Shortened-duration tacrolimus following nonmyeloablative, related donor BMT with high-dose posttransplantation cyclophosphamide

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Appendix
A. Acute GVHD Grading
B. Statistical Supplement

SCHEMA [a]

Days –6, –5 — **Fludarabine** 30 mg/m^2 IV daily (adjusted for renal function)
Cyclophosphamide 14.5 mg/kg IV daily
↓
Days –4, –3, –2 — **Fludarabine** 30 mg/m^2 IV daily
↓
Day –1 — **TBI** 200 cGy
↓
Day 0 — Infuse T-cell replete bone marrow
Begin antibiotic prophylaxis
↓
Days 3, 4 — **Cyclophosphamide** 50 mg/kg IV QD
Mesna 40 mg/kg IV QD in divided doses
↓
Day 5 Begin **tacrolimus** (1 mg IV QD for pts $\geq$ 18 y), **with planned duration as below** [b]
MMF 15 mg/kg PO TID (maximum 3 g/day)
(Immunosuppression must begin at least 24 hours after Cy completion)

Begin **GCSF** 5 µg/kg/d SC or IV, continue until ANC $\geq$ 1000/mm^3 over 3 days
↓
Day 28 (+/- 3 d) — Assess chimerism
↓
Day 35 — **Discontinue MMF** (optional if GVHD has occurred)
↓
Day 56 (+/- 3 d) — Assess chimerism, GVHD
Day 56 (+/- 3 d) — Evaluate disease
↓
Day 84 (+/- 5 d) — Assess chimerism, GVHD
↓
Day 112 (+/- 7 d) — Assess chimerism, GVHD
↓
Day 180 (+/- 7 d) — Assess chimerism, GVHD
Evaluate disease
↓
Day 270 (+/- 21 d) — Assess chimerism, GVHD
↓
1 yr (+/- 30 d) — Assess chimerism
1 yr (+/- 30 d) and beyond — Evaluate disease, GVHD

Shortened-course tacrolimus if eligible: up through Day 90 (starting regimen), Day 60, or Day 120 [c]
If ineligible: continue tacrolimus up through Day 180 [d]

a See Section 5.2 for complete dosing instructions, and Section 7.0 for required evaluations.
b See Section 5.281 for tacrolimus dosing in younger patients.
c See Section 5.283 for eligibility.
d Per Section 5.282.

1.0 INTRODUCTION

Recent advances in allogeneic blood or marrow transplant (BMT) platforms for hematologic malignancies have substantially lowered transplant-related morbidity both in the HLA-matched and partially HLA-mismatched settings. One of these major advances is the incorporation of high-dose posttransplantation cyclophosphamide (Cy) for prophylaxis of graft-versus-host-disease (GVHD) and graft rejection, as developed at Johns Hopkins.[1] For nonmyeloablative, related donor, HLA haploidentical or matched BMT, our postgrafting immunosuppression has standardly consisted of two doses of high-dose Cy, mycophenolate mofetil (MMF) for one month, and tacrolimus without taper until Day 180. However, there are several potential advantages to shorter-duration pharmacologic immunosuppression. The current study builds on our transplantation platform that incorporates high-dose posttransplantation Cy, by investigating the feasibility and safety of shorter planned durations of pharmacologic immunosuppression with tacrolimus.

1.1 Rationale for shorter-duration immunosuppression

With the advent of posttransplantation high-dose Cy, our nonmyeloablative allogeneic BMT platforms have been associated with acceptable rates of acute GVHD, graft failure, and nonrelapse mortality (NRM) that are similar to those seen with HLA-matched transplants.[2,3] However, relapse remains a major problem, and approaches that augment the anti-tumor efficacy of the transplant procedure are needed. Transplantation platforms that minimize the amount of pharmacologic immunosuppression, but that carry acceptable rates of severe GVHD and graft failure, are desirable for a number of reasons. Less pharmacologic immunosuppression has the potential to a) lower the risk of relapse by facilitating a graft-versus-tumor effect, and b) facilitate the development of immunotherapies in the transplant setting, including tumor vaccines. In addition, it has the potential to lower the risk of opportunistic infections, and to lower the likelihood or severity of drug toxicities.

Extensive published data demonstrate that allogeneic BMT can be associated with a clinically significant graft-versus-tumor (GVT) effect mediated by donor T cells specific for host histocompatibility antigens. Yet, the T cells that mediate a GVT effect may also cause clinically significant GVHD.
Tacrolimus, a calcineurin inhibitor (CNI), blocks T cell activation and the production of interleukin-2, a critical growth factor for T cells including regulatory T cells that control autoimmunity. CNI's are used to prevent acute GVHD, but they are associated with an increased incidence of renal dysfunction, hypertension, opportunistic infection, and other complications. Importantly, CNI's block T cell development in the thymus[16,17] resulting in delayed immunologic reconstitution, and by suppressing T cell activation may block the GVT effect and increase the risk of disease relapse after allogeneic BMT.[18-20]

Posttransplantation immunotherapy in order to augment the anti-tumor efficacy of the transplant is an area of ongoing research. In addition, pretransplantation vaccination of donor T cells against tumor-associated antigens has the potential to augment the anti-tumor effect of allogeneic BMT without increasing the incidence or severity of GVHD. A number of antigens specific to or highly expressed by hematologic malignancies have been identified, including myeloma idiotype,[4] the Wilms Tumor 1 antigen, PRAME, and proteinase-3 [5] in AML, and the bcr-abl fusion protein of CML.[6,7] Clinical trials of peptide-based tumor vaccines in the non-BMT setting have been conducted, with some promising immunologic and clinical outcomes.[8] The major obstacles to obtaining clinical responses to tumor vaccines are pre-existing T cell tolerance [9,10] as well as tumor [11]- or therapy-induced immunosuppression.

1.2 High-dose posttransplantation cyclophosphamide

The immunologic rationale for administering high-dose Cy after transplantation is that recently activated, alloreactive T cells (the cells most responsible for GVHD) are selectively sensitive to the toxic effects of this drug.[12] High-dose Cy, when administered in a narrow window after transplantation, depletes alloreactive T cells from the donor and host and can inhibit both GVHD and graft rejection.[12-17] As a form of drug-induced immunologic tolerance,[18] the strategy of giving high-dose Cy after transplantation takes advantage of the heightened cytotoxic sensitivity of proliferating, alloreactive T cells over non-alloreactive, resting T cells to being killed by a DNA-damaging agent.[19] Pre-clinical studies demonstrated that engraftment of major

histocompatibility complex (MHC)-mismatched bone marrow could be achieved by conditioning mice with pretransplantation fludarabine and low dose (200 cGy) total body irradiation (TBI), with posttransplantation Cy.[14] Additional studies demonstrated that posttransplantation Cy reduced the incidence and severity of GVHD in the setting of MHC-mismatched allogeneic BMT after myeloablative conditioning.[13]
After allogeneic BMT, standard regimens of GVHD prophylaxis consist of a CNI (cyclosporine or tacrolimus) in combination with either methotrexate, MMF, or sirolimus. However, a nonmyeloablative, partially HLA-mismatched (haploidentical), related donor BMT platform with high-dose posttransplantation Cy, MMF, and tacrolimus for GVHD and graft rejection prophylaxis has produced encouraging results [3]. This approach has been associated with rapid and stable engraftment in most patients.[3] Most importantly, this approach has carried acceptable rates of GVHD and NRM that parallel those seen with nonmyeloablative HLA-matched transplants (Figure 1).[2,3,20,21] Cy, when administered at high doses after myeloablative, HLA-matched, related or unrelated donor BMT, notably has been found to be effective single-agent prophylaxis against GVHD, obviating the need for CNI's in this setting (Figure 1).[22]

