Partially HLA-Mismatched Related Donor Hematopoietic Stem Cell Transplantation Using Killer Immunoglobulin Receptor and Human Leukocyte Antigen Based Donor Selection

PROTOCOL FACE PAGE FOR

Principal Investigator/Department:	Brian Shaffer, MD	Adult BMT
	Sergio Giralt, MD	Adult BMT
	Katharine C. Hsu, MD, PhD	Adult BMT
	Juliet Barker, MD, MBBS	Adult BMT
	Christina Cho, MD	Adult BMT
	David Chung, MD, PhD	Adult BMT
	Parastoo Dahi, MD	Adult BMT
	Arnab Ghosh, MD	Adult BMT
	Sergio Giralt, MD	Adult BMT
	Boglarka Gyurkocza, MD	Adult BMT
	Ann Jakubowski, MD, PhD	Adult BMT
	Scott James, MD, PhD	Adult BMT
	Oscar Lahoud, MD	Adult BMT
	Richard Lin, MD	Adult BMT
	Kate Markey, MBBS, PhD	Adult BMT
	Esperanza Papadopoulos, MD	Adult BMT
	Jonathan Peled, MD	Adult BMT
	Miguel-Angel Perales, MD	Adult BMT
	Ioannis Politikos, MD	Adult BMT
	Doris Ponce, MD	Adult BMT
	Craig Sauter, MD	Adult BMT
	Michael Scordo, MD	Adult BMT
	Gunjan Shah, MD	Adult BMT
	Melody Smith, MD	Adult BMT
	Roni Tamari, MD	Adult BMT
	Marcel van den Brink, MD, PhD	Adult BMT
	James Young, MD	Adult BMT
	Sean Devlin, PhD	Epidemiology/Biostatistics
	Scott Avecilla, MD, PhD	Laboratory Medicine
	Marci Andrejko, NP	Nursing
	Jason Batalha, NP	Nursing
	Kathleen Cavalier, NP	Nursing
	Naomi Cazeau, NP	Nursing
	Abigail Cohen, NP	Nursing
	Melissa Cortes, NP	Nursing
	Theresa Elko, NP	Nursing
	Shani Irby, NP	Nursing
	Rebecca Goldberg, NP	Nursing
	Carter Hibbs, NP	Nursing
	Stefanie Kelly, NP	Nursing
	Sheila Kenny, NP	Nursing
	Christina Kiss, NP	Nursing

	Nicole Lestrange, NP	Nursing
	Kathryn Mazzarrella, NP	Nursing
	Courtney McElrath, NP	Nursing
	Chelsea Mintz, NP	Nursing
	Dayna Palumbo, NP	Nursing
	Emily Patterson, NP	Nursing
	Natasia Rodriguez, RN	Nursing
	Megan Scott, NP	Nursing
	Allison Tucker, NP	Nursing
	Tara Wolff, NP	Nursing
Consenting Professional(s)/Department:	Juliet Barker, MD, MBBS	Adult BMT
	Christina Cho, MD	Adult BMT
	David Chung, MD, PhD	Adult BMT
	Parastoo Dahi, MD	Adult BMT
	Sergio Giralt, MD	Adult BMT
	Arnab Ghosh, MD	Adult BMT
	Boglarka Gyurkocza, MD	Adult BMT
	Ann Jakubowski, MD, PhD	Adult BMT
	Scott James, MD, PhD	Adult BMT
	Oscar Lahoud, MD	Adult BMT
	Richard Lin, MD	Adult BMT
	Kate Markey, MBBS, PhD	Adult BMT
	Esperanza Papadopoulos, MD	Adult BMT
	Jonathan Peled, MD	Adult BMT
	Miguel-Angel Perales, MD	Adult BMT
	Ioannis Politikos, MD	Adult BMT
	Doris Ponce, MD	Adult BMT
	Craig Sauter, MD	Adult BMT
	Michael Scordo, MD	Adult BMT
	Gunjan Shah, MD	Adult BMT
	Melody Smith, MD	Adult BMT
	Roni Tamari, MD	Adult BMT
	Marcel van den Brink, MD, PhD	Adult BMT
	James Young, MD	Adult BMT

