Official Title:	Haploidentical Allogeneic Peripheral Blood Transplantation: Clinical Trial and Laboratory Correlates Examining Checkpoint Immune Regulators' Expression
NCT number:	NCT03480360
Document Type:	Study Protocol
Date of the Document:	March 4, 2019

Haploidentical Allogeneic Peripheral Blood Transplantation: Clinical Trial and Laboratory Correlates Examining Checkpoint Immune Regulators' Expression

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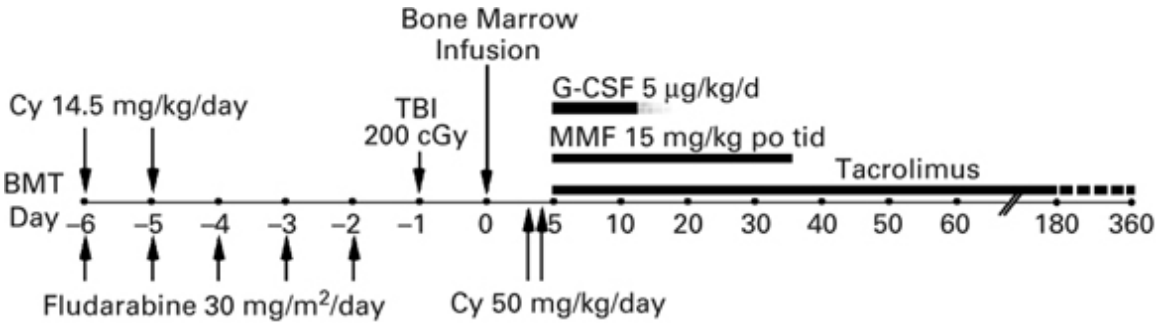
Rev#2: March 4, 2019

Treatment Schema



Adapted from BMT 2008, 42:523

Cyclophosphamide (Cy): 14.5 mg/kg for 2 days (days -6, -5) and then 50 mg/kg for two days (days 3, 4)
Fludarabine: 30 mg/m^2 daily for 5 days
Total Body Irradiation (TBI): 200 cGy for one day (day -1)
Immune suppression: will begin on day 5, 24 hours after the completion of the last dose of cyclophosphamide
Tacrolimus: 1 mg IV daily, (or the oral equivalent) adjusted to achieve a level between 5 and 15 ng/ml. If there is no evidence of GVHD, discontinue Tacrolimus by Day 180.
MMF (Cellcept): dose at 15 mg/kg po TID (maximum dose of 3 grams/day). Stop Cellcept at Day 35 following transplantation.
G-CSF (or biosimilar): 5 mcg/kg/d starting day 5 and continue until ANC > 1000/mcL for 3 days.

Day 0 = day of transplant; cell dose goal: ≤ 5 x 10^6 CD34+ cells/kg recipient weight

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SECTION 1.0 INTRODUCTION

Background

The types of allogenic hematopoietic stem cell transplantations have evolved over the past 30 years. With the improved understanding of the mechanisms of graft versus host disease (GVHD), what initially began using only related donors, has expanded to unrelated donors. Over the ensuing years, if neither a related nor unrelated donor was available, then further options developed to include cord blood transplantations or mismatched donor transplantations. With the limited supply of cord blood available and the inferior outcomes of mismatched donors, the use of a haplotype-matched donor, also called haploidentical transplant, was examined. Since patients share a haplotype with one of their parents or one of their children, donors can be identified for most, if not all patients. A major advantage of using a haplotype-identical donor is that almost all patients will have a readily available donor.

How do the outcomes of haploidentical transplants compare with other types of transplants?

Several large clinical trials of haploidentical trials demonstrate that haploidentical transplant for patients with hematologic malignancies result in similar outcomes, when compared with age-matched controls for HLA-matched related and unrelated donors (**1,2**) (**Table 1**). A large multicenter study confirmed these findings in AML patients and showed that the 3-year overall survival for haploidentical recipients was 45%, compared with 50% for matched unrelated donors and 44% for reduced intensity recipients using unrelated donors (**3**).

Table 1

Bashey et al	***Haplo (n=53)***	***MRD (n=117)***	***MUD (n=101)***
TRM at day 100	20%	19%	30%
OS at 2 years	64%	76%	67%
DFS at 2 years	60%	53%	53%
Acute GVHD (grade 3-4)	11%	8%	11%
Chronic GVHD (grade 3-4)	38%	54%	54%

TRM = treatment related mortality; MRD=matched related donor; MUD=matched unrelated donor

Johns Hopkins University has pioneered the efforts into haploidentical transplants and they have the largest experience to date. Their regimen, the regimen we propose to use, uses pre-transplant cyclophosphamide with fludarabine and one day of low dose total body irradiation. Post-transplant immune suppression is accomplished using high dose cyclophosphamide, tacrolimus and mycophenolate (Cellcept). Donor bone marrow is used as the source of stem cells. The Hopkins group identified a very low non-relapsed mortality, measuring 4% at 1 year and 15% at 2 years. These low rates were due to the low incidence of GVHD and the low rate of

infections. The addition of mycophenolate (Cellcept) at an increased dose for haploidentical transplants has also contributed to the lower incidence of GVHD. The graft rejection rate was 13% and the incidence of GVHD was 34% (**4**). These incidences of graft rejection and GVHD are similar to HLA-matched sibling donor transplant rates (**5,6**). It is interesting to note that there is a low incidence of infections (< 15%) in this patient population (**4**). Due to the administration of post-transplant cyclophosphamide, engraftment of neutrophils and platelets occur a little later than in other types of allogeneic transplants, at approximately Day 15 and Day 24, respectively (**4**). As with all types of transplants, relapse was the major reason for failure in this high-risk group of patients.

In summary, a review of the literature notes overall survival rates at 1 year ranging from 31% to 78% and at 2 years, 28-64%. Treatment-related mortality at Day 100 ranges from 4 % to 20% and at 2 years, from 10 to 42% (**Table 2**).

Table 2

Reference	***Number of patients***	***TRM at day 100***	***Survival at 1 year***	***DFS at 1 year***
Ciurea Blood 2015	n = 192	12%	61%	38%
Luznik BBMT 2008	n = 67	4%	52%	50%
Bashey JCO 2013	n = 53	20%	78% (approx.)	64%
Solomon BBMT 2012	n = 20	10%	69%	50%

TRM=treatment-related mortality; DFS=disease-free survival

Controversial areas of haploidentical transplantation

It is difficult to compare clinical outcomes for recipients of haploidentical transplantations since this is a new and evolving field. Many centers are using various treatment regimens that may or may not include TBI and may include a myeloablative, rather than reduced intensity chemotherapy regimen. Other centers are using various immune suppression regimens and other centers are using marrow, peripheral blood progenitor cells or a combination of both. Some of the early trials transplanted patients after failing numerus regimens while other centers transplanted patients in first remission. This area is thoroughly reviewed in many review articles (**3, 7 - 9**). We have summarized 4 of the most prominent studies to date below (**Table 2**).

The process of haploidentical transplantation continues to evolve. To date, the major source of cells has been donor *marrow*. Recently, a large study indicated that donor *peripheral blood* progenitor cells provide an alternative source without a marked increase in the incidence of GVHD and with similar outcomes (**1**). Unlike the Hopkins' regimen, we propose to use donor –derived peripheral blood progenitor cells, instead of marrow.

The ideal chemotherapy regimen is also unknown, as is the optimal immune suppression during the transplant. In addition, the regimen that was first proposed and developed by Hopkins is developing into a standard regimen, and this will be the regimen we will use, including the same chemotherapy and immune suppression. Finally, the best characteristics of the optimal donor is still uncertain (see below for details).