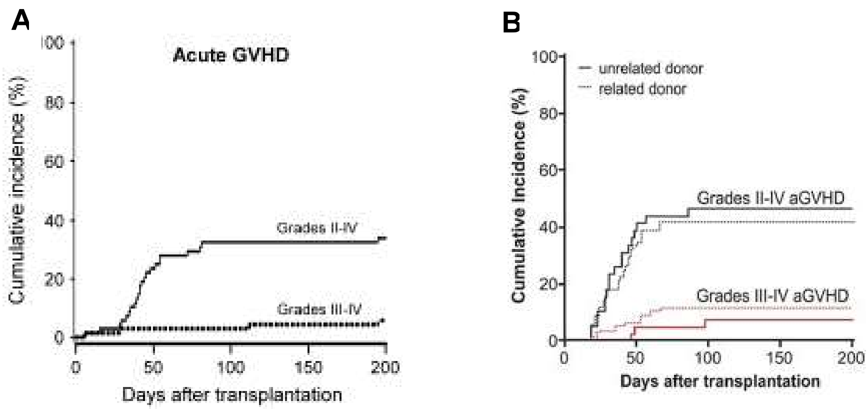

Figure 1. Acute GVHD after BMT incorporating high-dose posttransplantation Cy. A) nonmyeloablative BMT.[3] B) myeloablative, HLA-matched, related or unrelated donor BMT, with Cy as single-agent prophylaxis.[22]

1.3 <u>Nonmyeloablative BMT with fludarabine, TBI, and posttransplantation cyclophosphamide</u>

Independent clinical trials have evaluated or are evaluating a nonmyeloablative, partially HLA-mismatched (haploidentical), related donor BMT platform with high-dose posttransplantation Cy, tacrolimus, and MMF for GVHD and graft rejection prophylaxis. Conditioning in these studies has historically consisted of fludarabine, low-dose Cy, and 200 cGy TBI. The postgrafting immunosuppression regimen that underlies recent and ongoing research efforts at Johns Hopkins has been published.[3,21] A combined analysis of two independent clinical trials for poor-risk hematologic malignancies was originally reported in 2008 (40 patients at Johns Hopkins, 28 at Fred Hutchinson Cancer Research Center), evaluating the safety and efficacy of a high-dose posttransplantation Cy platform after outpatient nonmyeloablative conditioning and T-cell-replete BMT from partially HLA-mismatched, related donors (Figure 2).[3] Following transplantation, high-dose (50 mg/kg) Cy was administered on Day 3 (Seattle group), or on Days 3 and 4 (Hopkins). Pharmacologic prophylaxis of GVHD was initiated on the day following completion of posttransplantation Cy with MMF until Day 35, and tacrolimus which was tapered to off by Day 180 (Seattle) or continued at full dose until Day 180 (Hopkins). Filgrastim 5 μg/kg/day was administered until recovery of neutrophils to >1000/μL:

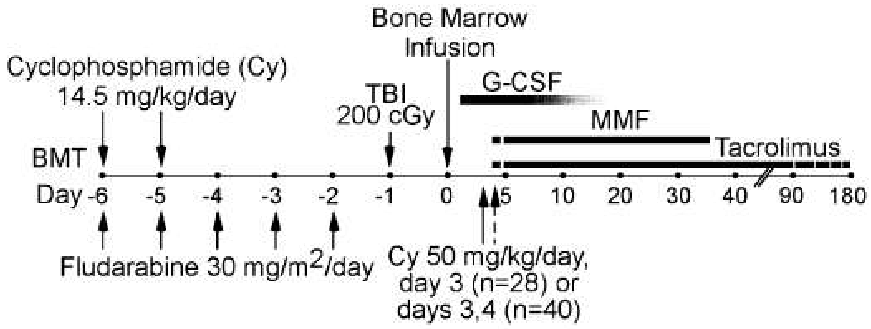

Figure 2. Treatment Schema in Previous Studies

Median times to recovery of neutrophils and platelets were 15 and 24 days, respectively. Graft failure occurred in 9 of 66 evaluable patients (12%); all but one patient with graft failure had recovery of autologous hematopoiesis with median times to neutrophil and platelet recovery of 15 days (range, 11-42) and 28 days (range, 0 – 395 days) respectively. Engrafting patients achieved full donor chimerism rapidly; with few exceptions, donor chimerism in patients with sustained engraftment was virtually complete (≥95%) by 2 months after transplantation. The cumulative incidences of acute grade II-IV GVHD and acute grade III-IV GVHD by Day 200 were <35% and <10%, respectively, on competing-risk analysis. The groups did not differ significantly in the incidence of acute grade II-IV or III-IV GVHD, although the risk of chronic GVHD appeared to be lower with two doses of Cy. The cumulative incidence of extensive chronic GVHD by 1 year was only 5% in the group with two doses of Cy. The cumulative incidences of relapse and NRM at 1 year were 51% and 15% respectively on competing-risk analysis; however the event-free survival (EFS) probability at 1 year was only 34%. Similar outcomes were seen in a recent analysis of 185 patients treated on these trials including follow-up phase II trial (J0457) of this approach.[21] Interestingly, although increasing degrees of HLA mismatch between donor and recipient have historically been associated with greater GVHD and inferior survival after allogeneic BMT, that analysis retrospectively found no such adverse effect of HLA mismatching using this approach in the haploidentical setting.[21]

On an updated analysis of 212 patients with advanced hematologic malignancies, uniformly treated at Johns Hopkins with related donor, partially HLA-mismatched BMT with fludarabine/Cy/TBI conditioning and postgrafting immunosuppression with 2 doses of Cy, MMF on Days 5-35, and full-dose tacrolimus on Days 5-180, the 1-year EFS was 44%, and cumulative incidence of NRM by competing risk analysis was 8% by Day 100 and 14% at 1 year. The cumulative incidence of acute grade II-IV GVHD was 28% by Day 200, cumulative incidence of severe GVHD was only 4%, and the cumulative incidence of chronic GVHD was 14% by competing risk analysis (unpublished data).

The same approach to nonmyeloablative BMT using partially HLA-mismatched, related donors has been adopted at Johns Hopkins for HLA-identical donor BMT. In a phase I trial (J0169) of nonmyeloablative, matched sibling donor BMT, Cy alone on Day 3, Cy alone on Days 3 and 4, or Cy on Days 3 and 4 with MMF was investigated with the latter being favored. Although the optimal postgrafting immunosuppression after high-dose Cy is not defined, MMF plus a CNI is the industry-standard GVHD prophylaxis for nonmyeloablative conditioning (i.e. omission of a CNI is nonstandard). At Johns Hopkins we have used the approach developed in over 200 nonmyeloablative haploidentical transplants described above in our nonmyeloablative matched unrelated donor (MUD) transplants, and have recently adopted this for our nonmyeloablative matched sibling transplants as well. This is similar to most centers that use the same nonmyeloablative conditioning and GVHD prophylaxis for matched sibs and MUDs.