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

OneMSK Sites
Manhattan

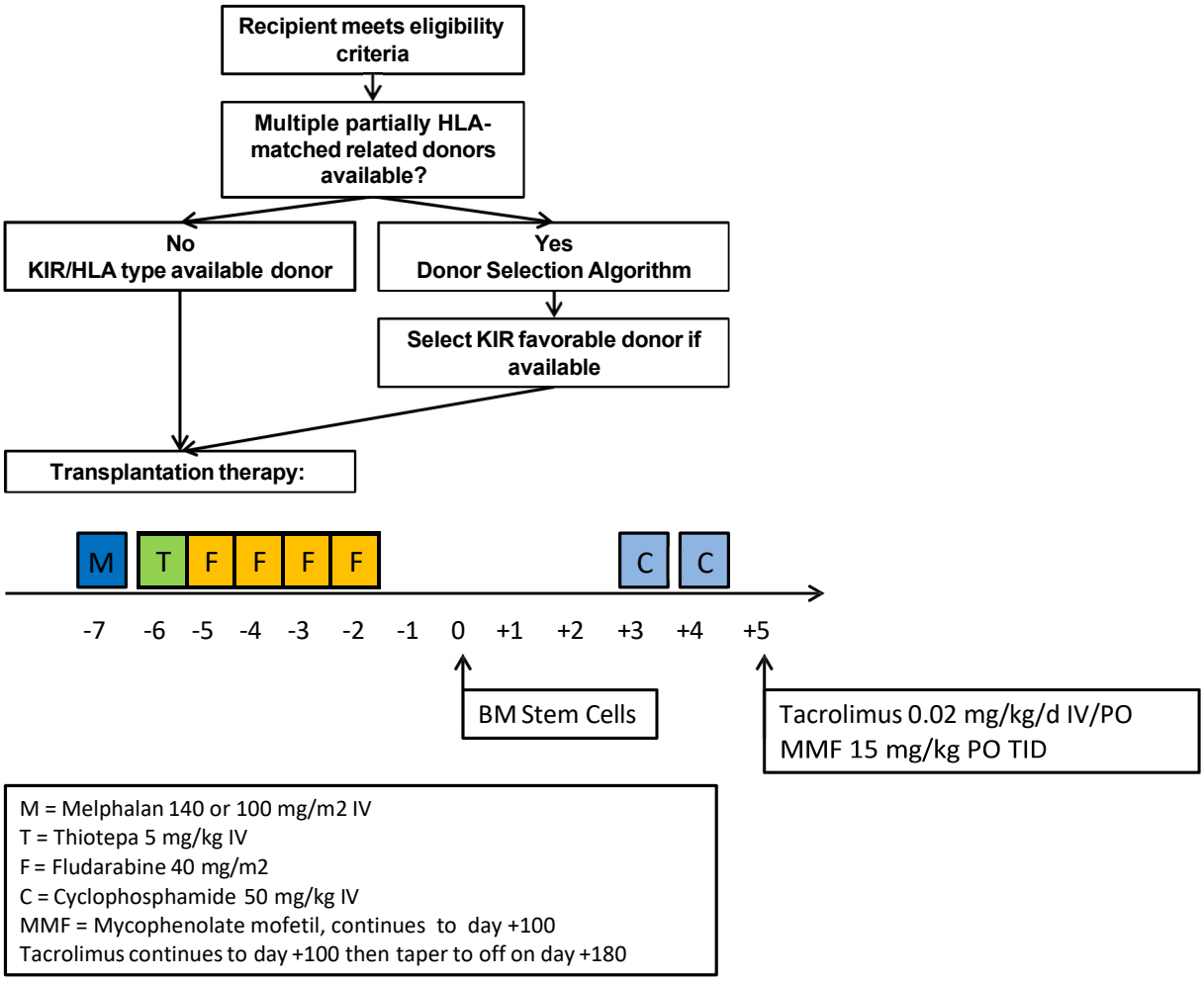
Table of Contents

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a pilot study evaluating a novel killer immunoglobulin-like receptor (KIR) and human leukocyte antigen (HLA) based donor selection algorithm in the context of a standard HLA-mismatched related donor allogeneic hematopoietic cell transplantation (allo HCT). Donor derived NK cells are known to play an important role in controlling malignancy after transplant. Engagement of inhibitory and activating KIR on the surface of the NK cell with their cognate ligand class 1 HLA on malignant cells governs effector function of the NK cell. We have previously demonstrated that donor KIR genotyping, when taken in the context of donor and recipient HLA allotype may be used to select "KIR advantageous" donors that yield lower rates of recipient relapse of AML after allo HCT. Importantly, NK cells are not associated with graft *versus* host disease (GVHD). Based on this, we hypothesize that KIR/HLA based selection may be used to increase NK mediated graft *versus* malignancy phenomenon without increasing GVHD. The purpose of this study is to estimate the proportion of patients who have a "KIR favorable" donor. Eligible candidates include persons aged 18-75 with a hematologic malignancy requiring allo HCT and who do not have a HLA-matched related or unrelated donor. Potential haploidentical HLA matched related persons, will be typed for HLA and KIR. Persons with at least one related donor matched for 4 to 7 of 8 HLA alleles (HLA-A, HLA-B, HLA-C, and HLA-DRB1) and who meet the eligibility criteria may be enrolled. If multiple donors are identified a KIR/HLA based donor selection algorithm, detailed below, will direct selection of the donor with greatest NK alloreactivity. Recipients will undergo a standard melphalan, fludarabine, and thiotepa based conditioning regimen. The hematopoietic stem cells will be derived from donor bone marrow and are infused in an unmodified fashion. Following stem cell infusion, post-transplant cyclophosphamide (PT-Cy), tacrolimus, and mycophenolate mofetil based graft *versus* host disease (GVHD) prophylaxis will be used. Patients will be monitored carefully after transplant for GVHD, infections, and other medical complications of the procedure. The targeted enrollment will be 50 ealuable patients over 2.5 years.

The primary objective of this protocol will be to estimate the proportion of patients undergoing partially HLA matched allo HCT who do and do not have a "KIR favorable" donor as defined by KIR/HLA donor selection algorithm. The secondary endpoint of the study will be to explore overall survival, relapse incidence, non-relapse mortality, engraftment failure, and GHVD for patients who do and do not have a KIR favorable donor.

1.1 Protocol Schema



2.0 OBJECTIVES AND SCIENTIFIC AIMS

2.1 Primary Objectives

- To estimate of the proportion of patients who have a KIR favorable donor while undergoing an allogeneic transplant with a partially HLA-matched related donor.

2.2 Secondary Objectives

- To explore the incidence of relapse, non-relapse mortality, and overall survival for patients who were and were not transplanted with a KIR favorable donor.
- To determine the incidence of acute and chronic graft *versus* host disease in patients treated with PT-Cy.
- To determine the cumulative incidence of engraftment failure at 100 days post transplantation.

2.3 Translational Objectives

- To describe NK cell surface activating and inhibitory receptor phenotype using multicolor flow cytometry on blood NK populations after haploidentical alloHCT using post transplant cyclophosphamide based GVHD prophylaxis in study participants.
- To determine NK cell cytotoxicity against HLA mismatched leukemia cell lines *in vitro* using post transplant blood cells obtained from study participants.

3.0 BACKGROUND AND RATIONALE

Allogeneic hematopoietic cell transplantation using partially HLA-matched related donors

Allogeneic hematopoietic cell transplantation (allo HCT) is a potentially curative therapy for persons with advanced hematologic malignancies. While matched sibling and unrelated donors remain the preferred stem cell sources for allo HCT, only 25-30% of eligible recipients will have a matched sibling donor. The probability of procuring a suitably matched unrelated donor (URD) for allo HCT varies widely. Among persons of Euro-Caucasian descent, the probability of having an available 8/8 HLA-matched unrelated donor (URD) is approximately 70%; however, persons from racial or ethnic minority groups have a <25-40% probability of finding such a donor. Therefore, development of an alternative stem cell source other than matched related or unrelated donors will expand access to allo HCT, particularly for persons in racial or ethnic minority groups.

Partially HLA-matched related donors have emerged as a viable alternative for persons who lack fully matched sibling or unrelated donor options.[1-4] Partially HLA-matched related donors, who are typically haploidentical matched, offer at least two advantages over unrelated donor sources: First, because parents and children share one HLA haplotype, and other family sources such as siblings or cousins may be haploidentical matched, there is a high likelihood that a suitable donor will be available. Second, collection of stem cells is more likely to proceed rapidly when obtained from a related donor as compared to an URD, where the average time to procurement is approximately 7 weeks.[5] This facilitates rapid allo HCT in persons who are not candidates for umbilical cord blood transplantation.

Post-allograft high dose cyclophosphamide for graft *versus* host disease prevention

The major historical barrier to haploidentical allo HCT has been a high rate of both acute and chronic GVHD secondary to recipient/donor HLA disparity. Historical series confirm a rate of moderate to severe acute graft *versus* host disease (GVHD) >50% and chronic GVHD >80% in recipients of <5-6/6 HLA-matched related donor allografts.[6] Infusion of high dose cyclophosphamide (CY) shortly after allo HCT has emerged as a viable mechanism to prevent GVHD in recipients of haploidentical allo HCT.[1, 7, 8] The rationale behind this approach is that donor lymphocytes activated by recipient major and minor histocompatibility antigens will enter cell division and become susceptible to Cy toxicity, while tolerant lymphocytes and hematopoietic stem cells remain at rest and are resistant to Cy.[7, 9] In an

initial report, O'Donnell and colleagues demonstrated that haploidentical allo HCT using fludarabine, Cy, and total body irradiation (TBI) based non-myeloablative (NMA) conditioning followed by infusion of 50 mg/kg of Cy on day +3 and +4 post allo HCT with cyclosporine and mycophenolate mofetil resulted in acceptable engraftment, toxicity, and GVHD.[1] In a follow-up study, Luznik et al reported the outcomes of 68 persons with hematologic malignancies treated in phase II study with haploidentical bone marrow allografts using fludarabine, Cy, and TBI based NMA conditioning followed by 50 mg/kg Cy on day +3 and +4 post allo HCT.[2] Traditional cyclosporine and mycophenolate mofetil GVHD prophylaxis was also used. The median time to neutrophil and platelet recovery was 15 and 24 days, respectively. Graft failure occurred in 9/66 persons. The incidence of grade III-IV acute GVHD was 6% and the incidence non-relapse mortality (NRM) was 15% at one year. The rate of relapse was 51% at one year, resulting in a two year survival of 36%.

In order to improve on these results, several groups tested the use of post allo HCT Cy (PT-Cy) with myeloablative or reduced intensity (RIC) preparative regimens. Raiola and colleagues reported their findings in 55 patients who underwent haploidentical allo HCT using myeloablative conditioning with busulfan, thiotepa, and fludarabine or fludarabine and TBI followed by PT-Cy, cyclosporine, and mycophenolate mofetil.[10] The incidence of grade II-IV acute GVHD was 12% and chronic GVHD was 10%. The rate of NRM was 18% and relapse rate was 26%, resulting in a 22 month disease-free survival rate of 68% for persons in remission and 37% for persons with active disease at the time of allo HCT. Ciurea and colleagues reported similar results using fludarabine, melphalan, and thiotepa based RIC followed by haploidentical allo HCT and PT-Cy in 28 persons with hematologic malignancies.[4] Here, 6/28 persons developed primary graft failure. In a follow-up, Ciurea and colleagues reported improved results with ablative dose fludarabine, melphalan, and thiotepa or TBI in 94 patients with hematologic malignancies treated with haploidentical allo HCT and post transplantation CY, tacrolimus, and mycophenolate mofetil. The median time to neutrophil engraftment was 18 days and 95% of persons achieved full donor hematopoiesis. The rate of grade II-IV acute GVHD was 45% and chronic GVHD was 15%. The NRM, relapse rate, and overall survival were 24%, 38%, and 45%, respectively.

In aggregate, these results suggest that HLA-mismatched related donor transplantation with PT-Cy confers acceptable rates of donor cell engraftment, acute and chronic GVHD, and overall survival. The most frequent cause of adverse outcomes after allo HCT remains relapse; therefore, methods to improve donor alloreactivity that do not result in greater incidence of GVHD have the greatest likelihood of improving outcomes.

Incidence of primary engraftment failure after post transplantation cyclophosphamide
Early reports of haploidentical allo HCT using PT-Cy describe an incidence of primary engraftment failure (PEF) of approximately 20-40%.[1, 2] Early preparative regimens were insufficiently lymphoablative to allow for donor engraftment in the setting of PT-Cy. More modern regimens including those based on fludarabine, melphalan, and thiotepa (FMT) conditioning as is proposed here are sufficiently lymphoablative to prevent host *versus* graft mediated rejection. The rate of PEF with FMT conditioning is approximately 5%.[11] An

important factor determining the likelihood of PEF is the presence of antibodies directed at donor specific HLA in the recipient. The majority of patients in early studies who developed PEF were found to have donor specific HLA antibodies at moderate or high titers. [12] For the purposes of this study all participants will undergo screening for donor specific HLA antibodies. If the recipient has antibodies against a specific donor HLA, this donor will be de-prioritized irregardless of their KIR status.