The role of post-transplant cyclophosphamide

In preparation for a haploidentical transplantation, a non-myeloablative chemotherapy or chemotherapy with irradiation regimen is used. Due to the HLA disparity between the patient and the HLA-haploidentical donor, the initial major concern was the potential for a high incidence of GVHD, as well as graft rejection. It was determined that the administration of POST-transplant immune suppression using cyclophosphamide markedly reduced graft rejection and GVHD (**10**). Cyclophosphamide was selected due to its potent immune suppressive effects. To prevent GVHD, two large doses of cyclophosphamide (50 mg/kg) are administered following transplant, on Days 3 and 4. Additional immune suppression is used, including Tacrolimus and CellCept to assist in suppressing GVHD.

The timing of administering post-transplant cyclophosphamide was found to be critical to prevent GVHD. Cyclophosphamide needs to be administered soon after the transplant and BEFORE starting any other immune suppression. It is hypothesized that cyclophosphamide induces T cell tolerance, by targeting alloreactive T cells, specifically in the allogeneic T cells that have been recently infused and activated. Since calcineurin inhibitors (i.e. tacrolimus) block cyclophosphamide-induced tolerance, tacrolimus started AFTER cyclophosphamide has been administered.

Selection of the appropriate donor

Patients undergoing haploidentical HSCT will often have more than one potential donor, and consideration for the optimal donor-recipient match should be addressed along a hierarchy of factors. However, in the haplo- identical setting, some of these factors remain controversial.

These donor-recipient factors and the donor selection is crucial to the success of the transplant. As with any type of allogeneic stem cell donor selection, the donor selected affects the donor cell yield, the risk for graft rejection, the incidence of GVHD (both acute and chronic), the transplant-related mortality, the graft-versus-tumor effect, relapse and overall survival.

The hierarchy of factors for algorithmic consideration for a potential donor includes the following:

- ***donor-specific antibodies***
- ***ABO matched donor-recipient status preferred*** (based on anticipated cell loss if cell processing is needed in the major ABO-mismatched setting)
- ***donor age*** (younger age donor preferred, to optimize cell yield and immuno-genicity; donor < 30 years is the optimal, donor ages 31-45 years is intermediate and donors > 46 years are the least optimal)
- ***gender*** (male donor preferred to avoid issues of increased allo-immunization, especially with multiparous female donors, including responses directed against the H-Y antigen in male recipients)
- ***cytomegalovirus serology*** (CMV serostatus match preferred).

Controversial factors demonstrating uncertain impact at this time on transplant outcome include the number of HLA mismatches, natural killer (NK) cell alloreactivity via killer

immunoglobulin-like receptor (KIR-KIR) ligand donor-recipient mismatching, and the presence of non-inherited maternal HLA antigens (NMAs).

Immune reconstitution following an haploidentical transplant

Research examining immune reconstitution following a haploidentical transplant remains in its infancy. As with all types of allogeneic transplantations, within the first 6 months following a haploidentical transplant, CD4+T cells, CD8+ naïve T cells and memory T Cells remain low in number and demonstrate depressed cellular function. (**11**). An extensive review of this topic states that future studies are critically needed to address this area so that we will better understand the mechanisms of immune reconstitution (**12**).

Laboratory Correlates: The role of Immune Check point regulators in transplantation

An active area of research is exploring ways to maximize the beneficial graft-versus-tumor (GVT) effect while minimizing GVHD. Murine models, and limited clinical case series in acute GVHD, demonstrate that immune checkpoint regulators, such as CTLA4 (cytotoxic T-lymphocyte-associated protein 4) and PD-L1 (programmed death-ligand 1) determine donor T-cell responses against minor HLA-antigens, on both normal host tissues in GVHD and tumor cells, in GVT **(13-15).** The expression of CTLA4 and PD1 ligand (PD-L1) on donor T cells protect against GVHD. For example, treatment of mice with a CTLA4 fusion protein (CTLA4-Ig) prevents acute GVHD without inhibiting the GVT **(16-17).** Both the prevalence of these Immune Checkpoint Regulators and the cell types that express them following allogeneic stem cell transplant are currently unknown.

Dr. Randy Noelle's lab has recently identified a new immune checkpoint regulator called VISTA (V-domain Ig Suppressor of T-cell Activation). VISTA plays a role in tumor immune surveillance and peripheral tolerance (**18-20**). VISTA's expression is restricted to hematopoietic cells. We recently showed that VISTA is expressed on myeloid-derived suppressor cells (MDSCs) and its expression enhances the function of MDSCs in several tumor models (**21**). Based on these findings, we postulate that through its expression on MDSCs, VISTA modulates GVHD and GVT effects.

In addition to immune checkpoint regulators, MDSC are known to suppress alloreactive T cells following allogeneic stem cell transplantation. In mouse models, the administration of donor-derived MDSCs at the time of transplantation prevented acute GVHD (**22,23).** This effect did not negate the beneficial GVT effect. The role of MDSCs in the setting of chronic GVHD is suggested by a study demonstrating that patients' GVHD improved as the number of MDSCs increased in response to extracorporeal photopheresis **(24).** These results suggest that MDSCs are a potential therapeutic target in GVHD.

To date, there are no studies evaluating the incidence, prevalence or function of immune checkpoint regulators following haploidentical stem cell transplantation or in the setting of GVHD. We hypothesize that VISTA may play a role in suppressing GVHD in through its expression on MDSCs. It is unknown which cell subsets express these immune checkpoint regulators and the prevalence of these regulators following allogeneic stem cell transplantation.

Summary

We plan to use the standard Johns Hopkins' regimen, with the use of donor peripheral blood stem cells, rather than marrow. We will define clinical outcomes while focusing our efforts on immune reconstitution focusing on immune checkpoint regulators after a related haploidentical stem cell transplant.

2.0 OBJECTIVES

We propose a clinical trial to define clinical endpoints, including engraftment, 100-day survival and one year survival (**Objective #1**). We will characterize the incidence, prevalence and function of immune checkpoint regulators in patients' blood and bone marrow following transplantation (**Objective #2**). We will correlate these laboratory results with clinical outcomes and the incidence of GVHD. As an exploratory aim, in those patients experiencing GVHD and requiring treatment, we will define the frequency/expression of checkpoint regulator expression and correlate these results with the patient's response to GVHD therapy.

2.1 Objective #1: The primary objective of this trial is to define the 100-day survival of patients being treated on this regimen.

Secondary objectives include: To define clinical endpoints using this myeloablative haploidentical transplant regimen, **including**:

- **2.1.1** Time to marrow engraftment (defined as absolute neutrophil count > 500/mm^3 and platelets > 20,000/mcl for three consecutive days (count first day as engraftment)
- **2.1.2** Response to treatment at 100 days
- **2.1.3** Response to treatment at one year
- **2.1.4** One year survival
- **2.1.5** Treatment-related mortality in the first 100 days
- **2.1.6** Toxicities associated with this treatment regimen
- **2.1.7** Incidence of acute and chronic GVHD
- **2.1.8** Donor-recipient chimerism following transplant at Days 30, 60 and 90.

2.2 Objective #2: To characterize the incidence, prevalence and function of immune checkpoint regulators (VISTA, CTLA-4, PD-1) during early immune recovery following an allogeneic stem cell transplant.

Since the presence of MDSCs may correlate with engraftment, the response to treatment, and the risk of developing GVHD, we will define MDSCs frequency and immune checkpoint regulators expression on MDSCs and on peripheral blood and bone marrow in allogeneic transplant recipients following transplantation.

We will correlate these laboratory results with clinical outcomes (described above) and the incidence of GVHD.

2.3 Exploratory aim # 2a) In those patients experiencing GVHD, we will define the MDSCs frequency and checkpoint regulator expression on MDSCs, peripheral blood

mononuclear cells and myeloid subsets. Blood samples will be drawn at the time of diagnosis and weekly for four weeks to evaluate changes in response to treatment.