In summary, HLA-haploidentical BMT after non-myeloablative conditioning and using 2 doses of posttransplantation Cy followed by MMF for one month and tacrolimus for up to 6 months is a generally well-tolerated procedure that can be administered largely in an intensified outpatient setting. The toxicity of the procedure compares favorably to the toxicity of non-myeloablative transplantation using unrelated or even HLA-identical sibling donors.[2] The major cause of treatment failure in this high-risk population is relapse, occurring in approximately 50% of patients by 1 year. Therefore, investigations of strategies that may lower the risk of relapse are needed. Towards this end, a strategy of reduced-duration pharmacologic immunosuppression is herein investigated.

1.4 Shortened-course immunosuppression

MMF plus a CNI (i.e. tacrolimus or cyclosporine) is an industry-standard GVHD prophylaxis for nonmyeloablative conditioning. However, optimal postgrafting immunosuppression after high-dose Cy is not defined, nor is the optimal type or duration of postgrafting immunosuppression fully defined in allogeneic BMT in general.

For example, in nonmyeloablative, HLA-matched BMT with postgrafting immunosuppression consisting solely of MMF + cyclosporine, investigators at the Fred Hutchinson retrospectively evaluated three durations of cyclosporine: taper from Days 35 to 56, Days 56 to 77, or Days 56 to 180.[23] Grafts were derived from peripheral blood stem cells. There was no significant association between cyclosporine duration and the rates of acute grade II-IV GVHD (57%, 43%, and 49% respectively), extensive chronic GVHD, or NRM; however, longer duration of cyclosporine was associated with a lower risk of acute severe (grade III-IV) GVHD and lower incidence of discontinuation of all systemic immunosuppression by 24 months (an indirect marker of the prevention and successful treatment of GVHD).

In an older randomized trial of myeloablative, HLA-identical or one-antigen mismatched BMT, where postgrafting immunosuppression consisted of methotrexate and cyclosporine with or without methylprednisolone, results suggested that cyclosporine could be stopped earlier (by Day 60) in patients without prior acute GVHD, whereas those with prior acute GVHD appeared to benefit from a longer course [24]. In another randomized trial of myeloablative, HLA-matched related or unrelated donor BMT, the risk of clinically extensive chronic GVHD and transplant-related mortality did not significantly differ in patients assigned to 6 months versus 24 months of cyclosporine.[25] Other nonrandomized studies of myeloablative, HLA-matched sibling transplant have suggested a benefit to longer duration cyclosporine in chronic GVHD prevention.[26] However, some studies have found an increased risk of relapse associated with higher doses of cyclosporine.[27,28]

The experiences with immunosuppression duration with other allogeneic BMT platforms cannot be directly extrapolated to the high-dose posttransplantation Cy platform. Immunosuppression must be sufficient to prevent graft failure and to prevent excessive rates of GVHD including severe GVHD; yet extended-course immunosuppression may increase the risk of infection, drug toxicity, and relapse. There are presently no published data on the minimum required duration of tacrolimus after nonmyeloablative BMT that includes high-dose Cy as part of postgrafting immunosuppression. The effectiveness of high-dose posttransplantation Cy in GVHD prevention, however, permits the investigation of this question.

2.0 OBJECTIVES

2.1 Primary objective

In nonmyeloablative, related donor, partially HLA-mismatched or HLA-matched BMT with post-grafting immunosuppression that includes high-dose cyclophosphamide and MMF, evaluate the safety and feasibility of reduced-duration tacrolimus (from Day 5 through either Day 90, Day 60, or Day 120).

2.2 Secondary objectives

1. In patients eligible for reduced-duration tacrolimus, estimate the incidences of acute grade II-IV GVHD, acute grade III or higher GVHD, chronic GVHD (overall and by extent), graft failure,

relapse, and NRM between the date of early tacrolimus cessation and Day 180, and beyond Day 180.
2. Estimate the cumulative incidence of acute grade II-IV GVHD, acute grade III-IV GVHD, chronic GVHD, relapse/progression, and NRM for the group overall.
3. Estimate the cumulative incidence of systemic steroid initiation, the cumulative incidence of non-steroid immunosuppression use, and the cumulative incidence of discontinuation of systemic immunosuppression for GVHD treatment by 1 year and 2 years after BMT for the group overall and for patients with shortened-duration tacrolimus; and describe the number and types of systemic immunosuppression used for GVHD treatment.
4. Estimate the event-free survival, progression-free survival, and overall survival after transplantation.
5. Describe the graft failure frequency, kinetics of neutrophil and platelet recovery, and kinetics of donor chimerism in unsorted and $CD3^+$ sorted peripheral blood.
6. Characterize immune reconstitution after transplantation and its relationship to duration of pharmacologic immunosuppression and clinical outcomes.
7. Describe major toxicities and complications associated with the transplantation procedure.
8. Evaluate selected patient and transplant characteristics in relation to transplantation outcomes.

3.0 SELECTION OF PATIENTS AND DONORS

3.1 Eligibility for transplantation

The following are eligibility for study entry and transplantation. Eligibility criteria for protocol-driven, early cessation of tacrolimus are designated in Section 5.283.

1. Patient age 0.5-75 years
2. Presence of a suitable first-degree related, HLA-haploidentical or HLA-matched bone marrow donor.
    a. The donor and recipient must be identical at at least one allele of each of the following genetic loci: HLA-A, HLA-B, HLA-Cw, HLA-DRB1, and HLA-DQB1. A minimum match of 5/10 is therefore required, and will be considered sufficient evidence that the donor and recipient share one HLA haplotype.
3. Eligible diagnoses:
    a. Low-grade non-Hodgkin's lymphoma or plasma cell neoplasm with either of the following, and with stable disease or better prior to transplantation:
        i. Progressed during multiagent therapy, failed at least two prior therapies (excluding single agent rituximab), or there is evidence of prior transformation
        ii. SLL or CLL with 11q or 17p deletion or with progression < 6 months after a purine analog-containing regimen
    b. Relapsed, refractory, or progressive aggressive non Hodgkin's lymphoma (including mantle cell lymphoma), with PR or better prior to transplantation, and autologous BMT is not recommended.

        Note: Patients with Burkitt's, atypical Burkitt's, or acute lymphoblastic lymphoma must be in CR.
    c. Relapsed, refractory, or progressive Hodgkin's lymphoma meeting one of the following criteria, and autologous BMT is not recommend:
        i. PR or better prior to transplantation.
        ii. Stable disease prior to transplantation, provided that the disease is low-volume and disease control is regarded as sufficient to proceed with BMT. Eligibility of such patients will be determined on a case-by-case basis with the PI or co-PI.
    d. One of the following poor-risk lymphomas or plasma cell neoplasms, in PR or better prior to transplantation:
        i. Transformed lymphoma
        ii. T-cell PLL

iii. Peripheral T-cell lymphoma
iv. NK or NK/T-cell lymphoma
v. Blastic/blastoid mantle cell lymphoma
vi. Plasma cell leukemia