Role of NK alloreactivity in haploidentical allogeneic hematopoietic cell transplantation

Allo HCT confers an immune-mediated graft *versus* tumor effect, whereby donor lymphocytes eradicate malignant cells, protect the recipient from relapse, and allow for long-term disease free survival.[13, 14] Natural killer (NK) cells have emerged as key mediators of this phenomenon. NK cells control innate immunity, are typically the first cells to recover after allo HCT, and have potent anti-neoplastic and anti-viral properties NK.[15-19] Importantly, there is no evidence that allogeneic NK cells mediate or contribute to GVHD, even in a highly HLA-mismatched environment.[20-23] Capturing and maximizing donor NK alloreactivity in allo HCT, therefore, is an attractive strategy to reduce the risk of malignancy relapse without increasing the risk of GVHD.

NK effector function is controlled by an array of cell surface receptors, among which the killer Ig-like receptors (KIR) stand out as key regulatory elements. NK cells are tolerized to autologous cells via engagement of inhibitory KIR with specific class I human leukocyte antigen (HLA) epitopes[24]: KIR2DL1 recognizes HLA-C alleles characterized by Asn77 and Lys80 (HLA-C2 group); KIR2DL2/3 recognizes HLA-C alleles characterized by Ser77 and Asn80 (HLA-C1 group); and KIR3DL1 recognizes HLA-A and -B alleles with the Bw4 epitope.[25] In addition to their inhibitory interaction, engagement of the same inhibitory KIR with self-HLA educates, or “licenses,” NK cells for higher effector function against neighboring cells lacking “self” HLA.[26, 27] Target cells lacking the self-class I HLA ligand to the NK KIR are sensitive to cytotoxicity unleashed by licensed NK cells, whose recognition of “missing self” in the target cell results in reactivity. In individuals who lack cognate class I ligands for their inhibitory KIR, NK cells expressing inhibitory KIR for non-self HLA are termed “unlicensed.” Although these cells exhibit decreased effector capacity *in vitro*, we have demonstrated that unlicensed NK cells can achieve significant levels of effector function when stimulated by cytokines or by CD16 engagement and can play an important role in tumor eradication.[28-31]

Ruggeri and colleagues described the relevance of inhibitory KIR signaling in the context of HLA mismatched AHST.[32] Expression of a KIR ligand (HLA-C1, HLA-C2, or HLA-Bw4) in the allo HCT donor that was absent in the recipient, so called “missing-self,” was associated with increased NK alloreactivity. Importantly, missing-self was not associated with increased graft rejection or GVHD. In a follow-up study of 112 patients with AML undergoing HLA-haploidentical ASHT, Ruggeri and colleagues demonstrated that transplantation from a donor/recipient pair with missing self (N = 51) results in a significant reduction in the risk of

relapse or death (relative risk 0.48; 95% confidence interval 0.29-0.78, P<0.001).[33] Single center and registry data further support that lack of expression of KIR ligand (missing ligand) in the context of HLA-matched ASHT is associated with decreased relapse incidence.[34, 35] These data would suggest that haploidentical transplantation may be optimized by preferential selection of donors to promote missing self. An important limitation to this strategy is that expression of KIR ligands in the recipient is fixed; therefore recipients who express all KIR ligands, or who lack a donor that does express the missing ligand, will not benefit from this strategy. In the context of the Ruggeri study, donor/recipient pairs with missing self were identified in approximately 45% of transplants. Therefore, strategies to promote NK alloreactivity in the absence of missing self are necessary.

Promotion of unlicensed NK cells in KIR-ligand matched allogeneic hematopoietic cell transplantation

Despite being HLA-mismatched, the majority of haploidentical donors are likely to be KIR-ligand matched with the transplant recipient. For example, because different HLA-C alleles may contain the C1 epitope, HLA-C mismatched donors may both express C1. This results in a scenario analogous to HLA-matched allo HCT, where NK alloreactivity may be inhibited by expression of cognate class I HLA KIR ligands on recipient neoplastic cells. In this scenario, KIR genes and KIR alleles may be used to minimize the degree of inhibitory signals and maximize the degree of activating signals delivered to donor NK cells via KIR.

Selection of donors with increased KIR mediated activating signals results in greater NK alloreactivity, such as those found in individuals homozygous for the partial centromeric KIR-haplotype B (cenBB), are more protective for AML relapse.[36, 37] We found that the most protective activating KIR for relapse is KIR2DS1, whose *in vitro* activity we originally reported to be diminished by high levels of its ligand HLA-C2.[38] In line with our *in vitro* studies, we subsequently demonstrated in a allo HCT cohort of 1277 transplants for AML that protection from relapse was restricted to donors with HLA-C1, and HLA-C2C2 minimizes any KIR2DS1 effect (Figure 1).[39] These findings have subsequently been confirmed in a recent publication by a separate group of investigators.[40] In this cohort, we found that the degree of relapse in donor/recipients who were mismatched at HLA-C was significantly reduced in the presence of donor KIR2DS1 expression (17.1 *versus* 35.6%, HR 0.40 [0.20-0.78], P = 0.007), Figure 1.

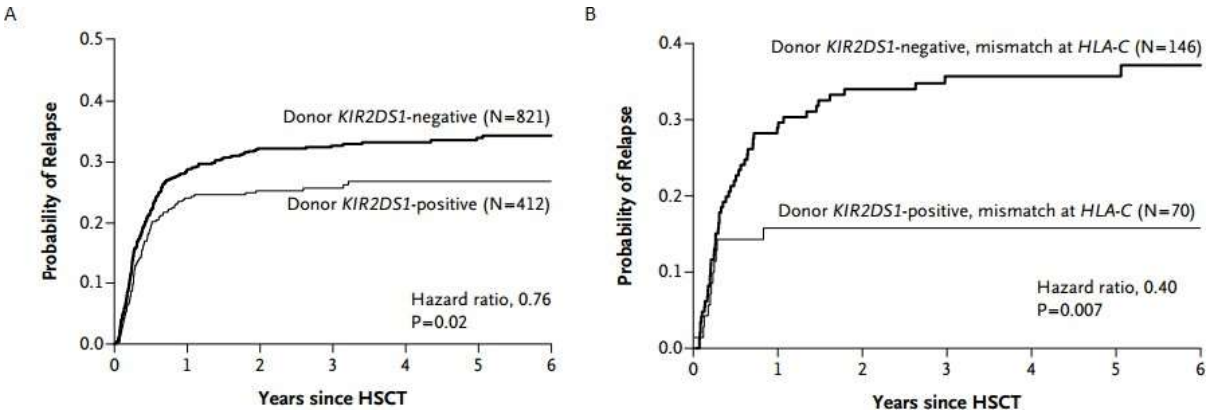


Figure 1: (A) Cumulative incidence of AML relapse in persons transplanted from a KIR2DS1 positive *versus* negative donor. (B) The benefit in relapse prevention was enhanced in patients transplanted from HLA-C mismatched donors.

Supporting these data, Oevermann and colleagues recently demonstrated that use of donors with the activating KIR rich haplotype B were associated a reduction in relapsed pediatric acute lymphoblastic leukemia (50.6% *versus* 29.5%, P = 0.033). [41] Mancusi and colleagues also note an association between donor KIR2DS1 expression and reduction in transplant related mortality after haploidentical allo HCT. [42] Approximately 30-40% of persons express KIR2DS1. Therefore, it is likely that a KIR2DS1 donor will be available among 2-3 haploidentical family members.