3.0 ELIGIBILITY CRITERIA

3.1 Age: ≤ 75 years

3.2 The patient must be approved for transplant by the treating transplant physician. This includes completion of their pre-transplant workup, as directed by standard DHMC SOPs **(DHMC SOP – Pre-transplant Evaluation of allogeneic recipient (Appendix).**

3.3 The patient must have a disease (listed below) with treatment-responsiveness that the treating transplant physician believes will benefit from an allogeneic stem cell transplant. The diseases include:

- 3.3.1 Acute leukemia – AML, ALL
- 3.3.2 Chronic leukemia – CML, CLL
- 3.3.3 Myelodysplasia
- 3.3.4 Myeloproliferative disorder
- 3.3.5 Myelofibrosis
- 3.3.6 Lymphoma – NHL or Hodgkin's disease
- 3.3.7 Plasma cell disorder, including myeloma, Waldenstrom's Macroglobulinemia

3.4 Donor availability- the patient must have an identified **RELATED** haplo-identical donor

3.5 No HIV infection or <u>active</u> hepatitis B or C

3.6 ECOG performance status: 0-2

3.7 DLCO ≥40% predicted

3.8 Left ventricular ejection fraction ≥ 40%

3.9 Serum bilirubin < 2x upper limit of normal; transaminases < 3x normal at the time of transplant

3.10 No active or uncontrollable infection

3.11 In female, a negative pregnancy test if experiencing menstrual periods

3.12 No major organ dysfunction precluding transplantation

3.13 No evidence of an active malignancy that would limit the patient's survival to less than 2 years. (If there is any question, the PI can make a decision).

3.2 EXCLUSION CRITERIA

3.2.1 Psychiatric disorder or a mental deficiency of the patient that is sufficiently severe to make compliance with the treatment unlikely, and making informed consent impossible.

3.2.2 Major anticipated illness or organ failure incompatible with survival from BMT.

3.2.3 History of refractory systemic infection

3.3 Donor eligibility

3.3.1 HLA haplo-identical matched related.

3.3.2 The donor must be healthy and must be willing to serve as a donor, based on standard NMDP guidelines and **DHMC SOP – Donor Evaluation (Appendix)**

3.3.3 The donor must have no significant co-morbidities that would put the donor at marked increased risk
3.3.4 There is no age restriction for the donor
3.3.5 Informed consent must be signed by donor

3.4 Donor Exclusion Criteria

3.4.1 The NMDP guidelines for exclusion criteria will be used (**Appendix**). In addition, the following donors are NOT eligible:
3.4.2 Pregnant or lactating donor
3.4.3 HIV or active Hep B or C in the donor
3.4.4 Donor unfit to receive G-CSF and undergo apheresis
3.4.5 A donor with a psychiatric disorder or mental deficiency that makes compliance with the procedure unlikely and informed consent impossible

4.0 REGISTRATION AND DATA SUBMISSION

This is a non- randomized trial with laboratory correlates. To enter eligible patients or to discuss a patient's eligibility, please contact Dr. Kenneth R. Meehan (or any of the Transplant physicians) at 603-650-4628 or the Clinical Research Associate (TBD at 603-650-4035). Informed consent must be signed prior to the initiation of treatment.

5.0 GENERAL WORKUP AND DIAGNOSTIC PROCEDURES

The Pre-study evaluation will follow the **DHMC SOP - Pre-transplant evaluation of allogeneic recipient (Appendix).**

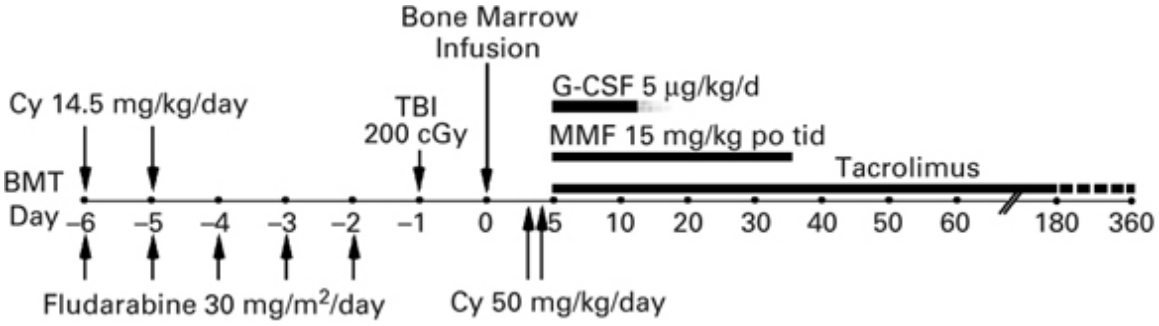
5.1 It is recommended that the pre-transplant evaluation be done within a reasonable amount of time prior to transplant, this time frame will vary, but it is recommended to be within 8 weeks of admission for transplantation. Exceptions are noted below.

5.1.1 HLA typing – HLA typing can be performed at any time prior to transplant
5.1.2 At some point during patient's treatment, a biopsy of the involved site or bone marrow biopsy is needed to document the malignant diagnosis. This could also include cytogenetics, molecular analysis and/or flow cytometry.
5.1.3 Disease remission status at the time of transplant (varies by disease – CIBMTR definitions will be used)
5.1.4 Serologic testing: CMV, EBV, HSV, VZV
5.1.5 Bone marrow aspirate and biopsy (within 8 weeks)
5.1.6 Antibody screen for HBV, HCV, HIV, HTLV I/II, CMV, EBV, toxoplasmosis, syphilis
5.1.7 Bloodwork: CBC, Comprehensive metabolic panel, TSH
5.1.8 Serum pregnancy test, if female with child-bearing potential
5.1.9 PSA is recommended in males $\geq$ 50 years of age (done within one year)
5.1.10 Pulmonary function tests with DLCO
5.1.11 Cardiac function tests to include an EKG and ECHO
5.1.12 Chest x-ray
5.1.13 Urinalysis
5.1.14 Dental Exam (as indicated - done within one year)
5.1.15 GYN exam w/ PAP smear (as indicated- done within one year)

5.1.16 Mammogram (as indicated- done within one year)

5.1.17 Informed written consent must be obtained. Patients must be able to give informed consent as a prerequisite to this procedure. The Informed Consent form will become part of the permanent record and a copy will be given to the patient.

6.0 TREATMENT PLAN



Adapted from BMT 2008, 42:523

Cyclophosphamide (Cy): 14.5 mg/kg for 2 days (days -6, -5) and then 50 mg/kg for two days (days 3, 4)
Fludarabine: 30 mg/m^2 daily for 5 days
Total Body Irradiation (TBI): 200 cGy for one day (day -1)
Immune suppression: will begin on day 5, 24 hours after the completion of the last dose of cyclophosphamide
Tacrolimus: 1 mg IV daily, (or the oral equivalent) adjusted to achieve a level between 5 and 15 ng/ml. If there is no evidence of GVHD, discontinue Tacrolimus by Day 180.
MMF (Cellcept): dose at 15 mg/kg po TID (maximum dose of 3 grams/day). Stop Cellcept at Day 35 following transplantation.
G-CSF (or biosimilar): 5 mcg/kg/d starting day 5 and continue until ANC > 1000/mcL for 3 days.

Day 0 = day of transplant; cell dose goal: ≤ 5 x 10^6 CD34+ cells/kg recipient weight

6.1 Details of the treatment regimen:

6.1.2 For each medication, actual body weight will be used.

6.2 Cyclophosphamide (Cy): 14.5 mg/kg IV for 2 days (days -6, -5); Following transplant, cyclophosphamide will be administer at 50 mg/kg IV over 90 mins for two days (days 3, 4). Cyclophosphamide will be administered with Mesna (80% dose of cyclophosphamide in 4 divided doses over 8 hours).

6.3 Fludarabine: 30 mg/m^2 IV daily for 5 days, on days -6 to day -2.

6.4 Total Body Irradiation (TBI): 200 cGy to be administered for one day (day -1).

6.5 Immune suppression: Will start on day 5, 24 hours after the completion of the last dose of cyclophosphamide.

6.6 Tacrolimus: initiated at a dose of 1 mg IV daily, adjusted to achieve a level between 5 and 15 ng/ml. This can be started as an oral medication at the appropriate dose or converted to oral form once the patient is clinically stable. If there was no evidence of GVHD, discontinue Tacrolimus by Day 180.