e. For patients with SLL, CLL, or PLL, ≤ 20% of bone marrow cellularity involved by this process (to lower risk of graft rejection).
f. Relapsed, refractory, or progressive acute leukemia in second or subsequent remission, with remission defined as <5% bone marrow blasts morphologically.
g. Poor-risk acute leukemia in first remission, with remission defined as <5% bone marrow blasts morphologically:
    i. AML with at least one of the following:
        - AML arising from MDS or a myeloproliferative disorder, or secondary AML
        - Presence of Flt3 internal tandem duplications
        - Poor-risk cytogenetics
        - Primary refractory disease
    ii. ALL (leukemia and/or lymphoma) with at least one of the following:
        - Poor-risk cytogenetics
        - Clear evidence of hypodiploidy
        - Primary refractory disease
    iii. Biphenotypic leukemia
h. MDS with at least one of the following poor-risk features:
    i. Poor-risk cytogenetics
    ii. IPSS score of INT-2 or greater
    iii. Treatment-related or secondary MDS
    iv. MDS diagnosed before age 21 years
    v. Progression on or lack of response to standard DNA-methyltransferase inhibitor therapy
    vi. Life-threatening cytopenias, including those requiring frequent transfusions
i. Interferon- or imatinib-refractory CML in first chronic phase, or CML in second or subsequent chronic phase
j. Philadelphia chromosome negative myeloproliferative disease (including myelofibrosis)
k. Chronic myelomonocytic leukemia
l. Juvenile myelomonocytic leukemia

4. One of the following, to lower risk of graft rejection:
    a. Cytotoxic chemotherapy, alemtuzumab, or an adequate course of 5-azacitidine or decitabine within 3 months prior to start of conditioning; or
    b. Previous BMT within 6 months prior to start of conditioning
        Note: Patients who have received treatment outside of these windows may be eligible if it is deemed sufficient to reduce graft rejection risk; this will be decided on a case-by-case basis by the PI or co-PI.
5. No active extramedullary leukemia or known active CNS involvement by malignancy. Such disease treated into remission is permitted.
6. Any previous BMT must have occurred at least 3 months prior to start of conditioning.
7. No previous allogeneic BMT (syngeneic BMT permissible).
8. Adequate end-organ function as measured by:
    a. Left ventricular ejection fraction ≥ 35% or shortening fraction > 25%
    b. Bilirubin ≤ 3.0 mg/dL (unless due to Gilbert's syndrome or hemolysis), and ALT and AST ≤ 5 x ULN
    c. FEV_1 and FVC ≥ 40% of predicted; or if unable to perform pulmonary function tests due to young age, oxygen saturation >92% on room air

9. ECOG performance status $\leq$ 2 or Karnofsky or Lansky score $\geq$ 60.
10. Not pregnant or breast-feeding.
11. No uncontrolled infection.

    Note: Infection is permitted if there is evidence of response to medication. Eligibility of HIV infected patients will be determined on a case-by-case basis.

3.2 Donor eligibility

1. Donors must be HLA-haploidentical or HLA-identical, first-degree relatives of the patient based on allele or allele group level typing as defined in Section 3.1. Half-siblings are not permitted.
2. Medically fit to and willing to donate
3. Lack of recipient anti-donor HLA antibody

    Note: In some instances, low level, non-cytotoxic HLA specific antibodies may be permissible if they are found to be at a level well below that detectable by flow cytometry. This will be decided on a case-by-case basis by the PI and one of the immunogenetics directors. Pheresis to reduce anti-HLA antibodies is permissible; however eligibility to proceed with the transplant regimen would be contingent upon the success of the desensitization.
4. Has not donated blood products to patient

3.3 Donor prioritization

Donors will be prioritized in the following order:

1. Fit to donate
2. HLA-matched prioritized over HLA-mismatched
3. Lack of major ABO incompatibility

    In order of priority:
    a. Compatible
    b. Minor incompatibility
    c. Major incompatibility
4. CMV serostatus: CMV negative donor preferred, if the patient is CMV negative; CMV positive donor preferred, if the patient is CMV is positive.

Other factors such as donor age and health history will be integrated into the donor selection process per standard practice and may be prioritized over HLA, ABO and CMV status.

4.0 REGISTRATION PROCEDURES

4.1 Registration requirements

Patients will be registered in the CRMS. The following are additionally required:

1. Signed and dated informed consent
2. Patient eligibility checklist(s)

A registration may be cancelled, provided that protocol treatment has not been begun.

4.2 Accrual goal

The goal is to transplant up to 55 patients per planned duration of immunosuppression (per Section 9.0), with up to 5 additional patients transplanted per regimen to replace unevaluable patients (per Section 5.286). Contingent on feasibility and safety outcomes, up to two reduced durations of tacrolimus will be investigated and thus a targeted maximum of 110 patients will be transplanted. The cap is 140 transplanted patients, to account for inevaluable patients or patients who are assigned to the Day 120 arm pending full evaluation of the Day 90 arm (per Section 9.11) . Additional patients may be screened, consented, and registered, in order to reach accrual goals.

Every effort will be made to recruit women and minorities to this study.

5.0 TREATMENT PLAN

5.1 Evaluations and procedures

Required evaluations are designated in Section 7.0.

5.2 Transplantation regimen

The preparative regimen in each case consists of fludarabine, Cy, and TBI, with posttransplantation high-dose Cy, MMF, and tacrolimus.

5.21 Fludarabine

Fludarabine 30 mg/m^2/day (adjusted for renal function) is administered over a 30-60 minute IV infusion on Days –6 through –2 (maximum cumulative dose, 150 mg/m^2).

The body surface area (BSA) for fludarabine dosing is based on actual body weight.

For decreased creatinine clearance (CrCl), fludarabine dosage is reduced as follows:

CrCl 46-60 mL/min, fludarabine = 24 mg/m^2
CrCl 31-45 mL/min, fludarabine = 22.5 mg/m^2
CrCl 21-30 mL/min, fludarabine = 19.5 mg/m^2
CrCl $\leq$ 20 mL/min, fludarabine = 15 mg/m^2

For patients $\geq$ 18 years old, CrCl will be estimated by the Cockcroft Formula, based on ideal body weight (IBW):

$$\text{CrCl} = \frac{(140 - \text{age}) \times \text{IBW (kg)}}{P_{Cr} \times 72} \times 0.85 \text{ for females}$$

For patients <18 years old, CrCl will be estimated by the Schwartz equation:

Schwartz equation: CrCl (mL/min/1.73m^2)= [length (cm) x k] / serum creatinine

k = 0.45 for infants 1 to 52 weeks old
k = 0.55 for children 1 to 13 years old
k = 0.55 for adolescent females 13-18 years old
k = 0.7 for adolescent males 13-18 years old

A measured CrCl or a glomerular filtration rate may be substituted to determine CrCl.

CrCl may change during the days fludarabine is given. The dose should be adjusted accordingly; however a fludarabine dose may be given based on the preceding day's estimated CrCl .

5.22 Pretransplantation cyclophosphamide

Cy 14.5 mg/kg/day is administered as a 1-2 hour IV infusion on Days –6 and –5 after hydration. Mesna 11.6 mg/kg IV daily on Days –6 and –5 is not required, but may be given.

Cy and mesna are dosed according to IBW, unless the patient weighs less than IBW, in which case dose drug according to actual weight.

5.23 Total body irradiation

200 cGy TBI is administered in a single fraction on Day –1. Radiation sources, dose rates, and shielding follow institutional practice.