In addition to the activating KIR2DS1, we have also examined the role of inhibitory KIR in determining transplant outcomes. Among the inhibitory KIR, KIR3DL1 and HLA-Bw4 are the most polymorphic of the KIR/KIR ligand pairs, and different pairings of each have been described to result in substantial differences in strength of inhibition, suggesting an important role in NK biology.[43-46] Highly expressed KIR3DL1 alleles ($KIR3DL1^{hi}$) interact strongly with HLA-Bw4 alleles containing an isoleucine at position 80 ($Bw4\text{-}I^{80}$) to produce a strong licensing/inhibition signal, while the poorly expressed KIR3DL1 alleles ($KIR3DL1^{lo}$) have similar interactions with HLA-Bw4 containing threonine at position 80 ($Bw4\text{-}T^{80}$).[43-47] In persons infected with HIV, these 3DL1/HLA combinations predictive of higher licensing [48] result in a reduced incidence of progression to AIDS.[49] The strong interactions that produce higher licensing, however, also predict for easier inhibition. In collaboration with the National Marrow Donor Program (NMDP) and Center for International Blood & Marrow Transplant Research (CIBMTR), which provided sequencing -based donor KIR allele typing, we completed a pilot study of donor KIR3DL1-Bw4 allele combinations for 299 AML patients undergoing HCT from largely HLA-matched unrelated donors.[50, 51] We found that donors with poorly inhibitory KIR3DL1-Bw4 allele combinations were associated with significantly lower relapse and higher survival following HCT, and the highly licensed, but highly "inhibitable" combinations were associated with higher relapse (Figure 2). Importantly, there was no association with relapse protection when considering donor KIR3DL1 allele independent of HLA. These data are consistent with our model that HLA expression on tumor

cells leads to inhibition of licensed NK cells, an outcome avoided by unlicensed NK cells (Bw6/Bw6) and NK cells with poor or no KIR3DL1-Bw4 interaction.[28, 31, 52]

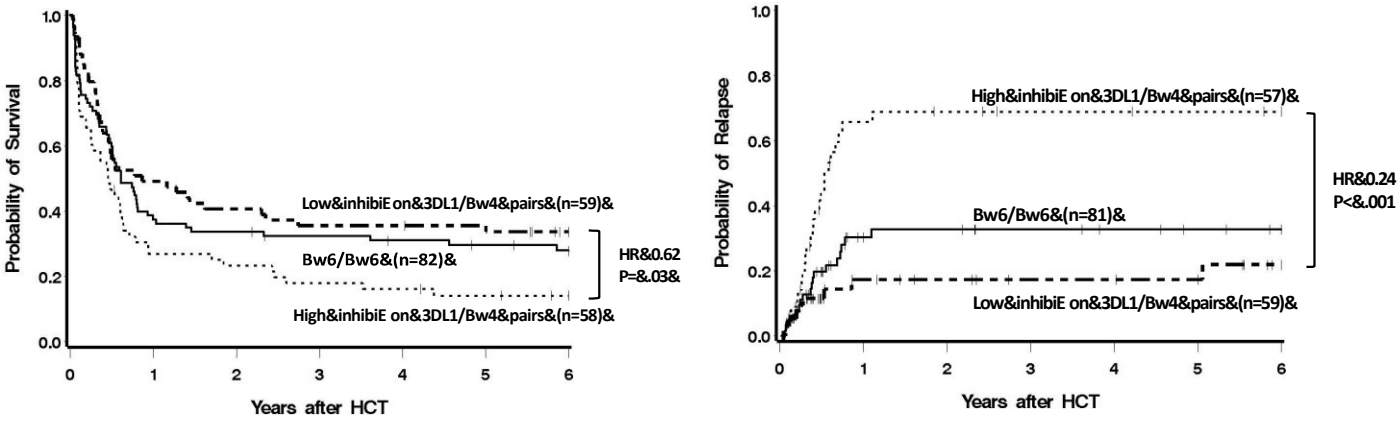


Figure 2: Relapse and survival in AML recipients of 9/10 or 10/10 URD allo HCT, stratified by with donors with high inhibitory KIR3DL1/Bw4 combinations (3DL1 ^{hi}+Bw4-I^{80} or 3DL1^{lo}+Bw4-T^{80}), low inhibitory combinations (3DL1^{hi}+Bw4-T^{80} or 3DL1^{lo}+Bw4-I^{80}), or lack of inhibition altogether (Bw6/Bw6).

Based on the strength of these univariate results, we devised and validated (99.5% concordance with allele typing by sequencing) a novel high through-put PCR-SSP method for “intermediate-resolution” identification of the most frequent and functionally defined KIR3DL1 allele groups.[53] Collectively, the allele groups represent 97.5% alleles found in a sequenced population of 426 individuals. Applying this method to an expanded cohort of 1328 AML patients undergoing HCT from 9/10 and 10/10 HLA-matched unrelated donors, we have confirmed the beneficial effect of no/low-inhibitory KIR3DL1-Bw4 allele combinations on relapse in a multivariate analysis [HR 0.72 (95%CI 0.57-0.90), p=0.0035), even after adjustment for KIR2DS1/HLA-C1 (HR 0.74 (95%CI 0.58-0.95), p=0.018) and cenBB (HR 0.72 (95%CI 0.57-0.92), p=0.009), (Preliminary Results).

Rationale for the proposed study

Taken together, these results indicate that NK alloreactivity plays an important role in disease control after allotransplantation, particularly in patients with myeloid malignancies. The role of NK mediated alloreactivity against lymphoid malignancies appears to be enhanced in the HLA mismatched environment. Whether the KIR advantageous donors are favorable for these individuals is unclear but they do not appear to result in any significant harm to these patients, therefore, it is reasonable to include persons with lymphoid malignancies in this pilot study.

Because of the relatively low rate of treatment related mortality seen with PT-Cy based GVHD prophylaxis, reduction in relapse is now the most important determinant of post transplant outcomes. Therefore mechanisms to increase donor alloreactivity that are specifically targeted to malignant cells are key to improve this transplant platform. The proposed study will use a novel KIR donor selection algorithm developed at MSKCC to select haploidentical

donors for allo HCT. This study will estimate the probability of finding a KIR advantageous donor in this setting. The secondary endpoints will address other primary transplant outcomes.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a pilot study evaluating a KIR/HLA based haploidentical donor selection for persons requiring allogeneic hematopoietic cell transplantation for hematologic malignancy. The goal of the study is to estimate the proportion of patients with a KIR-favorable donor. Eligible patients will be identified at the weekly service rounds of the adult BMT service. Recipients who are identified as suitable for transplantation but who lack a matched sibling or unrelated donor option will undergo a search for a suitable haploidentical matched related donor. If at least one eligible related donor matched at 4-7/8 HLA alleles (HLA-A, -B, -C, -DRB1) is identified and the patient provides informed consent this person may be enrolled onto the study. If multiple potential donors are identified, a donor selection algorithm will be used to select the donor predictive to result in the greatest NK alloreactivity versus the recipient malignancy. This selection process is outlined above and in section 9. Briefly, donors are prioritized to maximize the missing ligand phenomenon, whereby donor KIR fail to interact with their cognate ligand on recipient cells, inducing alloreactivity. Donors/recipient pairs with missing self (donor expresses KIR ligand absent in the recipient) are prioritized. If none are available, KIR/HLA gene and allele based selection will be used to select donors with a high degree of licensing via the KIR associated with the missing recipient ligand. In recipients who express all KIR ligands, KIR/HLA gene and allele based selection may be used to minimize inhibitory signaling through KIR2DL1 and KIR3DL1 and to select donors who express the stimulatory KIR2DS1. An exception to this algorithm is allowed for recipients with high titers of donor-specific anti-HLA antibodies. Donors will undergo eligibility evaluation and subsequent bone marrow stem cell harvest per FACT and MSKCC instructional guidelines.

The trial is anticipated to enroll 50 evaluable patients over 2.5 years. The study will assess whether KIR/HLA based donor selection is feasible and practical in the context of haploidentical related donor allo HCT. Outcomes will be assessed in persons transplanted with and without a KIR-favorable donor. The study will employ a stopping rule considering treatment related mortality ≥30% before 100 days as an unacceptable risk.

4.2 Intervention

All study participants will receive similar therapy. Transplant conditioning will consist of: melphalan (140 mg/m^2 IV on day -7), fludarabine (40 mg/m^2/d on days -5 through -2), and thiotepa (5 mg/kg IV on day -6). Melphalan must be reduced to 100 mg/m^2 for recipients ≥55 years old or for persons with significant cardiac comorbidities precluding full dose conditioning. The donor stem cell product will be derived from the bone marrow with a target cell infusion of 400 million total nucleated cells per recipient kg. GVHD prophylaxis will consist

of cyclophosphamide (50 mg/kg IV on day +3 and +4), mycophenolate mofetil (15 mg/kg PO/IV TID), and dose adjusted tacrolimus.

Supportive care will include standard antimicrobial prophylaxis programs as outlined by the adult BMT service handbook. These will include prophylaxis for Pneumocystis *jiroveci*, Herpesviridae spp., fungal infections, and Toxoplasmosis *gondii* when appropriate. Other supportive care measures will include treatment of emergent infections, blood product support, prophylaxis for tumor lysis syndrome and sinusoidal obstructive syndrome, and management of acute medical complications during transplantation therapies.