6.7 MMF (Cellcept): dose Cellcept at 15 mg/kg po TID (maximum dose of 3 grams/day). Discontinue Cellcept on day 35 following transplantation (without a taper).

6.8 Mesna

6.8.1 **Pre-transplant:** On Days – 6 and – 5, Mesna (3 mg/kg) will be administered intravenously over 15 minutes prior to each dose of cyclophosphamide and repeated at 4 hours and 8 hours after the start of each cyclophosphamide dose.

6.8.2 **Post-transplant:** On Day + 3, Mesna (10 mg/kg) will be administered intravenously prior to the first dose of cyclophosphamide. Mesna (30 mg/kg every 12 hours) will then be administered as a continuous infusion every 12 hours x 4 doses (total of 48 hour infusion).

6.5.1 Further modifications of tacrolimus doses will be based on tacrolimus trough concentrations (target trough of 5-10 ng/mL; not to exceed 15 ng/mL).

6.5.2 Tacrolimus taper should begin approximately between Day 90 and 120 with a goal of stopping by Day 180.

6.10 Supportive Care Recommendations

6.10.1 **Infectious prophylaxis** will include Fluconazole, Acyclovir and Levaquin (Recommendations from DHMC SOP addressing antimicrobial prophylaxis, and adapted from *BBMT* 2009;15:1143-1238). The following are recommended:

Fluconazole: 400 mg PO daily beginning on day 0.

Levofloxacin: 750 mg PO daily from day 0 until ANC is greater than 500/mm^3 post-engraftment or broad spectrum antibiotics are started.

Acyclovir: 800 mg PO twice daily beginning on admission.

Sulfamethoxazole 800 mg/trimethoprim 160 mg (Bactrim DS) one tablet PO once daily from admission until and including day - 2. Discontinue after day - 2 dose given. As an alternative, Pentamidine (300 mg inhalation) can be used once on admission.

At day 30, PJP prophylaxis will resume using sulfamethoxazole 800mg / trimethoprim 160mg using one of the various recommended regimens. IF ANC is less than 500 and/or platelets are less than 20,000 without transfusion on day +30 do NOT restart sulfamethoxazole/trimethoprim, instead consider atovaquone, dapsone or pentamidine.

6.11 **CMV monitoring**- using standard guidelines and the DHMC SOP addressing monitoring of CMV, it is recommended that CMV PCR be tested once every 1-2 weeks,

starting approximately day 28 until day 100. CMV testing could continue after this time point if the patient remains on immunosuppression. Treatment for a positive result will be done according to standard guidelines as outlined in the DHMC SOP.

6.12 **EBV monitoring**- using standard guidelines and the DHMC SOP addressing monitoring of EBV, it is recommended that EBV PCR be tested approximately every 2-3 weeks after transplant starting approximately day 30 until day 100. EBV testing could be checked periodically after this time point if the patient remains on immunosuppression. Treatment for a positive result will be done according to standard guidelines as outlined in the DHMC SOP.

6.13 Veno-Occlusive Disease (VOD) Prophylaxis: Day -7 until discharge or through Day +30:

For those patients believed to be at increased risk for the development of VOD, one of the following regimens may be used until the time of discharge or up until day +30, based on the treating physician's level of concern:

6.13.1 Regimen #1: *Ongoing* Enoxaparin with platelet transfusion

Enoxaparin: 40 mg subcutaneous daily. ***Maintain platelet count ≥ 10 x10^3/mcL.***
Ursodiol: 300 mg PO twice daily for body weight ≤ 90 kg
OR
Ursodiol : 300 mg PO every AM and 600 mg PO every PM for body weight > 90 kg

6.13.2 Regimen #2 *Discontinue* Enoxaparin once platelets decrease

Enoxaparin: 40 mg subcutaneous daily. ***Discontinue once platelet count decreases to 20 x10^3/mcL.***
Ursodiol: 300 mg PO twice daily for body weight ≤ 90 kg
OR
Ursodiol : 300 mg PO every AM and 600 mg PO every PM for body weight > 90 kg

6.15 Filgrastim or the generic equivalent (G-CSF): G-CSF will be administered once daily beginning on day +7 and continue until ANC > 1000/mcL for 3 days. NMDP guidelines will define the final dose, as listed below (based on patient's actual weight):
6.15.1 G-CSF Doses:

Patient's weight (kilograms)	Daily G-CSF dose (mcg/day)
< 60	300
61 - 99	480
100 - 130	600
>130	780

6.16 Collection of Allogeneic Stem Cells from Donors

6.16.1 Haplo-identical donors

Donors will be relatives (parent, child, sibling etc.). Donors will be mobilized following NMDP and institutional guidelines **(DHMC SOP Mobilization of allogeneic donor – Appendix).** G-CSF (or the generic equivalent) will be administered daily as a

subcutaneous injection. The final dose used will be based on NMDP guidelines, as noted below (using the donor's actual weight). The collected cells may be stored frozen or collected fresh and infused.

Donor weight (kilograms)	Daily G-CSF dose (mcg/day)
45 - 60	600
61 - 78	780
79 - 90	900
91 - 96	960
97 - 108	1080
>109	1200

6.16.2 Target CD34+ cell doses will be based on DHMC guidelines. A minimum of 2 x 10^6 CD34+ cells/kg of recipient weight will be infused.

6.17 Allogenic stem cells – Infusion into recipients

On day 0 of treatment, a minimum of 2 x 10^6 CD34+ cells/kg of recipient weight will be infused. Standard infusion procedure will be followed using DHMC SOP.

Based on the above considerations, the hierarchy of factors for algorithmic consideration for a potential donor includes the following:

- ***donor-specific antibodies***
- ***ABO matched donor-recipient status preferred*** (based on anticipated cell loss if cell processing is needed in the major ABO-mismatched setting)
- ***donor age*** (younger age donor preferred, to optimize cell yield and immuno-genicity; donor < 30 years is the optimal, donor ages 31-45 years is intermediate and donors > 46 years are the least optimal)
- ***gender*** (male donor preferred to avoid issues of increased allo-immunization, especially with multiparous female donors, including responses directed against the H-Y antigen in male recipients)
- ***cytomegalovirus serology*** (CMV serostatus match preferred).

7.0 POTENTIAL TOXICITIES AND DOSE MODIFICATIONS

7.1 Evaluation of toxicities (other than Hematologic) will be graded using NCI Common Toxicity Criteria, specifically CTC AE 4.0. Life-threatening toxicities must be reported to the principal investigator, the clinical research assistant and the IRB. The following laboratory test abnormalities and adverse events should be captured on the non-serious AE CRF page or SAE Report Form (paper or electronic) as appropriate:

- Any laboratory test result/adverse reaction that is ≥ Grade 4 or meets the definition of an SAE
- Any laboratory test result/adverse reaction abnormality that required the subject to have study drug discontinued or interrupted

- Any laboratory test result/adverse reaction abnormality that required the subject to receive specific corrective therapy.
- Any laboratory test result/adverse reaction deemed related to the treatment that required medical intervention, either by requiring an office/infusion room visit, treatment (ranging from medication administration, hydration or transfusion).

7.2 Monitoring of Toxicities after discharge from the hospital

Monitoring of all acute toxicities will occur until the time of discharge. After discharge (approximately day 17-20 following transplant), transplant patients often experience a number of medical issues. As a result, after discharge until day 100, only serious toxicities (> grade 4) will be recorded. Of course, should any life threatening event possibly be linked to the study at any time, the investigator shall use his/her discretion is reporting the event to the appropriate committees.

7.3 Fludarabine

Consider 20% dose reduction of fludarabine for patients with a creatinine clearance < 70 ml/min.

7.4 Tacrolimus

The dose of tacrolimus will be adjusted to achieve levels between 5 – 10 ng/ml.

