble memory T cells.* Proceedings of the National Academy of Sciences of the United States of America, 1993. **90(24):** p. 11787-11791.
9. Felix, N.J. and P.M. Allen, *Specificity of T-ce/1 alloreactivity.* Nat Rev Immunol, 2007. 7(12): p. 942-953.

10. Silva-Santos, B., K. Serre, and H. Norell, *[gamma][delta] T cells in cancer.* Nat Rev Immunol, 2015. **15(11):** p. 683-691.
11. Jensen, K.D., et al., *Thymic selection determines gammadelta T cell effector fate: antigen-naive cells make interleukin-17 and antigen-experienced cells make interferon gamma.* Immunity, 2008. **29(1**): p. 90-100.
12. Vantourout, P. and A. Hayday, *Six-of-the-best: unique contributions of [gamma][delta] T cells to immunology.* Nat Rev Immunol, 2013. **13(2):** p. 88-100.
13. Khairallah, C., et al., *?? T Cells Confer Protection against Murine Cytomegalovirus (MCMV).* PLoS Pathog, 2015. **11(3):** p. e1004702.
14. Dechanet, J., et al., *Major expansion of gammadelta T lymphocytes following cytomegalovirus infection in kidney allograft recipients.* J Infect Dis, 1999. **179(**1): p. 1-8.
15. Hammerich, L. and F. Tacke, *Role of gamma-delta T cells in liver inflammation and fibrosis.* World Journal of Gastrointestinal Pathophysiology, 2014. **5(2):** p. 107-113.
16. Perko, R., et al., *Gamma delta T cell reconstitution is associated with fewer infections and improved event-free survival after hematopoietic stem cell transplantation for pediatric leukemia.* Biol Blood Marrow Transplant, 2015. **21(1):** p.130-6.
17. Schumm, M., et al., *Depletion of T-cell receptor alpha/beta and CD19 positive cells from apheresis products with the CliniMACS device.* Cytotherapy, 2013. **15(10):** p. 1253-1258.
18. Bertaina, A., et al., *HLA-haploidentical stem cell transplantation after removal of a/3+ T and B cells in children with nonmalignant disorders.* Blood, 2014. **124(5):** p. 822-826.
19. Bertaina, A., et al., *Children with Acute Leukemia Given Hematopoietic Stem Cell Transplantation (HSCT) from an HLA-Compatible Sibling, or an Unrelated Donor (UD) or an HLA-Haploidentical Relative after Alpha/Beta T-Cell Depletion Have* a *Comparable Outcome.* Blood, 2015. **126(23):** p. 195-195.
20. Offer, K., et al., *Efficacy of tacrolimus/mycophenolate mofetil as acute graft-versus-host disease prophylaxis and the impact of subtherapeutic tacrolimus levels in children after matched sibling donor allogeneic hematopoietic cell transplantation.* Biol Blood Marrow Transplant, 2015. **21**(3): p. 496-502.
21. Mori, T., et al., *Effect of early posttransplantation tacrolimus concentration on the development of acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation from unrelated donors.* Biol Blood Marrow Transplant, 2012. **18(2):** p. 229-34.
22. Dominietto, A., et al., *In vivo 8-cell depletion with rituximab for alternative donor hemopoietic SGT.* Bone Marrow Transplant, 2012. **47(1**): p. 101-106.
23. Roberts, K.G., et al., *Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia.* N Engl J Med, 2014. **371(11):** p. 1005-15.
24. Dohner, H., et al., *Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel.* Blood, 2017. **129(4):** p. 424-447.
25. Filipovich, A.H., et al., *National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report.* Biol Blood Marrow Transplant, 2005. **11(12):** p. 945-56.
26. Rowlings, P.A., et al., *IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade.* Br J Haematol, 1997. **97(4):** p. 855-64.

20.0 APPENDICES

Appendix A – Personalized rabbit ATG[3] dosing (P-rATG[3]); Target: Post – alloHCT <20Au*day/ml

Body weight (kg)	Lymphocyte count (x10^9/kg)	Cumulative dose (mg/kg)	Starting day	Number of doses	Daily dose (mg/kg)
5	0.1	10	-12	3	3.3
5	0.5	10	-12	3	3.3
5	1	10	-12	3	3.3
5	2	10	-12	3	3.3
5	3	10	-12	3	3.3
5	4	10	-12	3	3.3
7.5	0.1	10	-12	3	3.3
7.5	0.5	10	-12	3	3.3
7.5	1	10	-12	3	3.3
7.5	2	10	-12	3	3.3
7.5	3	10	-12	3	3.3
7.5	4	10	-12	3	3.3
10	0.1	10	-12	3	3.3
10	0.5	10	-12	3	3.3
10	1	10	-12	3	3.3
10	2	10	-12	3	3.3
10	3	10	-12	3	3.3
10	4	10	-12	3	3.3
15	0.1	10	-12	3	3.3
15	0.5	10	-12	3	3.3
15	1	10	-12	3	3.3
15	2	10	-12	3	3.3
15	3	10	-12	3	3.3
15	4	10	-12	3	3.3
20	0.1	8	-12	3	2.7
20	0.5	10	-12	3	3.3
20	1	10	-12	3	3.3
20	2	10	-12	3	3.3
20	3	10	-12	3	3.3
20	4	10	-12	3	3.3
25	0.1	8	-12	3	2.7
25	0.5	10	-12	3	3.3
25	1	10	-12	3	3.3
25	2	10	-12	3	3.3
25	3	10	-12	3	3.3
25	4	10	-12	3	3.3
30	0.1	7	-12	3	2.3
30	0.5	9	-12	3	3
30	1	10	-12	3	3.3
30	2	10	-12	3	3.3
30	3	10	-12	3	3.3
30	4	10	-12	3	3.3
35	0.1	7	-12	3	2.3
35	0.5	8	-12	3	2.7
35	1	9	-12	3	3
35	2	10	-12	3	3.3
35	3	10	-12	3	3.3
35	4	10	-12	3	3.3
40	0.1	6	-12	3	2.0
40	0.5	7	-12	3	2.3
40	1	8	-12	3	2.7
40	2	10	-12	3	3.3
40	3	10	-12	3	3.3
40	4	10	-12	3	3.3
50	0.1	5.5	-12	3	1.8
50	0.5	6	-12	3	2
50	1	8	-12	3	2.7
50	2	10	-12	3	3.3
50	3	10	-12	3	3.3
50	4	10	-12	3	3.3
60	0.1	5	-12	2	2.5
60	0.5	5.5	-12	3	1.8
60	1	6	-12	3	2.0
60	2	8	-12	3	2.7
60	3	10	-12	3	3.3
60	4	10	-12	3	3.3
70	0.1	5	-12	2	2.5
70	0.5	5	-12	3	1.7
70	1	6	-12	3	2.0

70		8	-12	2.7
70		10	-12	3.3
70	4	10	-12	3.3
80	0.1	4.5	-12	2.3
80	0.5		-12	1.7
80			-12	2.0
80		8	-12	2.7
80		10	-12	3.3

For patient above 80Kg (peds and adults) according to ALC only: starting day -12

ALC before ATG dosing:

0.1	360mg in 2 days (180mg per day)
0.5	400mg in 2 days (200mg per day)
1	480mg in 3 days (160mg per day)
2	::::e640mg in 3 days (215mg per day)
3	::::e800mg in 3days (270mg per day)
4	::::e800mg in 3 days (270mg per day)