Patients will undergo donor/recipient bone marrow chimerism at 30 and 100 days post allo HCT and thereafter at the discretion of the treating clinician. Disease restaging will be performed at 3, 6, 9, 12, 18, and 24 months and as otherwise clinically indicated by the treating physician.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Melphalan (Alkeran®)

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

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

Solution preparation:

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

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

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

5.2 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: up to 10mg/ml.
3. IV piggyback volume: 50 cc.

Clinical considerations:
1. Hydration: during 500 cc saline.
2. Emetic potential: low.
3. Supportive medications: none.

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

5.3 Cyclophosphamide (Cytoxan®)

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.

5.4 Mesna (Mesnex®)

Supplied as: 200 mg/2 ml ampule, 1 gram/10 ml multi-dose vials
Reconstitution: not applicable.
Storage and stability:
1. Store vials at room temperature.
2. Multi-dose vials may be stored and used for up to 8 days after initial entry.
3. Infusions prepared in D5W are stable for 48 hours when stored under refrigeration or at room temperature.

Solution Preparation:
1. Must be diluted prior to infusion
2. Standard IV fluid: D5W.
3. IV piggyback volume: 50ml D5W

Usual Dosage and administration: 100% of the total cyclophosphamide dose divided into 3 doses, and administered at 30 minutes prior to and 4 and 8 hours after the start of the chemotherapy. Given as IVPB over 15 minutes.
Clinical Considerations: mesna functions solely for uroprotection to prevent hemorrhagic cystitis and has no cytotoxic activity.
Toxicities: none.

Incompatibilities: Carboplatin, Cisplatin, and Epirubicin.

5.5 Mycophenolate Mofetil (CellCept®)

Supplied as: 500 mg vial of powder for reconstitution.
Reconstitution: reconstitute each 500 mg vial with 14 ml of D5W only. Gently shake the vial to dissolve the drug. The vial will contain 500 mg of mycophenolate in approximately 15 ml.
Storage and Stability: Store at 15 -30°C. Drug compatible with D5W only. A final concentration of 6mg/ ml must be achieved prior to administration. Reconstituted vials and IV preparations are stable for up to 4 hours after preparation.
Solution Preparation:

1. Reconstitute each 500 mg vial with 14 ml of D5W.
2. Gently shake the vial to dissolve the drug.
3. Drug must be further diluted to a final concentration of 6 mg/ml. A 1000 mg dose should be placed in 140 ml of D5W.
4. MMF vials are stable for 4 hours at room temperature after reconstitution.
5. Doses of MMF may begin infusion into the patient up to 4 hours after initial reconstitution of the vials.

Clinical Considerations: administer only with D5W, over at least 2 hours. Mycophenolate is mutagenic, carcinogenic, and teratogenic. Precautions must be taken when handling this product. If medication comes in contact with skin, wash thoroughly with soap and water.
Incompatibilities**:** Only compatible with D5W.

5.6 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 D5W.
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 D5W, filgrastim is stable for 7 days.
5. Stability (plastic syringe) filgrastim is stable for two weeks in BD 1 ml plastic TB syringes at 2-8°C.

5.7 Tacrolimus (Prograf®)

Supplied as: Capsules (1 and 5 mg) for parenteral administration or as a sterile solution in 1 mL ampules containing 5 mg anhydrous tacrolimus per mL, in boxes of 10 ampules, for intravenous administration.
Storage and Stability: Tacrolimus injection ampules are stored between 5º-25ºC. Prograf capsules are stored at room temperature (15º-30ºC).
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.

5.8 Thiotepa

Supplied as: 15 mg vial lyophilized powder that must be diluted prior to infusion.

Storage and Stability: Thiotepa vials are stored in a refrigerator protected from light. Once reconstituted in saline, thiotepa solution is stable for 14 days in refrigeration or 7 days at room temperature.

Solution Preparation: Thiotepa powder is dissolved in normal saline to achieve a final concentration up to 5 mg/mL and delivered in a final volume of 500 mL.

Clinical Considerations: Thiotepa infusion should be through IMED 2200 tubing.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

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 riskfor relapse including:
        - t(9;22) or detected BCR-ABL1 translocation by genomic methodologies
        - BCR-ABL1-Like B-ALL [54] 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)
        - CNS or other extramedullary involvement
        - WBC count ≥ 100,000 cells/μL at diagnosis

    - Relapsed ALL, biphenotypic/bilineal leukemia, or AML with ≤ 10% blasts in the bone marrow prior to transplantation.

    - Acute biphenotypic or bilineal leukemia in first or greater complete remission.

- Acute myeloid leukemia (AML) in CR1 with intermediate or high risk features including:
    - Cytogeneic abnormalities associated with myelodysplastic syndrome including abnormalities of chromosome 5 or 7
    - History of anti-neoplastic therapy (radiation or chemotherapy)
    - Extramedullary involvement
    - WBC count ≥100,000 cells/μL at diagnosis
    - Rearrangements or mutations of 11q23 (MLL)
    - Abnormalities of chromosome 3
    - TP53 mutation or loss of 17p
    - Complex or monosomal karyotype
    - Normal karyotype with mutations of FLT3, RUNX1, or ASXL1

- Myelodysplastic syndrome, myeloproliferative neoplasms, or MDS/MPN overlap syndrome with:
    - International prognostic scoring system risk score of INT-2 or high risk at the time of transplant evaluation.
    - Any risk category if life-threatening cytopenia exists.
    - 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,

- Myelofibrosis with DIPSS scores of INT-2 or high risk or any risk category if life threatening cytopenias are present.

- Chronic myelomonocytic leukemia (CMML)

- Chronic myeloid leukemia (CML) who have 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 <20% blasts after therapy.

- Hodgkin lymphoma:
    - Relapsed disease with progression after autologous bone marrow transplant or are ineligible for this procedure..
    - Responding to therapy prior to enrollment

- Non-Hodgkin lymphoma:
    - Responding to therapy prior to enrollment.

        - Progression after autologous bone marrow transplant or are ineligible for this procedure
    - Chronic lymphocytic leukemia with high risk disease as defined by the EBMT consensus criteria.

- Patients aged 18 through 69 years old are eligible.
- Patients aged 70-75 with HCT-CI of 0-1 are eligible.
- Patients must have Karnofsky performance status ≥70%.
- Cardiac left ventricular ejection fraction ≥50% at rest.
- Total bilirubin ≤ 2 mg/dL, except for patient's with Gilbert's syndrome
- AST and ALT ≤ 5x ULN unless thought to be disease related.
- Estimated or measured creatinine clearance > 50 mL/min.
- Hemoglobin adjusted pulmonary DLCO ≥50% of predicted, if Hgb is within normal range, unadjusted DLCO must be ≥ 50%..

6.2 Subject Exclusion Criteria

6.2.1 Persons with a HLA matched sibling donor.

6.2.2 Female patients who are pregnant or breast-feeding.

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

6.2.4 Persons who are seropositive for HIV.

6.2.5 Persons with uncontrolled central nervous system malignancy.

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

6.2.7 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.

6.2.8 Prior diagnosis of non-hematologic malignancy within 5 years of planned protocol therapy EXCEPT:

- Diagnosis of breast ductal carcinoma *in situ* treated with curative intent

- Diagnosis of prostate adenocarcinoma with Gleasons score ≤ 6 treated with curative intent
- Non-melanomatous skin cancer

6.3 Donor Inclusion Criteria

6.3.1 Partially HLA-mismatched relative (allele level matched at 4 to 7 of 8 HLA loci: -A, -B, -C, and -DRB1).

6.3.2 Meets criteria outlined in the FACT-approved SOP for "DONOR EVALUATION AND SELECTION FOR ALLOGENEIC TRANSPLANTATION" in the Blood and Marrow Transplant Program Manual, document E-1. (Link to URL: https://one.mskcc.org/sites/pub/corp/bmt/Documents/D2_SOP_Donor%20Selection%20and%20Evaluation_04_2015.pdf)

6.3.3 Donor must be willing to undergo general anesthesia and bone marrow stem cell harvest.

6.3.4 Donor must be ≥14 years old.

6.4 Donor Exclusion Criteria

6.4.1 Evidence of infection not responding to antibiotic therapy.

6.4.2 Chronic viral infection including active hepatitis B, hepatitis C, HIV, or HTLV-1/2.

6.4.3 Factors that place the donor at increased risk of general anesthesia and bone marrow harvest, such as congenital or acquired bleeding disorders, intolerance or allergy to anesthesia, prior serious surgical complications, or uncontrolled cardiac or pulmonary disorders.