7.6 GVHD

The diagnosis of GVHD is based on clinical signs (known as "clinical GVHD") and/or a tissue biopsy ("pathologic GVHD"). The clinical signs and symptoms include rash, diarrhea, increased liver function tests (LFTs), or nausea/vomiting. If a tissue biopsy is obtained (skin, GI tract, Liver), this provides additional histopathologic evidence for the diagnosis of GVHD.

The treatment of GVHD varies greatly, and it is recommended to follow the DHMC SOP for treatment of GVHD (**DHMC SOP – Grading and Treatment of GVHD – Appendix**). For reporting purposes, the following tables should be used.

TABLE 2 Clinical grading of *acute* GVHD

Extent of organ involvement			
Stage	**Skin**	**Liver (bilirubin)**	**Gut (stool output per day)**
0	No GVHD rash	<2 mg/dL	<50 mL/day or persistent nausea (child: <10 mL/kg/day)
1	Maculopapular rash <25% BSA	2-3 mg/dL	500-999 mL/day (child: 10-19.9 mL/kg/day) or persistent nausea, vomiting or anorexia, with a positive upper GI biopsy
2	Maculopapular rash 25-50% BSA	3.1-6 mg/dL	1000-1500 mL/day (child: 20-30 mL/kg/day)
3	Maculopapular rash >50% BSA	6.1-15 mg/dL	Adult: >1500 mL/day (child: >30 mL/kg/day)
4	Generalised erythema plus bullous formation	>15 mg/dL	Severe abdominal pain with or without ileus
Grade	**Skin**	**Liver (bilirubin)**	**Gut (stool output per day)**
I	Stages 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2-3 or	Stages 2-4
IV	Stage 4 or	Stage 4	-

Abbreviations: BSA = body surface area; GI = gastrointestinal; GVHD = graft-versus-host disease.

TABLE 3 Overall grading of *acute* GVHD

Typically, acute GVHD occurs until day 100. GVHD occurring after day 100 is considered chronic GVHD.

Clinical grade of *acute* GVHD			Skin	GI Tract	Liver
I			1 – 2	None	None
II			3	1	1
III			NA	2 - 4	2 – 3
IV			4	NA	4

Abbreviations: NA = not applicable

7.9 Clinical grading of ***chronic*** GVHD

7.9.1 GVHD occurring after Day 100 is considered chronic GVHD. Chronic GVHD will be defined as "limited" or "extensive".

7.9.2 Limited Chronic GVHD

1. localized skin

AND/OR

2. Liver dysfunction due to chronic GVHD

7.9.3 Extensive Chronic GVHD

1. generalized skin involvement

OR

2.Localized skin and/or liver dysfunction due to GVHD

AND

1. Involvement of eye – clinically
2. Involvement of oral mucosa or salivary glands
3. Involvement of any other target organs

8.0 DRUG FORMULATION, AVAILABILITY AND TOXICITY

8.1**Fludarabine Monophosphate**

8.1.1 **Availability:** Fludarabine is commercially available.

8.1.2 **Administration:** Fludarabine is administered as an IV infusion over 30 minutes.

8.1.3 **Toxicities**: The dose-limiting toxicity is myelosuppression. Additional toxicities include, fever, mild nausea and/or vomiting, skin rashes, myalgia, fatigue, autoimmune hemolytic anemia (may be life-threatening), peripheral neuropathy and pulmonary toxicity (both pneumonia and pulmonary hypersensitivity reactions have been reported; fatal pulmonary toxicity has been described, especially when fludarabine was used in combination with pentostatin). Severe or fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status has been described primarily after high doses of fludarabine.

8.1.4 **Rare complications** - include transfusion-associated graft versus host disease, thrombotic thrombocytopenia purpura and liver failure. Tumor

lysis syndrome has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed.

8.2Cyclophosphamide

8.1**Mechanism of action:** Cyclophosphamide is a pro drug that requires activation. Following hepatic and cellular activation, phosphoramide mustard and acrolein are formed. Phosphoramide mustard is the alkylating agent that demonstrates cytotoxic effects. Acrolein binds to proteins but does not contribute to the anti-tumor effects.

8.2**Toxicities***:* Potential toxicities include: fever and/or chills, nausea and vomiting, anemia, oliguria/anuria, diarrhea, mental status changes, sinus tachycardia, elevated bilirubin, thrombocytopenia, BUN elevation, serum creatinine elevation, elevated transaminase, elevated alkaline phosphatase, pulmonary congestion, fatigue/weakness/malaise, dyspnea, pruritus, edema, erythema leukopenia, stomatitis, anorexia, rash, infection, weight gain (≥10%), arrhythmias, hypomagnesemia, acidosis, hypocalcemia, dizziness, dry skin, exfoliative dermatitis, GI bleeding, sensory dysfunction, jaundice, pulmonary edema, proteinuria, hypophosphatemia, headache, coagulation disorders. In addition, heart problems can occur including a heart attack, the development of fluid around the heart or inflammation of the heart or the tissue surrounding the heart. Acrolein is toxic to the bladder and is associated with the development of hemorrhagic cystitis.

8.3**How supplied**: cyclophosphamide is commercially available

8.5 Tacrolimus

8.5.1 Availability: Tacrolimus is commercially available as an injection (5 mg/mL) and as oral capsules.

8.5.2 Administration: Oral therapy will be administered twice a day. Intravenous tacrolimus may be administered in divided doses every 12 hours.

8.5.3 Toxicities: Most of the adverse event information described comes from studies of tacrolimus in solid organ transplantation. Patients can experience anemia, leukocytosis, thrombocytopenia, mild to moderate hypertension, chest pain. Antihypertensive therapy may be required. The most common adverse effects of tacrolimus have involved the central nervous system, and include headache, tremors, insomnia, paresthesias and dizziness. Less common side effects include agitation, anxiety, confusion, seizures, depression, hallucinations, myoclonus, neuropathy, psychosis, incoordination, and abnormal dreams. Metabolic abnormalities include hyperkalemia, hypokalemia, hypophosphatemia and hypomagnesaemia. In addition, hirsutism occurs only rarely. Gastrointestinal adverse effects include nausea, vomiting, anorexia, constipation and diarrhea. Overt nephrotoxicity is usually seen early after transplantation and is characterized by an increased serum creatinine and a decrease in urine output. Hematuria has been reported in greater than 3% of tacrolimus-treated patients. Abnormal liver function tests have been reported in 6% to 36% of patients; ascites was reported in 7-27% of these

patients. Other miscellaneous effects that have occurred in clinical trials include pain, fever, back pain, peripheral edema and hyperglycemia. Other rare effects include peritonitis, photosensitivity and very rarely anaphylaxis.

8.5.4 Drug Interactions: Tacrolimus is metabolized by cytochrome P450. Drugs that are inhibitors (e.g., Iitraconazole) or inducers (e.g., phenytoin) of 3A4 might increase or decrease tacrolimus concentrations respectively. This could result in increased or decreased effect of tacrolimus. Tacrolimus dose will be adjusted based on blood levels.

8.7 G-CSF or Filgrastim (or biosimilar)

8.7.1 Availability: G-CSF is commercially available.

8.7.2 Administration: The dose for recipients is 5 mcg/kg/day as a subcutaneous injection. The dose for donor stem cell collection is 10 mcg/kg/day given subcutaneously. We will be using doses based on NMDP guidelines (as listed within the protocol).

8.7.3 Toxicities: Toxicities can include chills, nausea, anorexia, myalgias, bone pain, local injection site pain or inflammation, abnormal liver function tests, thinning of hair, and enlargement of the spleen. Rarely fluid retention and pericardial effusions occur. All of these are generally reversible when the drug is discontinued.

9.0 REQUIRED DATA and CORRELATIVE LABORATORY STUDIES

9.1The "Treatment Evaluation" required for this trial is listed in Section 5.0, "General Workup and Diagnostic Procedures".