5.24 Day of rest

A day of rest, i.e. after preparative regimen completion and prior to bone marrow infusion, is not routinely scheduled. Up to two days of rest may be added in this window based on logistical considerations or clinically as indicated. For one day of rest, fludarabine would be administered on Days –7 through –3, pretransplantation Cy on Day –7 and Day –6, and TBI on Day –2. For two days of rest, fludarabine would be administered on Days –8 through –4, pretransplantation Cy on Day –8 and Day –7, and TBI on Day –3.

5.25 Bone marrow transplantation

On Day 0, bone marrow is infused. Donor bone marrow will be harvested with a target yield of 4 x 10^8 nucleated cells/kg recipient IBW. Peripheral stem cell harvest is not permitted because of the increased risk of GVHD. Sample of the product to be infused will be sent for flow cytometry to determine the content of $CD34^+$ and $CD3^+$ cells. Graft dose including total nucleated cells infused/kg, $CD34^+$ cells infused/kg, and $CD3^+$ cells infused/kg will be recorded.

The graft will not be manipulated to deplete T cells. Processing for ABO incompatibility follows institutional practices. Guidelines for bone marrow infusion are established and outlined in the ABO compatible/minor mismatched allogeneic BMT or the ABO incompatible allogeneic BMT standing orders.

5.26 Posttransplantation cyclophosphamide

Hydration prior to Cy, management of volume status, and monitoring for hemorrhagic cystitis will follow institutional standards. Intravenous hydration will be started at least 2 hours prior to Cy and continued for at least 8 hours post-Cy. Urine output should be at least 3 mg/kg (actual body weight) before Cy is administered.

Mesna is given in divided doses IV 30 minutes pre- and at 3, 6, and 8 hours post-Cy, unless patients are treated in the Children's Center in which case mesna is dosed per pediatric oncology standard (e.g., divided doses IV 30 minutes pre- and at 3, 6 and 9 hours post-Cy). The total daily dose of mesna is equal to 80% of the total daily dose of Cy.

Cy and mesna are dosed according to IBW, unless the actual body weight is less, in which case dose drugs according to actual body weight.

Cy 50 mg/kg IV, over approximately 1-2 hours (depending on volume), is given on Day 3 posttransplantation (ideally between 60 and 72 hours after marrow infusion) and on Day 4 (approximately 24 hours after Day 3 Cy).

It is crucial that no systemic immunosuppressive agents are given from Day 0 until at least 24 hours after the completion of the posttransplantation Cy. This includes corticosteroids as anti-emetics.

5.27 Mycophenolate mofetil

MMF begins on Day 5, at least 24 hours after completion of posttransplantation Cy. The MMF dose is 15 mg/kg PO TID (actual body weight) with total daily dose not to exceed 3 grams (i.e. maximum 1 g PO TID). Doses are rounded to the nearest strength tablets. Equivalent IV dosing (1:1 conversion) may instead be given. Guidelines for dose modification are provided in Section 8.15. MMF prophylaxis is discontinued after the last dose on Day 35, or may be continued if there is GVHD.

5.28 Tacrolimus

5.281 Tacrolimus initiation and dosing

Tacrolimus begins on Day 5, at least 24 hours after completion of posttransplantation Cy. Duration of tacrolimus is designated in Section 5.282.

For patients ≥ 18 years old, the tacrolimus starting dose is 1 mg IV QD. Tacrolimus can be changed to a PO BID dosing schedule once a stable therapeutic level is achieved and the patient can tolerate PO medications. Dose is adjusted to maintain a serum trough level of **10 – 15 ng/mL,** with a minimum acceptable trough level of 5 ng/mL.

For patients < 18 years old, the starting dose of tacrolimus is 0.015 mg/kg IV Q12 hours, based on IBW unless the actual body weight is less. Tacrolimus can be changed to a PO BID dosing schedule once a stable therapeutic level is achieved and the patient can tolerate PO medications. Dose is adjusted to maintain a serum trough level of **10 – 15 ng/mL**, with a minimum acceptable trough level of 5 ng/mL.

In the case of prohibitive toxicities to calcineurin inhibitors (e.g., PRES), other immunosuppression may be given after case-by-case discussion with the PI or co-PI.

5.282 Tacrolimus duration

The tacrolimus duration is assigned prospectively in cohorts of patients before initiation of the preparative regimen. Up to two of the following three regimens of shortened-duration tacrolimus will be investigated, beginning with tacrolimus up through and including Day 90. The sequence and evaluation of regimens is specified in Section 9.0.

Stop tacrolimus after last dose on Day 90
or
Stop tacrolimus after last dose on Day 60
or
Stop tacrolimus after last dose on Day 120

Eligibility for protocol-driven, early tacrolimus discontinuation is provided in Section 5.283. All of these eligibility criteria must be met in order to stop tacrolimus at the prespecified time point.

In patients ineligible for protocol-specified early tacrolimus cessation, tacrolimus is discontinued after the last dose on Day 180; however in these patients, tacrolimus may be continued beyond Day 180 if GVHD has occurred or may be discontinued earlier in the context of relapse, progression, graft failure, or prohibitive toxicity. Patients with suspected graft failure should remain on tacrolimus until at least the ~Day 56 chimerism assessment, although earlier discontinuation is permissible after discussion with the PI or co-PI.

5.283 Eligibility for protocol-driven, early cessation of tacrolimus

In order to be eligible for protocol-specified shortened-course tacrolimus, patients must meet all of the following criteria by the scheduled date of tacrolimus cessation:

a. No documented graft failure
b. Presence of at least 5% donor T cell chimerism in peripheral blood and/or bone marrow at ~Day 56 evaluations and beyond
c. No acute, clinical grade II-IV GVHD, whether active or resolved

Note: Eligible patients with acute grade I GVHD by the time of scheduled tacrolimus discontinuation will stop tacrolimus early as planned.

d. No chronic GVHD, whether active or resolved, with the exception of asymptomatic or minimally symptomatic chronic GVHD limited to the oral mucosa.
e. No documented disease progression or relapse
f. No receipt of prohibited preemptive posttransplantation therapy (per Section 5.4)

Evaluations that are required before the protocol-driven, early cessation of tacrolimus are provided in Section 7.0. Early cessation of tacrolimus when required evaluations for eligibility are pending is discussed in Section 5.284.

5.284 <u>Early tacrolimus cessation in the context of pending evaluations</u>

If results of scheduled evaluations are pending at the time tacrolimus is due to stop early, tacrolimus should be stopped according to schedule and the duration of tacrolimus reevaluated when results become available.

For logistical reasons, stopping tacrolimus up to 5 days after the scheduled stop date is permissible in patients for whom early, protocol-specified tacrolimus cessation is planned. Stopping tacrolimus more than 5 days later than the scheduled stop date constitutes a protocol violation and is further discussed in Section 5.286.

5.285 <u>Changes in planned tacrolimus duration</u>

If a patient begins study treatment but, prior to the planned tacrolimus discontinuation date, the target number of patients have been evaluated for safety or stopping criteria are met, the assigned tacrolimus duration for that patient may be changed as appropriate.

5.286 <u>Evaluability for the primary endpoint</u>

For protocol-driven, early cessation of tacrolimus, stopping tacrolimus up to 5 days after the scheduled stop date is permissible for logistical reasons. Such patients will be considered evaluable for both the safety and feasibility of early tacrolimus cessation.