6.4.4 Pregnant or breast-feeding.

6.4.5 Unwilling or unable to provide informed consent.

6.4.6 Unable to provide a bone marrow allograft product.

7.0 RECRUITMENT PLAN

Eligible patients are identified via the weekly BMT/Leukemia Patient Review Conference. Patients that do not have a matched sibling or unrelated adult donor option and who do have a haploidentical matched related donor and meet the other eligibility criteria will be offered participation in this study. Both the patient and potential donor must meet the eligibility criteria before enrollment in the study.

This protocol will take due notice of NIH/ADAMHA policies concerning inclusion of women and minorities in clinical research populations. We expect that the study population will be fully

representative of the range of patients referred for transplant without exclusion as to age, gender, or ethnic background. 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 antibodies against CMV, , Hepatitis B (core antibody, surface antibody), Hepatitis C, EBV, and Toxoplasmosis, HIV, and HTLV-1/2
- Pregnancy test for women of childbearing potential
- Bone marrow aspirate (biopsy if clinically indicated)
- Urinalysis
- Electrocardiogram and echocardiogram
- Spirometry and pulmonary diffusion capacity
- Donor-specific anti-HLA antibody titers

9.0 TREATMENT/INTERVENTION PLAN

9.1 Regimen for Cytoreduction

All drugs are administered relative to the date of stem cell infusion (day 0).

9.1.1 Conditioning program

- Melphalan 100-140 mg/m^2 (adjusted as per section 9.1.2) on day -7.
- Fludarabine 40 mg/m^2 on day -5, -4, -3, -2.
- Thiotepa 5 mg/kg on day -6.

9.1.2 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 weight.

Persons ≥55 years old or those with any cardiac comorbidies (including known cardiovascular disease, history of recurrent or chronic arrythmia, or history of chemotherapy induced cardiomyopathy) receive melphalan 100 mg/m^2. Patients who are not felt to be fit for higher dose therapy or who have a hematopoietic cell comorbidity index >3 receive melphalan 100 mg/m^2.

9.1.3 Fludarabine administration

Fludarabine is administered intravenously over 30 minutes. Persons with creatinine clearance < 50 mL/min/1.73 m^2 BSA will receive a 25% reduction in dose (30 mg/m^2).

9.1.4 Adjustment of chemotherapy based on adjusted body weight

Patients who are >125% of ideal body weight will receive melphalan based on adjusted body weight. Fludarabine will be dosed based on actual body weight. Ideal and adjusted body weights are calculated as below:

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

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

9.2 Selection of Haploidentical Donors

9.2.1 Up to 5 potentially haploidentical donors will be typed for HLA alleles, KIR genes, and KIR 3DL1 alleles.

9.2.2 If the recipient produces high titers of anti-HLA antibodies to a HLA antigen expressed in any donor, this donor will automatically be prioritized last in the donor selection process.

9.2.3 Otherwise eligible donors are prioritized as follows:

IF the recipient is lacking a KIR ligand that is expressed in a potential donor (missing self) this donor will be prioritized.

IF the recipient expresses all KIR ligands or the available donors are also missing the absent KIR, donors will be prioritized in the following fashion:

a. Donors with KIR3DL1 non-engaging (combination of KIR3DL1-null and/or KIR3DS1).
b. Donors with predicted low inhibitory KIR3DL1 interactions.
c. Donors that express KIR2DS1 AND HLA-C1.
d. Donor who is centromeric B haplotype homozygous (cenBB).

Donors may be de-prioritized at the discretion of the treating physician if extenuating circumstances make them unlikely to be suitable stem cell donors. Examples include advanced donor age, donor comorbidities, or social/supportive care factors that may limit a donor's ability to participate in the protocol. The protocol will capture prospectively whether the preferred KIR donor was used.

Potential donors who decline participation will be deprioritized.

Reason for the selection of the donor used for transplantation will be gathered prospectively. The donor selected and reason for selection will be documented in Appendix A – KIR Donor Selection form.

9.3 Bone Marrow Harvest and Stem Cell Infusion

9.3.1 Bone marrow progenitor cells are harvested according to the MSKCC Blood and Marrow Transplant Program Manual Document F-1.

9.3.2 G-CSF mobilized peripheral blood progenitor cells may be used for patients at risk of engraftment failure who are receiving melphalan 100 mg/m^2.

9.3.3 Appropriate reduction in red cell content will be performed according to MSKCC Cytotherapy Laboratory guidelines for persons with a donor-recipient major ABO incompatibility.

9.3.4 The target cell dose will be 4 x 10^8 nucleated cells per kg recipient body weight.

9.4 Post-transplant GVHD Prophylaxis

9.4.1 Post-transplant cyclophosphamide

All recipients will receive post-transplant cyclophosphamide as follows:

- Cyclophosphamide will be administered at a dose of 50 mg/kg recipient IBW IV on days +3 and +4 according to institutional infusion guidelines.
- Mesna will be administered at 100% of the dose of cyclophosphamide intravenously in three divided doses per day on days +3 and +4.
- All recipients will peri-cyclophosphamide hydration as per adult BMT service guidelines..
- Corticosteroid administration is forbidden prior to infusion of the second dose of cyclophosphamide except for the treatment of life-threatening emergencies.

9.4.2 Tacrolimus

- Tacrolimus will be administered at a dose of 0.02 mg/kg/day continuous IV infusion beginning on day +5. Patients ≥70 years old may start at 0.015 mg/kg/day based on institutional guidelines.
- Dosing will be adjusted to obtain a serum concentration between 5-15 ng/mL
- Patients may be converted to twice daily oral therapy when oral intake resumes after transplantation.
- Tacrolimus taper will begin on day 100. The dose may be reduced by 5-10% per week to off at day +180-270. The investigator may increase or suspend the tacrolimus taper if clinical symptoms of GVHD are present.

9.4.3 Mycophenolate mofetil (Cellcept™)

- Mycophenolate mofetil will be administered at a dose of 15 mg/kg three times daily (max 1 gram per dose) starting day +5 and continuing through day +100 post-transplantation. There will be no taper. The clinician may adjust the dose of mycophenolate mofetil if clinically significant adverse side effects to this medication occur.

9.5 Supportive Therapy

9.5.1 Tumor lysis prophylaxis

Patients may receive tumor lysis syndrome prophylaxis at the discretion of the treating physician. Tumor lysis syndrome prophylaxis includes allopurinol 300 mg PO/IV on day -5 then 300 mg/day until day 0 with or without normal saline 100 mL/hour continuous IV infusion from day -5 until day -1.

9.5.2 Infection prophylaxis

- Infection prophylaxis will be administered according to institutional guidelines.
- Specific infection prophylaxis will be administered for Pneumocystis *carinii*, Herpesviridae, and fungal infections to all recipients and for Toxoplasmosis *gondii* to seropositive persons/recipients.

9.5.3 Growth factor support

Patients will receive G-CSF beginning on day +7 at a dose of 5 mcg/kg/day subcutaneously (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.

9.5.4 Sinusoidal obstructive syndrome prophylaxis

Patients will receive ursodiol 300-600 mg PO BID beginning on day -8 and continuing at least until discharge from the inpatient unit. If a patient is unable to take oral medications ursodiol may be discontinued at that time.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

10.1 Post transplantation evaluation

Post transplantation evaluations are summarized in the following table. Additional testing may be performed as is clinically indicated. Scheduled evaluations on day +30 and +100 may occur ±7 days from the schedule time point. Long term follow up at 9 months and 12 months may be done ±14 days from the scheduled time point. Follow up at 12 months, 18 months and 24 months may be done ±30 days from the scheduled time point.

	7	14	21	30	42	49	56	70	84	100	6 mo.	9 mo.	12 mo.	18 mo.	24 mo.
Window	± 3	± 3	± 3	± 7	± 3	± 3	± 3	± 7	± 7	± 7	± 14	± 14	± 30	± 30	± 30
History and Physical	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC with diff, CMP, Mg	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Disease Specific Monitoring Studies[1]										X	X	X	X	X	X
Total Bone Marrow Chimerism				X						X					
Research Blood[2]				X						X	X				
Research BMA[2]				X											
Lymphocyte Subsets[3]											X	X			
GvHD Evaluation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity	X	X	X	X	X	X	X	X	X	X					

1. May include but is not limited to the following studies: bone marrow aspirate and/or biopsy for patients with MDS, acute or chronic leukemia, and CLL. Patients with lymphoma or extramedullary leukemia will undergo CT scans.
2. Research collection for translational objectives will consist of three 10 mL heparinized green top tubes of blood on the dates described above and one bone marrow aspiration (2-3 mL, heparinized green top tube) on day +30. Bone marrow aspirate collected in lavender EDTA tubes are acceptable.
3. Flow cytometry-7 Markers Short BMT Panel (Lymphocyte Subsets) should be ordered during 6 month and 9 month windows per institutional guidelines.