9.1.1During the inpatient hospital stay for the transplant, toxicity will be monitored (Section 7.0, "Potential Toxicities and Dose Modifications").

9.1.2We will identify the following clinical endpoints:

9.1.1 Time to Marrow engraftment: Standard definitions of engraftment are being used. Engraftment of each cell line will be defined as an absolute neutrophil count (ANC) of > 500/mm^3 for three days (count first day) and a platelet count of 20,000/mm^3 untransfused (count first day).

9.1.2 Response to treatment at 100 days – using standard international response criteria, based on CIBMTR definitions.

9.1.3 Response to treatment at one year – using standard international response criteria, based on CIBMTR definitions.

9.1.4 100 day survival

9.1.5 One year survival

9.1.6 Treatment-related mortality in the first 100 days

9.1.7 Toxicities associated with this treatment regimen during the inpatient stay

9.1.8 Incidence of acute and chronic GVHD

9.1.9 Donor-recipient chimerism following transplant at Days 30, 60 and 90.

9.1 Transplant Evaluation –during the in-patient hospital course

Daily: During the inpatient hospital course, the patient will be evaluated daily using physical exam and lab work as needed.

9.2 Out-Patient Evaluation – Post-Transplant

Patients will be seen in clinic by the treating transplant attending. The recommended follow-up is weekly for 4 weeks. Evaluation during each clinic visit should include pertinent history and physical, CBC with diff and platelets, comprehensive metabolic profile panel. Other labs can be requested at the discretion of treating physician. Of course, extensive follow-up is required for each patient. The follow-up clinic schedule will be directed by the treating transplant physician.

9.3 Re-staging Evaluations

Tumor restaging will include bone marrow aspirate and biopsy on the following days:

Day 30
Day 60 (not mandatory)
Day 90-100
One year

The biopsy can be done within 21 days of designated dates, based on patient's clinical course, toxicities, and side effects.

It is possible that the patient may not require blood or marrow assessment at these time points, if their clinical course deems this not necessary. For example, in the setting of relapsed disease, ongoing treatment, the presence of GVHD or concurrent treatment of a co-morbid condition, the performance of a marrow biopsy may be contraindicated OR the results of the marrow may have no clinical significance, In these cases, a marrow (and/or blood work) is not necessary.

When a biopsy is done, it is recommended to check marrow for chimerism.

9.4 Since GVHD can be diagnosed clinically, if the treating physician believes a biopsy of a specific organ is needed to confirm a diagnosis, this may be done.

9.5 **Response to treatment**

Response to treatment will be assessed at approximately Day 100 (+/- 21 days) and at approximately one year (+/- 21 days) (as required by Foundation for the Accreditation of Cellular Therapy and the Center for International Blood and Marrow Transplant Research). Standard International Criteria for responses for each disease will be used, based on CIBMTR criteria. ***After one year, each patient will only be followed for survival or the development of GVHD.***

9.6 **Patient Samples**

Objective #2 focuses on the correlative laboratory evaluations. The goal is to demonstrate *in vivo* changes with this regimen. The following research specimens will be needed:

9.7 **Blood and marrow patient samples**

Per the protocol design, each patient will have blood and marrow assessments at Days 30, 60 (not mandatory), and day 90 - 100 (+/- 21 days). For each patient, at the time of a blood draw approximately an additional 8 cc will be obtained for research purposes. For each patient, at the time of a bone marrow aspirate approximately, an additional 5-6 cc will be obtained for research purposes. Baseline blood samples should be obtained within 14-21 days of admission.

It is possible that the patient may not require blood or marrow assessment at these time points, if their clinical course deems this not necessary. For example, in the setting of relapsed disease, ongoing treatment, the presence of GVHD or concurrent treatment of a co-morbid condition, the performance of a marrow biopsy may be contraindicated OR the results of the marrow may have no clinical significance, In these cases, a marrow (and/or blood work) is not necessary.

SAMPLES	**Time of engraftment**	**Day 30**	**Day 60** (not mandatory)	**Day 90**
Blood	X	X	X	X
Bone Marrow		X	X	X

9.8 **Processing of patient samples**

Patients' blood and marrow samples will be processed and analyzed by the Immune Monitoring Laboratory or by Dr. Mabaera. Each sample will be labeled with the trial number and provided with a unique patient number (UPN). No individual patient samples will be identifiable. The laboratory tests will be conducted by the Immune Monitoring Lab or by Dr. Mabaera (within Dr. Randy Noelle's laboratory). A copy of the results will remain in the laboratory and with the PI. The PI will be the only individual with access to the UPN and patient identity, so that lab results can be correlated with clinical outcomes.

Because early marrow recovery is associated with release of immature cells into the peripheral blood, including increased numbers of MDSCs, we plan to evaluate the frequency and phenotype of MDSCs during bone marrow recovery. Blood samples will be obtained at the time of neutrophil engraftment, approximately between days 12 – 18 following transplant. A sample will be obtained on day 21 if criteria for neutrophil engraftment are not met. Because MDSC frequency and activity are likely to be important predictors during early immune recovery, we will evaluate MDSC and immune checkpoint regulator expression on blood and marrow samples on approximately days 30, 60 and 90-100 following transplant, since patients undergo these evaluations as part of their routine care at these time intervals.

9.9 **Use of patient samples**

The patient samples will be used as outlined below:

9.5.1 To characterize incidence, prevalence and function of MDSCs and immune checkpoint regulators (VISTA, CTLA-4, PD-L1) during early immune recovery following an allogeneic stem cell transplant.

Since the presence of MDSCs may correlate with engraftment, the response to treatment, and the risk of developing GVHD, we will define MDSCs

frequency and immune checkpoint regulators expression on MDSCs and on peripheral blood and bone marrow in allogeneic transplant recipients following transplantation.

We will correlate these laboratory results with clinical outcomes and the incidence of GVHD.

9.5.2 Exploratory aim # 2a) In those patients experiencing GVHD, we will define the MDSCs frequency and checkpoint regulator expression on MDSCs, peripheral blood mononuclear cells and myeloid subsets. Blood samples will be drawn at time of diagnosis and weekly for four weeks to evaluate changes in response to treatment.

9.10 **Flow Cytometry for MDSCs and VISTA expression**

We will define the two human subsets of MDSCs using the following antibodies: $CD11b^{+}$, $CD33^{+}$ (subset), $HLA\text{-}DR^{-/low}$; with two subsets of granulocytic (CD15+) and monocytes (CD14+). In the limited studies available that have examined MDSCs following transplant, suppressive function has been associated with arginase-1 (Arg1) and indoleamine 2,3-dioxygenase-1 (IDO1) expression.

To evaluate MDSC subsets and immune checkpoint regulators, a portion of the sample will undergo red blood cell lysis and then be directly labeled for 8-color flow cytometry using the following antibodies: anti-CD15-BV421, anti-HLA-DR-BV510, anti-CD11b-FITC, anti-CD33-PE, anti-CTLA4-PerCP-Cy5.5, anti-CD14-PE-Cy7, anti-VISTA-APC, and anti-PD-L1-APC-Cy7. To determine MDSC mediators of MDSC suppressive activity, a second panel with anti-IDO1-AF647, anti-Arg1-APC, CellROX (reactive oxygen species) will be used in place of anti-CTLA4, anti-VISTA, and anti-PD-L1.

To evaluate immune checkpoint regulators' expression in T cells, blood will be run on ficoll-gradient and then stained with the following antibodies: anti-CD3, anti-CD4, anti-CD8, anti-CD11b, anti-FoxP3, anti-CTLA4, antiPD-L1 and anti-VISTA. All flow cytometry will be performed with appropriate staining (CD-Chex plus) and isotype controls. The data will be analyzed using FlowJo software.

9.11 Correlating the lab results to the clinical results

Potential correlation between lab findings and clinical parameters will be evaluated using descriptive methods.