Patients who should stop tacrolimus within this time frame (per protocol-driven criteria), but do not for logistical reasons, are unevaluable for safety; whether they remain evaluable for feasibility depends on the cause. If tacrolimus is not stopped in the allowable window because of pending evaluations, feasibility will be based on the results of those evaluations, provided they were done no later than 5 days past the prespecified date of tacrolimus cessation. Cases in which eligible patients do not stop tacrolimus early because of physician discretion will count *against* feasibility. If there are other logistical reasons for not stopping tacrolimus in the designated time frame, or if eligibility evaluations for early tacrolimus cessation are performed more than 5 days past the planned stop date, patients will be considered unevaluable for safety and feasibility.

Patients who develop a prohibitive toxicity to tacrolimus resulting in its earlier than scheduled discontinuation will be considered unevaluable for the primary endpoint. Patients who receive preemptive posttransplantation therapy that is not permitted on this study (Section 5.4) may be unevaluable for the primary endpoint.

Patients who are not evaluable for the primary endpoint may, if needed, be replaced but will continue on study unless consent is withdrawn.

5.29 Growth factors

GCSF (filgrastim) begins on Day 5 at a dose of 5 mcg/kg/day (actual body weight) IV or subcutaneously (rounding to the nearest vial dose is allowed), until the absolute neutrophil count (ANC) is $\geq$ 1,000/mm^3 over the course of three days. Additional GCSF may be administered as warranted. Pegfilgrastim (Neulasta®) and GM-CSF are not permitted.

5.3 Supportive care

Patients will receive transfusions, nutritional support, infection prophylaxis and treatment, and other supportive care according to standard of care and institutional guidelines.

5.31 Anti-ovulatory treatment

Menstruating females should begin an anti-ovulatory agent before starting the preparative regimen.

5.32 Intravenous access

A double lumen central venous catheter is required for administration of IV medications and blood products.

5.33 Infection prophylaxis

Patients will receive infection prophylaxis and treatment according to institutional guidelines. Infection prophylaxis should include agents or strategies to prevent herpes simplex, CMV (e.g., CMV PCR screening and preemptive therapy), Pneumocystis jirovecii, fungal infections, and infections from oral flora secondary to mucositis. Posttransplantation immunizations will be given per institutional standard.

5.34 Antiemetics

Note that steroids should not be used as an antiemetic agent after the graft is infused, until at least 24 hours after the completion of all posttransplantation Cy. The use of steroids as antiemetics after this time frame is discouraged in the absence of relapsed/progressive disease.

5.4 Posttransplantation therapies

5.41 Donor lymphocyte infusion (DLI)

Prophylactic posttransplantation DLI (e.g., for persistent detectable malignancy, prophylaxis in the absence of detectable malignancy, or mixed donor chimerism) is not permitted before Day 200, as this carries a high risk of GVHD. The use of DLI will be recorded and such patients will be censored for analysis of disease and graft failure outcomes, GVHD, and related transplant-related toxicity outcomes. Analysis of outcomes without such censoring is also planned.

5.42 Posttransplantation systemic therapy

Preemptive systemic chemotherapy or biologic therapy (e.g., DNA-methyltransferase inhibitor for MDS, tyrosine kinase inhibitor for Philadelphia chromosome + malignancy, flt3 inhibitor for AML) is permitted after transplantation, with the exception of anti-CD20 antibody therapy such as rituximab. The latter is not permitted because phase II data suggest that posttransplantation rituximab may increase the incidence of acute GVHD (unpublished data). Intrathecal chemotherapy is permitted.

5.43 Posttransplantation radiation

Consolidative radiation therapy is permitted after transplantation.

The use of preemptive therapy will be recorded. Patients who receive such therapies will not be censored for analysis of disease outcomes at that time, except as stated in Section 5.41.

5.5 Posttransplantation follow-up

Required evaluations are designated in Section 7.0.

More frequent monitoring of disease status, vital status, and toxicities may be performed for study purposes including through collection of outside records and patient and physician contact. Patients who relapse or progress will continue to be followed on study unless consent is withdrawn.

Patients will be followed primarily at Johns Hopkins at least until the ~ Day 56 evaluations, then periodically thereafter as designated in Section 7.0. In the event that it is not possible or practical for a patient to come back to Johns Hopkins for required evaluations, clinical and laboratory evaluations performed though a local oncologist may fulfill study requirements. This will be decided on a case-by-case basis by the PI or co-PI; however chimerism assessments must be performed at Johns Hopkins.

6.0 MEASUREMENT OF EFFECT AND ENDPOINTS

6.1 Hematologic parameters

6.11 Neutrophil recovery: Post-nadir ANC $\geq$ 500/mm^3 for three consecutive measurements on different days. The first of the three days will be designated as the day of neutrophil recovery.

6.12 Platelet recovery: Sustained platelet count $\geq$ 20,000/mm^3 or $\geq$ 50,000/mm^3 with no platelet transfusions in the preceding seven days. The first of three consecutive measurements on different days will be designated as the day of initial platelet recovery.

6.13 Donor chimerism: Mixed donor chimerism is defined as $\geq$ 5%, but < 95%, donor. Full donor chimerism is defined as $\geq$ 95% donor.

Prior to transplantation, a sample of peripheral blood from the patient, and either harvested bone marrow or blood from the donor, are collected for genetic studies to establish a baseline for subsequent chimerism assays.

Chimerism determinations from T cells (CD3$^+$ sorted) and whole blood (total nucleated cells) will be made from peripheral blood per Section 7.0, and more frequently as indicated. Methods may include (i) PCR analysis of variable number of tandem repeats (VNTR) in PBMC if informative, (ii) restriction fragment length polymorphism (RFLP) if the donor and recipient RFLPs are informative, (iii) fluorescence in-situ hybridization (FISH) for Y-chromosome markers on PBMC if the donor is male and patient is female, (iv) cytogenetic analysis, (v) flow cytometric analysis of HLA-A, B or DR on lymphocytes in the peripheral blood if haploidentical and suitable reagents exist. Chimerism may also be determined from the bone marrow.

6.14 Graft failure: < 5% donor chimerism in blood and/or bone marrow on ~Day 28 or after and on all subsequent measurements.

- Primary graft failure: < 5% donor chimerism in blood and/or bone marrow by ~ Day 56
- Secondary graft failure: achievement of $\geq$ 5% donor chimerism, followed by sustained <5% donor chimerism in blood and/or bone marrow.

< 5% donor T cell chimerism, but with $\geq$ 5 % donor chimerism in total leukocytes, is not considered graft failure.

6.2 Graft-versus-host disease

6.21 **Acute GVHD**: Acute GVHD is graded by standard criteria (Appendix).[29] All suspected cases of acute GVHD must be confirmed histologically by biopsy of an affected organ (e.g., skin, liver, or gastrointestinal tract). Date of symptom onset, date of biopsy confirmation of GVHD, maximum clinical grade, sites affected, and dates and types of treatment will be recorded. Dates of symptom onset of grade II or higher GVHD and grade III-IV GVHD will be recorded.

The cumulative incidences of acute grade II-IV and grade III-IV GVHD will be determined through competing risk analysis. Treatment of relapse/progression, graft failure, and death are considered competing risks for GVHD for study purposes. In addition, GVHD will be reported with only graft failure and death regarded as competing risks.