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.

Toxicities and adverse events will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03. All event reporting will be done per the MSKCC Adult Bone Marrow Transplant SAE SWP.

11.1 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 • Second malignancies such as myelodysplastic syndrome or acute myelogenous leukemia • Veno-occlusive disease of the liver • Injury to the kidneys, causing kidney failure • Allergic reaction to the infusion of cyclophosphamide

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, blurred vision, loss of hearing, peripheral neuropathy) • Severe hepatic injury causing hepatic failure • Hemolytic anemia • Phlebitis • Deep venous thrombosis

11.3 Thiotepa

COMMON, SOME MAY BE SERIOUS In 100 people receiving thiotepa 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)

OCCASIONAL, SOME MAY BE SERIOUS In 100 people receiving thiotepa from 4 to 20 may have:
• Redness or irritation of the skin • Headache • Allergic reaction to infusion (fever, hives, itching) • Abnormal liver function studies (increased ALT, AST, or alkaline phosphatase)

RARE, AND SERIOUS In 100 people receiving cyclophosphamide 3 or fewer may have:
• Anaphylaxis infusion reaction (hypotension, allergic symptoms) • Interstitial pneumonitis • Severe hepatic injury or hepatic failure

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

11.7 Transplant Related Risks

11.7.1 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 narcotics. 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 FAHCT 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.

11.7.2 Bone marrow stem 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.

11.7.3 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

11.7.4 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.

11.7.5 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 if found to have an infection. Patients are watched closely for bleeding and given platelet transfusions to prevent serious bleeding, but minor bleeding may occur.

11.7.6 Risk of a secondary cancer

Secondary cancers may occur after chemotherapy. The risk of developing a secondary cancer of the skin, cervix, etc is less than 5%.

11.7.7 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.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

12.1 Disease response criteria

- Disease will be staged to standard response criteria.

12.2 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 35 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.

12.3 Donor chimerism

- The percentage of donor contribution to hematopoiesis (chimerism) will be determined using short tandem repeat (STR) based analysis of the blood at monthly intervals after transplantation until full donor chimerism is obtained for two consecutive measurements. Full donor chimerism is defined as ≥95% donor.
- Chimerism may be obtained in the blood or bone marrow at any time other than above if it is determined necessary by the treating investigator.

12.4 Graft *versus* Host Disease

- Acute and chronic GVHD are defined by standard criteria.[55, 56]

12.4 Survival

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

Treatment related mortality: Death from any cause in the absence of disease progression or relapse. Persons who die for any reason after disease progression or relapse are considered to have experienced disease related mortality.

12.5 KIR-favorable donor

- A KIR favorable donor is defined as a donor who meet any of the following criteria:
    - Donor/recipient pair with missing self as defined by expression of one or more KIR ligand (HLA-C1, HLA-C2, HLA-Bw4) in the donor that is absent in the recipient.
    - Donor KIR3DL1 low or no inhibitory interaction with recipient Bw4.
    - Donor KIR2DS1 and HLA-C1+ in the absence of highly inhibitory KIR3DL1 interactions.

13.0 CRITERIA FOR REMOVAL FROM STUDY

If at any time the patient is found to be ineligible for the protocol as designated in the section on patient/subject eligibility the patient will be removed from the study. Subjects with relapsed or progressive disease may pursue alternative transplantation outcomes and will be monitored for survival. Patients may remove themselves from the study. The PI may remove patients from the study for noncompliance. In all instances of study removal, supportive care will continue as is appropriate for the patient.

Donors may be removed from consideration to donate if, during the workup, they are found to not meet standard NMDP criteria for allogeneic hematopoietic cell donors.

14.0 BIOSTATISTICS

The primary aim of this pilot study is to estimate the proportion of patients undergoing an allo HCT transplant who have a KIR favorable donor. KIR favorable donor is defined in section 12.5.

A total of 50 patients will accrue. After all patients have undergone allo HCT, the proportion along with the corresponding 95% exact confidence interval will be estimated. The table below provides the total width of the exact 95% confidence interval for different potential observed proportions.

	Observed Proportion with KIR Favorable Donor				
	0.125	0.25	0.5	0.75	0.875
Width of C.I.	0.25	0.32	0.36	0.32	0.25

In order to reduce patient risk, the study design includes early termination in event that patients have high rates of treatment-related mortality. The boundaries are based on the accrual of 50 evaluable patients. The stopping 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**
Treatment-Related Mortality (day+100)	3 in the first 8 patients 4 in the first 14 patients 5 in the first 21 patients	0.10	0.10
	6 in the first 27 patients 7 at any point	0.30	0.93

There are a number of secondary objectives included in this study. All endpoints will be descriptive and interpreted cautiously due to the small sample size. These include:

- To explore the incidence of relapse, non-relapse mortality, and overall survival for patients who were and were not transplanted with a KIR favorable donor. Relapse and non-relapse mortality will be estimated using cumulative incidence functions treating death in the absence of relapse and relapse as competing risks, respectively. Overall survival will be estimated using Kaplan-Meier methods.
- To determine the incidence acute and chronic graft *versus* host disease. Cumulative incidence functions will be used for the analysis, treating relapse and death without GVHD as competing events. This endpoint will be evaluated for all patients combined.
- To determine the cumulative incidence of engraftment failure at 100 days post transplantation. Disease progression and death without engraftment failure are considered competing events for this analysis. This endpoint will be evaluated for all patients combined.

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 that the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

15.2 Randomization

There is no randomization in this study.

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%20Data%20and%20Safety%*

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

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

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

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. O'Donnell, P.V., et al., *Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide.* Biol Blood Marrow Transplant, 2002. **8**(7): p. 377-86.
2. Luznik, L., et al., *HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide.* Biol Blood Marrow Transplant, 2008. **14**(6): p. 641-50.
3. Burroughs, L.M., et al., *Comparison of outcomes of HLA-matched related, unrelated, or HLA-haploidentical related hematopoietic cell transplantation following nonmyeloablative conditioning for relapsed or refractory Hodgkin lymphoma.* Biol Blood Marrow Transplant, 2008. **14**(11): p. 1279-87.