10.0 CRITERIA FOR RESPONSE AND TREATMENT ENDPOINTS

10.1**Objective #1 is to define clinical endpoints using this haploidentical transplant regimen**, **including**:

10.1Time to Marrow engraftment (defined as absolute neutrophil count > 500/mm^3 and platelets > 20,000/mcl for three consecutive days (count first day as engraftment)

10.2 Response to treatment at 100 days

10.2.1 Response to treatment at one year

10.2.2 100 day survival

10.2.3 One year survival

10.2.4 Treatment-related mortality in the first 100 days
10.2.5 Toxicities associated with this treatment regimen
10.2.6 Incidence of acute and chronic GVHD
10.2.7 Donor-recipient chimerism following transplant at Days 30, 60 and 90-100.

10.2 Response Criteria

At day 100 and at one year (+/- 21 days at each time point), the patient will be evaluated for response using standard CIBMTR criteria for each disease. See "Response to Treatment" for additional details.

10.3 Monitoring of Toxicities after discharge from the hospital

Monitoring of all acute toxicities (≥ grade 3) will occur until the time of discharge. After discharge (approximately day 15-17 following transplant), transplant patients often experience a number of medical issues. As a result, after discharge until day 100, only serious toxicities (≥ grade 4) will be recorded. Of course, should any life threatening event possibly be linked to the study at any time, the investigator shall use his/her discretion is reporting the event to the appropriate committees.

10.3 Defining "Off Therapy"

Patients will be considered "off therapy" at day 100 following transplant. After day 100, patients are far enough out from transplantation that any toxicity is not related to the transplant process. ***After day 100, patients will be followed for survival and the development of GVHD.***

10.4 Defining "Off Study"

10.4.1 Three year time point: Since three-year post-transplant remission is felt to predict for an extended disease-free survival, patients will be considered "off study" three years from the date of their transplant.

10.4.2 Relapsed or progressive disease: If the patient progresses or relapses at any time, the patient will be removed from study, since any intervening treatment will influence the endpoints and objectives of the study. The patient will be monitored for overall survival.

10.4.3 Development of a new malignancy: If the patient develops a new malignancy, the patient will be removed from study. The patient will be monitored for overall survival.

11.0 REMOVAL OF PATIENTS FROM PROTOCOL and STOPPING RULES

11.1 Any patient may request removal from the trial at any time. In addition, the treating physician may remove a patient from the trial at any time during treatment. If a patient requests withdrawal, the patient's samples will not be used for analyses.

11.2 Monitoring of Toxicities after discharge from the hospital

Monitoring of all toxicities will occur until the time of discharge. After discharge, transplant patients often experience a number of medical issues. As a result, after discharge, only serious toxicities (> grade 4) will be recorded. Of course, should any life

threatening event possibly linked to the study at any time, the investigator shall use his/her discretion is reporting the event to the appropriate committees.

11.3 Stopping Rules

All high dose chemotherapy regimens will cause significant toxicity. Thus, toxicity will be monitored closely. The Stopping rules for this protocol will address five areas, including:

- oPatient's death before Day 100
- oEngraftment
- oPersistent > Grade 4 toxicities
- oRejection of transplanted marrow
- oIncidence of GVHD

11.4 Patient's death before Day 100

Given the high-risk nature of this treatment and the often poor prognosis of these diseases, there is an expected 4 - 20% mortality rate until day 100. As a result, the trial will be suspended if ≥ 25% of patients die due to treatment-related toxicities before day 100. If a patient dies before day 100, the trial will be suspended until the PI and the required committees have had an opportunity to review the patient's clinical course and medical records to determine if the trial should continue.

11.5.2. Engraftment

In this cohort of patients, we anticipate a 10 - 13 % of patients could experience failure to engraft. As a result, the trial will be suspended if ≥ 20% patients fail to engraft. Engraftment will be defined as an absolute neutrophil count of < 500 cells/mcl by day 60 or platelet transfusion-dependence (to keep count > 20,000/mm^3) by day 60.

Of note, marrow engraftment following a haploidentical transplant is typically delayed when compared to a myeloablative related transplant. For example, the absolute neutrophil counts typically recovers on day 15 for haploidentical transplants compared with day 11-13 for a matched related donor. In addition, platelets engraft around day 24 for haploidentical recipients compared with day 12-15 for a matched related donor undergoing a myeloablative transplant.

11.5.3 Persistent ≥ Grade 4 toxicities

Due to the high-risk nature of this therapy and the anticipated side effects and toxicities, if similar grade 4 or greater toxicities occur in a consistent fashion, the PI and responsible committee members will review the patients' records and clinical course to determine if the clinical trial should continue.

11.5.4 Rejection of transplanted marrow

Following an allogeneic transplant, some patients engraft their donor cells, but lose their graft three or more months out from transplant. Based on the literature review of this trial and similar trials, the marrow rejection rate is 13 %. If 20 % or more of patients reject the marrow, the PI and the responsible committees will review the patient's medical records and clinical course to determine if the clinical trial should continue.

11.5.4 Incidence of GVHD

In general, the incidence of acute GHVD (grade 2-4) is 25-50% for recipients of a related donor transplant. The incidence of chronic GVHD is 30-50% for patients who received a related donor. If the incidence of acute GVHD is > 60% for related donors, the PI and the responsible committees will review the patient's medical records and clinical course to determine if the clinical trial should continue. If the incidence of chronic GVHD is > 80%, the PI and the responsible committees will review the patient's medical records and clinical course to determine if the clinical trial should continue.

12.0 REPORTING of SERIOUS ADVERSE EVENTS and UNANTICIPATED PROBLEMS

12.1 Any adverse event is any undesirable event occurring with the use of these study procedures. Adverse events will be graded according to the NCI Common Toxicity Criteria Version 4.0. A copy of the CTC version 4.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). All appropriate treatment areas should have access to a copy of the CTC version 4.0. Adverse events and unanticipated problems will be reported to The Dartmouth Committee for the Protection of Human Subjects (CPHS) as per their statement found at http://www.dartmouth.edu/~cphs/docs/aedsmmemo.pdf using their Serious Adverse Event (SAE) Reporting Form or their Reporting Form for An Unanticipated Problem Involving Risks to Subjects or Others (UPR)for Clinical Trials found at http://www.dartmouth.edu/~cphs/tosubmit/forms/.

Adverse events to be reported to the CPHS are: any adverse experience, defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research), that is considered:

- Serious: Death; a life-threatening adverse drug experience; inpatient hospitalization or prolongation of existing hospitalization; a persistent or significant disability or incapacity; or a congenital anomaly or birth defect; and
- Unexpected: Any adverse experience, the specificity or severity of which is not consistent with the current investigator brochure or consent form; and
- Possibly related: There is a reasonable possibility that the incident, experience, or outcome may have been associated with the procedures involved in the research; and is experienced by a participant in a trial open at a site subject to review by the CPHS.

The following definitions will be used to assess causality:

No: The clinical adverse event is definitely unrelated to study procedures (e.g., does not follow a reasonable temporal sequence from study procedure, present prior to procedure, etc.)

Unlikely: The study procedures do not have any reasonable association with the observed experience; however, relationship cannot be definitely excluded.

Possibly: The connection with study procedures appears feasible, but cannot be excluded with certainty (e.g., follows a reasonable temporal sequence from procedure, but may also be related to other known factors).

Probably: The clinical experience appears related to the study procedures with a high degree of certainty (e.g., follows a reasonable temporal sequence from procedure and abates upon termination of the procedure, cannot be reasonably explained by known characteristics of the patient's clinical state or other modes of therapy administered to the patient, etc.)

An unanticipated problem involving risks to subjects or others is defined as any incident, experience, or outcome that meets each of the following criteria:

- Unanticipated in terms of nature, severity, or frequency given: (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and consent document; and (b) the characteristics of the subject population being studied; and
- Possibly related to participation in the research means there is a reasonable possibility that the incident, experience, or outcome may have been associated with research participation; and
- The problem suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, emotional, economic, legal, or social harms) than was previously known or recognized.
- Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

13.0 STATISTICAL CONSIDERATIONS

The primary objective of this clinical trial is to define clinical endpoints for the proposed haploidentical transplant regimen, including engraftment, 100-day survival and one year survival. Time to event data will be summarized with Kaplan-Meir curves for censored data along with confidence intervals (**Objective #1**).