6.22 **Chronic GVHD:** Chronic GVHD is graded by both NIH consensus criteria[30] and Seattle criteria.[31] Date of onset, date of biopsy confirmation (if any), dates and types of treatment, and extent will be recorded. The cumulative incidence of chronic GVHD (overall and according to extent) will be determined through competing risk analysis.

6.3 Disease and survival endpoints

6.31 **Progression-free survival**: Interval from Day 0 to date of first objective disease progression or relapse, death from any cause, or last patient evaluation. Patients who have not progressed or died will be censored at the last date they were assessed and deemed free of relapse or progression. Disease persistence in the absence of progression is not included in this analysis.

6.32 **Event-free survival**: Interval from Day 0 to date of first objective disease progression or relapse, an unplanned therapeutic maneuver for disease persistence, death from any cause, or last patient evaluation. Patients without events will be censored at the last date they were assessed and deemed event-free.

6.33 **Overall survival**: Interval from Day 0 to date of death from any cause or last patient contact.

6.34 **Nonrelapse mortality**: Death without evidence of disease progression or relapse. Relapse/progression is a competing risk for NRM.

6.35 **Relapse or progression**: Defined per the following response criteria:

a. Lymphoma: 2007 International Working Group (IWG) criteria for lymphoma [32]
b. CLL: 2008 International Workshop criteria [33]
c. Multiple myeloma: International Myeloma Working Group uniform response criteria [34]
d. Acute leukemia: 2010 European LeukemiaNet criteria,[35] based on 2003 IWG criteria [36]
e. MDS: 2006 IWG criteria [37]

Designation of disease status in other histologies will also follow standard criteria. NRM is a competing risk for relapse/progression.

6.35 **Minimal residual disease (MRD)**: MRD is defined by the sole evidence of malignant cells by flow cytometry, FISH, PCR or other techniques, in absence of morphological or cytogenetic evidence of disease in blood or marrow. Since the frequency and sensitivity of testing for MRD are variable, evidence of MRD will not be sufficient to meet the definition of relapse or progression in this study, but will be captured in the case report forms along with data on changing management in response to MRD detection.

6.4 Correlative studies

Patients' peripheral blood specimens will be collected per Section 7.0 and banked for future research on immune reconstitution and on clinical outcomes including GVHD. Planned studies include but are not limited to characterization of humoral and cellular immune reconstitution at ~ 2 months and ~ 6 months posttransplantation, and analysis of cellular immunity including T regulatory cell subpopulations in relation to the development of GVHD.

7.0 STUDY PARAMETERS

The following table summarizes the minimum testing and clinical assessments required for study purposes. This is in addition to other testing and assessments indicated as standard of care, which may be collected for study purposes.

Table: Study Parameters

	Baseline [1,2]	D28 +/- 3 d	D56 +/- 3 d	D84 +/- 5 d	D112 +/- 5 d	D180 +/- 7 d	D270 +/- 21 d	D365 +/- 30 d [c]
Standard pre/post transplant evaluations [a, b]								
History and physical exam	X		X	X [m]	X [m]	X	X [m]	X
Performance status	X							
CBC / differential [d]	X	X	X	X [m]	X [m]	X [m]	X [m]	X [m]
Comprehensive metabolic panel (CMP) [e]	X		X	X [l]	X [l]	X [l]	X [l]	X [l]
Infectious disease evaluations [f]	X							
Serum HCG (if applicable)	X							
LV ejection fraction	X							
Pulmonary function tests	X							
Bone marrow biopsy and aspirate with flow cytometry and relevant cytogenetic and molecular studies [g]	X		X, with chimerism analysis [h]			X, with chimerism analysis [h]		X, with chimerism analysis [h]
CT of sinuses	X							
CT, PET/CT, or MRI of chest, abdomen, and pelvis (lymphoma and CLL only)	X		X			X [m]		X [m]
Response assessment to last therapy [i]	X							
HLA typing	X							
Lymphocytotoxic antibody screen	X							
Donor marrow or blood for VNTR or RFLP analysis [j]	X							
Patient blood for baseline VNTR or RFLP analysis [j]	X							
Peripheral blood chimerism, both total leukocyte (unsorted) and T-cell sorted [j]		X	X	X [l]	X [l]	X [l]	X [l]	X [l]
GVHD and other morbidity assessments [k]			X	X [l]	X [l]	X [l]	X [l]	X [l]
Additional evaluations								
Patient research blood			X [n]			X [n]		

[a] Baseline evaluations should occur ≤ 1 month before initiation of conditioning therapy, with the exception of the following: cardiac and pulmonary evaluations may occur ≤ 8 weeks prior, and the HLA typing and baseline studies for chimerism determinations may occur at any point prior. Results of evaluations performed before study entry as standard of care may be used for research purposes and to fulfill study requirements.
[b] Demographics and baseline characteristics will be captured. Characteristics to be recorded include: age, gender, race/ethnicity, performance status, disease type, remission status, prior treatments including prior transplantation and type, donor age, donor relationship to patient, donor gender, type of transplant (HLA-matched or mismatched), CMV serostatus of patient and donor, ABO compatibility.
[c] Patients should continue to follow-up at Johns Hopkins at least yearly on study, per institutional standard of care. Follow-up data may be captured more frequently for study purposes. Data that will continue to be recorded beyond 1 year include disease status until first relapse/progression, vital status, major transplant-related toxicities, and GVHD.

[d] At minimum, CBC/differential should also be performed twice a week from start of preparative regimen, until ANC is ≥1000/uL over course of 3 days, then weekly until 12 weeks posttransplantation, and periodically thereafter; those need not be captured in the CRF.
[e] CMP includes: BUN, creatinine, sodium, potassium, chloride, AST, ALT, total bilirubin, alkaline phosphatase. At minimum, these should be performed weekly until 12 weeks posttransplantation, then periodically until off immunosuppression; those need not be captured in the CRF.
[f] Infectious disease evaluations follow institutional standard of care. Minimum evaluations are CMV IgG, HSV IgG, VZV IgG, hepatitis panel (Hep B surface antigen, Hep B core antibody, Hep C antibody), HIV antibody (unless known to be HIV positive).
[g] Flow cytometry in diseases other than Hodgkin's lymphoma. Follow-up studies should include relevant cytogenetics and molecular markers to detect residual disease, i.e. repeat of studies found to be positive at baseline.
[h] For leukemia (including CLL), MDS, myeloproliferative disease, and myeloma; for lymphoma patients, required if bone marrow was positive on baseline (pretransplantation) evaluation. May be omitted if there is documented disease progression or relapse before scheduled assessment.
[i] Include comparison of pre- and post-treatment scans with bidimensional measurements where relevant.
[j] Collect 10 cc lavender top.
[k] GVHD and other morbidity assessments are also standardly performed weekly at Johns Hopkins until at least ~Day 60. Results of these and subsequent assessments may be collected for research purposes. Patients may be asked to complete GVHD questionnaires.
[l] Day 84 and later CMP, chimerism, and GVHD evaluations may be omitted in patients with documented graft failure. CBC/differential, H & P, and morbidity assessment at these time points will be obtained.
[m] May be omitted in patients who receive treatment for disease progression or relapse. The dates of treatment initiation and DLI will be recorded.
[n] Collect 60 cc in green top (heparinized) tubes. Deliver at room temperature to Human Immunology Core for cryopreservation. May be omitted at PI or co-PI discretion.