4. Ciurea, S.O., et al., *Reduced-intensity conditioning using fludarabine, melphalan and thiotepa for adult patients undergoing haploidentical SCT.* Bone Marrow Transplant, 2010. **45**(3): p. 429-36.
5. Barker, J.N., et al., *Searching for unrelated donor hematopoietic stem cells: availability and speed of umbilical cord blood versus bone marrow.* Biol Blood Marrow Transplant, 2002. **8**(5): p. 257-60.
6. Tomonari, A., et al., *Using related donors other than genotypically HLA-matched siblings in allogeneic hematopoietic stem cell transplantation for hematologic disease: a single institution experience in Japan.* Int J Hematol, 2002. **76**(4): p. 354-9.
7. Luznik, L., et al., *Posttransplantation cyclophosphamide facilitates engraftment of major histocompatibility complex-identical allogeneic marrow in mice conditioned with low-dose total body irradiation.* Biol Blood Marrow Transplant, 2002. **8**(3): p. 131-8.
8. Luznik, L., et al., *Durable engraftment of major histocompatibility complex-incompatible cells after nonmyeloablative conditioning with fludarabine, low-dose total body irradiation, and posttransplantation cyclophosphamide.* Blood, 2001. **98**(12): p. 3456-64.
9. Jones, R.J., et al., *Assessment of aldehyde dehydrogenase in viable cells.* Blood, 1995. **85**(10): p. 2742-6.
10. Raiola, A.M., et al., *Unmanipulated haploidentical bone marrow transplantation and posttransplantation cyclophosphamide for hematologic malignancies after myeloablative conditioning.* Biol Blood Marrow Transplant, 2013. **19**(1): p. 117-22.
11. Ciurea, S.O., et al., *Improved Early Outcomes Using a T Cell Replete Graft Compared with T Cell Depleted Haploidentical Hematopoietic Stem Cell Transplantation.* Biology of Blood and Marrow Transplantation, 2012. **18**(12): p. 1835-1844.
12. Ciurea, S.O., et al., *High Risk of Graft Failure in Patients with Anti-HLA Antibodies Undergoing Haploidentical Stem Cell Transplantation.* Transplantation, 2009. **88**(8): p. 1019-1024.
13. 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.
14. Schmid, C., et al., *Donor Lymphocyte Infusion in the Treatment of First Hematological Relapse After Allogeneic Stem-Cell Transplantation in Adults With Acute Myeloid Leukemia: A Retrospective Risk Factors Analysis and Comparison With Other Strategies by the EBMT Acute Leukemia Working Party.* Journal of Clinical Oncology, 2007. **25**(31): p. 4938-4945.
15. Campbell, K.S. and J. Hasegawa, *Natural killer cell biology: an update and future directions.* J Allergy Clin Immunol, 2013. **132**(3): p. 536-44.
16. Velardi, A., et al., *Clinical impact of natural killer cell reconstitution after allogeneic hematopoietic transplantation.* Semin Immunopathol, 2008. **30**(4): p. 489-503.
17. Shilling, H.G., et al., *Reconstitution of NK cell receptor repertoire following HLA-matched hematopoietic cell transplantation.* Blood, 2003. **101**(9): p. 3730-40.
18. Petersen, S.L., et al., *A comparison of T-, B- and NK-cell reconstitution following conventional or nonmyeloablative conditioning and transplantation with bone marrow or peripheral blood stem cells from human leucocyte antigen identical sibling donors.* Bone Marrow Transplant, 2003. **32**(1): p. 65-72.
19. Yokoyama, W.M., M. Altfeld, and K.C. Hsu, *Natural killer cells: tolerance to self and innate immunity to viral infection and malignancy.* Biol Blood Marrow Transplant, 2010. **16**(1 Suppl): p. S97-s105.
20. Curti, A., et al., *Successful transfer of alloreactive haploidentical KIR ligand-mismatched natural killer cells after infusion in elderly high risk acute myeloid leukemia patients.* Blood, 2011. **118**(12): p. 3273-9.

21. Bachanova, V., et al., *Allogeneic natural killer cells for refractory lymphoma.* Cancer Immunol Immunother, 2010. **59**(11): p. 1739-44.
22. Geller, M.A., et al., *A phase II study of allogeneic natural killer cell therapy to treat patients with recurrent ovarian and breast cancer.* Cytotherapy, 2011. **13**(1): p. 98-107.
23. Kumar, V., et al., *Role of murine NK cells and their receptors in hybrid resistance.* Curr Opin Immunol, 1997. **9**(1): p. 52-6.
24. Storkus, W.J., et al., *Reversal of natural killing susceptibility in target cells expressing transfected class I HLA genes.* Proc Natl Acad Sci U S A, 1989. **86**(7): p. 2361-4.
25. Lanier, L., *NK cell recognition.* Annu Rev Immunol, 2005. **23**: p. 225-274.
26. Anfossi, N., et al., *Human NK cell education by inhibitory receptors for MHC class I.* Immunity, 2006. **25**(2): p. 331-42.
27. Yu, J., et al., *Hierarchy of the human natural killer cell response is determined by class and quantity of inhibitory receptors for self-HLA-B and HLA-C ligands.* J Immunol, 2007. **179**(9): p. 5977-89.
28. Tarek, N., et al., *Unlicensed natural killer cells target neuroblastoma following anti-GD2 antibody treatment.* J Clin Invest, 2012. **122**: p. 3260-3270.
29. Jonsson, A.H. and W.M. Yokoyama, *Natural Killer Cell Tolerance: Licensing and Other Mechanisms*, in *Advances in Immunology*, W.A. Frederick, Editor. 2009, Academic Press. p. 27-79.
30. Yokoyama, W. and S. Kim, *Licensing of natural killer cells by self-major histocompatibility complex class I.* Immunol Rev, 2006. **214**: p. 143-154.
31. Hsu, K., et al., *Improved outcome in allogeneic hematopoietic stem cell transplantation in acute myelogenous leukemia (AML) predicted by donor KIR genotype and recipient HLA genotype in T-cell depleted HLA-identical sibling transplants.* Blood, 2005. **105**: p. 4878-4884.
32. Ruggeri, L., et al., *Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation.* Blood, 1999. **94**(1): p. 333-9.
33. Ruggeri, L., et al., *Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value.* Blood, 2007. **110**(1): p. 433-40.
34. Hsu, K.C., et al., *KIR ligands and prediction of relapse after unrelated donor hematopoietic cell transplantation for hematologic malignancy.* Biol Blood Marrow Transplant, 2006. **12**(8): p. 828-36.
35. Miller, J.S., et al., *Missing KIR ligands are associated with less relapse and increased graft-versus-host disease (GVHD) following unrelated donor allogeneic HCT.* Blood, 2007. **109**(11): p. 5058-61.
36. Cooley, S., et al., *Donors with group B haplotypes improve relapse-free survival after unrelated hemaotpoietic transplantation for acute myeloid leukemia.* Blood, 2009. **113**: p. 726-732.
37. Cooley, S., et al., *Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia.* Blood, 2010. **116**: p. 2411-2419.
38. Chewning, J., et al., *KIR2DS1-positive NK cells mediate alloresponse against the C2 HLA-KIR ligand group in vitro.* J Immunol, 2007. **179**: p. 854-868.
39. Venstrom, J., et al., *HLA-C-dependent prevention of leukemia relapse by donor activating KIR2DS1.* New England Journal of Medicine, 2012. **367**: p. 805-816.
40. Cooley, S., et al., *Donor killer cell Ig-like receptor B haplotypes, recipient HLA-C1, and HLA-C mismatch enhance the clinical benefit of unrelated tansplantation for acute myelogenous leukemia.* J Immunol, 2014. **192**: p. 4592-4600.
41. Oevermann, L., et al., *KIR B haplotype donors confer a reduced risk for relapse after haploidentical transplantation in children with ALL.* Blood, 2014. **124**(17): p. 2744-2747.

42. Mancusi, A., et al., *Haploidentical hematopoietic transplantation from KIR ligand–mismatched donors with activating KIRs reduces nonrelapse mortality.* Blood, 2015. **125**(20): p. 3173-3182.
43. Yawata, M., et al., *Roles for HLA and KIR polymorphisms in natural killer cell repertoire selection and modulation of effector function.* J Exp Med, 2006. **203**: p. 633-645.
44. O'Connor, G., et al., *Functional polymorphism of the KIR3DL1/S1 receptor on human NK cells.* J Immunol, 2007. **178**: p. 235-241.
45. Carr, W., M. Pando, and P. Parham, *KIR3DL1 polymorphisms that affect NK cell inhibition by HLA-Bw4 ligand.* J Immunol, 2005. **175**: p. 5222-5229.
46. Yawata, M., et al., *MHC class I-specific inhibitory receptors and their ligands structure diverse human NK cell repertoires towards a balance of missing-self response.* Blood, 2008. **112**: p. 2369-2380.
47. Gardiner, C., et al., *Different NK cell surface phenotypes defined by the DX9 antibody are due to KIR3DL1 gene polymorphism.* J Immunol, 2001. **166**: p. 2992-3001.
48. Boudreau, J., J.L. Luduec, and K. Hsu, *KIR3DL1 and HLA-Bw4 allotypes predict the extent of NK cell licensing.* Blood 2013. **122 (Abstract)**: p. 1043.
49. Martin, M.P., et al., *Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1.* Nat Genet, 2007. **39**(6): p. 733-40.
50. Giglio, F., et al., *Donor KIR3DL1 and HLA-B allotypes control leukemia relapse after allogeneic hematopoietic stem cell transplantation.* Blood (Abstract), 2012.
51. Vierra-Green, C., et al., *Allele-level haplotype frequencies and pairwise linkage disequilibrium for 14 KIR loci in 506 European-American individuals.* PLoS ONE, 2012. **7**: p. e47491.
52. Yu, J., et al., *Breaking tolerance to self, circulating natural killer cells expressing inhibitory KIR for non-self HLA exhibit effector function after T cell-depleted allogeneic hematopoietic cell transplantation.* Blood, 2009. **113**(16): p. 3875-84.
53. Boudreau, J., J. LeLuduec, and K. Hsu, *Development of a novel multiplex PCR assay to detect functional subtypes of KIR3DL1 alleles.* PLoS ONE, 2014. **In press**.
54. 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.
55. 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.
56. 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: KIR Donor Selection Assessment