13.1 Sample size. The patient sample size is based on determining the 100 day survival for patients treated on this trial. **The trial proposes that 20 evaluable patients will be needed to define the 100 day survival rate. The analysis for the primary objective of 100 day survival rate will be based on the Intent to Treat population, so that all patients will be evaluable for this objective.**

Based on haploidentical transplant clinical trials (see Introduction), the 100-day overall survival in these trials ranged from 80% to 96%. We estimate the 100-day survival for this clinical trial will be $\geq$ 70%. Based on exact confidence intervals for binomial proportions, we expect the half width of our confidence intervals to be approximately 20 % for 100-day survival.

13.2 Survival analysis. All patients will be followed for 100 day survival, one year survival and overall survival. The trial proposes that 20 evaluable patients will be needed to define the 100 day survival rate. **The analysis for the primary objective of 100 day survival rate will be based on the Intent to Treat population, so that all patients will be evaluable for this objective.** With a projected accrual rate of 10-12 patients per year, we estimate that the study will be completed in approximately 24 months. Each patient will be followed for at least one year. ***After one year, each patient will be followed only for survival and the development of GVHD.*** Duration of response and survival will be analyzed

using the product-limit method for censored time to event data to estimate the primary outcomes of 100 day and one year survival.

13.3 Engraftment will be evaluated in all treated patients. The rate of engraftment will be determined as the ratio of the number of patients with successful engraftment to the total number of patients treated. An exact 95% confidence interval will be computed based on the binomial distribution.

13.4 Toxicity will be tabulated according to type and grade. Rates of toxicity will be determined as the ratio of the number of patients experiencing the toxicity to the total number of patients receiving any treatment. Toxicity rates will be computed according to type and grade. In addition, the rate of any grade 3 or worse toxicity that occurs during the transplant hospital course or any grade 4 or greater toxicity that occurs out until day 100 will be computed.

13.5 Laboratory analysis. Statistical analysis will focus on the results of myeloid-derived suppressor cells (MDSCs) and the presence and function of Immune Checkpoint Regulators. Since minimal information is known about the presence of MDSCs following allogeneic stem cell transplantation, we will define their presence or absence within one patient over time. We will also compare the results between patients. It is impossible to compare the results to "baseline" results, since MDSC cell subsets are dynamic and change over time. In addition, the patient's or donor's baseline MDSCs will not be representative of the MDSCs identified following myeloablative chemotherapy and immune suppressive therapy.

13.6 Immune Checkpoint Regulators. We will identify their presence or absence on MDSCs. These results will be monitored over time in individual patients and results will also be compared between patients. Appropriate descriptive summary statistics (e.g., means and standard errors, medians) and graphical displays will be generated for each measure at baseline and during treatment and follow-up. Graphical displays will show changes over time for continuous endpoints both in terms of the absolute measures as well as percent change from baseline. We will use Wilcoxon signed-rank tests to evaluate changes from baseline (absolute change and percent change).

13.7 Correlation analysis. We will evaluate the correlation between biological endpoints and clinical outcomes using appropriate techniques. We will examine the presence/absence of MDSCs in each patient and compare time to bone marrow engraftment, 100 day survival, one year survival and incidence of GVHD using log rank tests for censored time-to-event data and Chi-square test for dichotomous outcomes. These methods will also be used to determine if a correlation exists between the presence/absence of GVHD or Immune Checkpoint Regulators on survival. Where necessary,

we will use transformations (e.g., logarithm and square root) to adjust for skewed distributions for lab measurements. Associations among lab results will be evaluated using Pearson or Spearman correlation analysis. In particular, we will construct a correlation matrix for the immune parameters. We will use 2-dimensional scatter plots to examine the distributions of the measures.

13.8 Stopping Rules. (See above section "**REMOVAL OF PATIENTS FROM PROTOCOL and STOPPING RULES".**

The Transplant Program performs a monthly Quality Assurance Meeting with presentation of all data on Transplant Inpatients and completed transplants.

14.0 STUDY MONITORING, AUDITING AND INSPECTION

14.1 Safety and Data Monitoring

This study will be monitored by the Data Safety Monitoring and Accrual Committee (DSMAC) of the Norris Cotton Cancer Center. The Committee meets quarterly to review accrual rates and information of all studies that have accrued participants. The Clinical Cancer Review Committee (CCRC) determines the frequency of DSMAC review. The DSMAC has the authority to suspend or to recommend termination to the CCRC of all research activities that fall within its jurisdiction. In the event that a study is suspended or terminated by the DSMAC, that information will be forwarded to the CPHS (Dartmouth IRB) office.

The DSMAC will monitor this trial. In particular, areas that will be monitored include accrual to the trial, toxicity associated with the treatment and patient outcomes (the "Objectives"). We will closely monitor for any side effects or toxicities. Stopping rules will be followed, as outlined in Section 11.

14.2 On-site monitoring

Clinical research monitoring for regulatory compliance and data integrity will be conducted according to the NCI-approved NCCC Data and Safety Monitoring Plan. Internal monitoring is conducted by appropriately trained staff of the NCCC Office of Clinical Research and Dartmouth-Hitchcock Medical Center Clinical Trials Office who are not involved in the study. This monitoring will include periodic assessment of the regulatory compliance, data quality, pharmacy records and study integrity. Study records will be reviewed and directly compared to source documents and the conduct of the study will be discussed with the investigator. Monitors may request access to all regulatory documents, source documents, CRFs, and other study documentation for on-site inspection. Direct access to these documents is guaranteed by the investigator, who must provide support at all times for these activities.

14.3 Auditing and Inspection

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable Dartmouth research compliance and quality assurance offices. The investigator will permit study protocol related audits and inspections by the Dartmouth CPHS, government regulatory bodies, and the Dartmouth compliance and quality assurance groups of all study related documents (e.g., source documents, regulatory documents, data collection instruments,

study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.).

14.4 Record Retention

Following closure of the study, the investigator will maintain all site study records in a safe and secure location. The records are maintained to allow easy and timely retrieval when needed (e.g., audit or inspection) and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Upon completion of study analysis, research information is stored in Dartmouth College Records Management off-site storage located at 6218 Etna Road, Hanover, NH 03755. Documents are shredded on site after 50 years of storage.

Electronic case report forms, participant, and study information will be kept in the Velos eResearch password-protected database (or equivalent) indefinitely.

15.0 HUMAN SUBJECTS

15.1 This is a non-randomized study. To enter eligible patients or discuss a patient's eligibility, please contact Dr. Kenneth R. Meehan or the Clinical Research Associate (TBD at 603-650-4035). No patients shall be entered on study without consultation with the Principal Investigator or CRA.

15.2 Patient care and status will be monitored by a Transplant/Hematology Physician and a designated Data Manager. All data will be maintained on a closed database with each subject identified by a unique patient number (UPN) with access to patient names only for those involved in clinical care. All patients will provide informed consent prior to study entry. Necessary information will be forwarded to the Center for International Blood and Marrow Transplant Registry (CIBMTR). Each patient will be fully informed concerning this study, including pertinent adverse reactions. All institutional or other Federal regulations and guidelines concerning informed consent will be fulfilled.

15.3 Gender and Minority Inclusion for Research Involving Human Subjects: The clinical trial is open to all patients with the hematologic malignancies as outlined within the "Eligibility Criteria" of the trial. All races and both genders are eligible. Given the geographic location of Dartmouth-Hitchcock Medical Center and the Norris Cotton Cancer Center, we anticipate the majority of patients will be Caucasian with an equal distribution of males and females.

15.4 Participation of Children: Stem cell transplants in pediatric patients are not performed at Dartmouth.

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