8.0 RISKS AND REPORTING REQUIREMENTS

8.1 Drug information

8.11 Cyclophosphamide (Cytoxan®)

Cyclophosphamide is an alkylating agent whose metabolites form cross-links with DNA resulting in cell cycle-nonspecific inhibition of DNA synthesis and function. Cyclophosphamide side effects include: nausea, vomiting, diarrhea, headache, dizziness, hemorrhagic cystitis, fluid weight gain/edema, SIADH, transaminitis, cardiomyopathy, pericarditis, rash, mucositis, alopecia, cytopenias, sterility, and rarely, secondary myelodysplastic syndrome and anaphylaxis.

Dose adjustments for cyclophosphamide will not be made.

8.12 Mesna (sodium-2-mercapto ethane sulphonate)

Mesna is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxasophosphorines (cyclophosphamide and ifosphamide). It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxasophosphorines, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxasophosphorines.

The total daily dose of mesna is equal to 80% of the total daily dose of cyclophosphamide.
At the doses used for uroprotection, mesna is virtually non-toxic. However, potential adverse effects include nausea and vomiting, diarrhea, abdominal pain, altered taste, rash, urticaria, headache, joint or limb pain, hypotension, and fatigue.

8.13 Fludarabine (Fludara)

Fludarabine is a purine analog antimetabolite. Side effects of fludarabine include:

a. Neurotoxicity: Agitation or confusion, blurred vision, loss of hearing, peripheral neuropathy or weakness have been reported. Severe neurologic effects, including blindness, coma, and death may occur; severe CNS toxicity is rarely seen with doses in the recommended range for nontransplant therapy. The dose used in this study is approximately 1.5 times the usual one-course dose given in non-transplant settings. Doses and schedules similar to those used in this study have been used in adult and pediatric patients without observed increase in neurotoxicity.

b. Anemia: Life-threatening and sometimes fatal autoimmune hemolytic anemia has been reported after one or more cycles of therapy in patients with or without a previous history of autoimmune hemolytic anemia or a positive Coombs' test and who may or may not be in remission. Corticosteroids may or may not be effective in controlling these episodes. The majority of patients re-challenged developed a recurrence of the hemolytic process.

c. Cardiovascular: Deep venous thrombosis, phlebitis, transient ischemic attack, and aneurysm (1%) are reported.

d. Fever: 60% develop fever.

e. Rash: 15% develop a rash, which may be pruritic.

f. Digestive: Gastrointestinal side effects include: nausea/vomiting (36%), diarrhea (15%), stomatitis (9%), anorexia (7%), GI bleeding and esophagitis (3%), mucositis (2%), liver failure, abnormal liver function test, constipation, dysphagia (1%) and mouth sores.

g. Some other effects include: Chills (11%), peripheral edema (8%), myalgias (4%), osteoporosis (2%), pancytopenia, arthralgias (1%), dysuria (4%), urinary tract infection and hematuria (2%); renal failure, abnormal renal function test, and proteinuria (1%); and, very rarely, hemorrhagic cystitis and pulmonary toxicity.

Dose adjustments of fludarabine are required for renal insufficiency (see Section 5.21).

8.14 Total Body Irradiation (TBI)

TBI can cause: nausea and vomiting, diarrhea, parotitis (rapid onset within 24-48 hours, usually self-limited), generalized mild erythema (usually within 24 hours, resolving in 48-72 hours) , hyperpigmentation, fever, mucositis, alopecia, and pancytopenia. Late effects include: cataracts (10-20%), hypothyroidism, nephropathy, interstitial pneumonitis, veno-occlusive disease, carcinogenesis, and sterility.

8.15 Mycophenolate Mofetil (MMF, Cellcept®)

MMF is an ester prodrug of the active immunosuppressant mycophenolic acid (MPA).
Side effects include: pancytopenia, infection (including sepsis, CMV, HSV, VZV, and Candida), nausea, vomiting, diarrhea, allergic reactions, hypertension, headache, dizziness, insomnia, hyperglycemia, electrolyte imbalances, rash, and leg cramps/bone pain.

Drug interactions: MMF activity is decreased with oral antacids and cholestyramine. There are no pharmacokinetic interactions with cotrimoxazole, oral contraceptives, or cyclosporine. Acyclovir or ganciclovir blood levels may increase due to competition for tubular secretion. High doses of salicylates or other highly protein-bound drugs may increase the free fraction of MPA and exaggerate the potential for myelosuppression.

Dose adjustments: No dose adjustments are required for liver dysfunction. For renal insufficiency, MMF dosing should not be modified unless dialysis is needed, in which case MMF can be reduced to 25-50% of the starting dose.

8.16 Tacrolimus (FK-506, Prograf®)

Tacrolimus is a macrolide immunosuppressant that inhibits lymphocytes through calcineurin inhibition.

Toxicities: There is a spectrum of well-described toxicities of tacrolimus. Toxicities include renal insufficiency, hypertension, hyperglycemia, hypomagnesemia, hypokalemia, nausea, diarrhea, headache, neurologic toxicity including tremor and leukoencephalopathy, infection, and rarely thrombotic thrombocytopenic purpura (TTP).

Drug interactions: Tacrolimus is well absorbed orally. Tacrolimus is extensively metabolized by the cytochrome P-450 (CYP3A4) system and metabolized products are excreted in the urine. Drugs that may increase tacrolimus levels include tri-azole drugs (especially voriconazole and posaconazole), nephrotoxic drugs, calcium channel blockers, cimetidine and omeprazole, metoclopramide, macrolide antibiotics, quinupristin/dalfopristin, danazol, ethinyl estradiol, methylprednisolone, and HIV protease inhibitors. Drugs that may decrease tacrolimus levels include some anticonvulsants (phenobarbital, phenytoin, carbamezepine), caspofungin, rifamycins, and St. John's wort.

Dose adjustments: The tacrolimus dose is adjusted to maintain a serum trough level of 5-15 ng/mL, with a target of 10-15 ng/mL. Patients with hepatic or renal insufficiency should receive doses at the lower end of therapeutic concentrations. No dose adjustments are required in patients undergoing hemodialysis.

Due to extreme interactions with voriconazole and posaconazole, the tacrolimus dose should be empirically lowered when these azoles are initiated at steady state levels of tacrolimus. Guidelines are provided in the table below. Dose adjustments for therapy with other azoles may be indicated. However, the initial tacrolimus dose (on Day 5) remains fixed.

Dosing considerations with concurrent azole therapy: Triazole antifungal medications are expected to increase serum CNI levels; therefore dosages of CNI's should be adjusted accordingly. Guidelines are provided in the table below. Of note, reversal of azole-mediated inhibition of CYP3A4 (and others) and P-glycoprotein is gradual when azoles are stopped. Therefore, immediate significant dose increases in tacrolimus are not advised when azoles are stopped. Rather, tacrolimus dose increases should be cautious and based on more frequent monitoring of levels as appropriate.

Table: Suggested preemptive dose reduction of tacrolimus when azoles are initiated at steady state levels of tacrolimus

Antifungal	**Tacrolimus**	
	Dose ↓	**Comment**
Voriconazole	67%	Strongly advised
Posaconazole	67%	Advised
Itraconazole	50%	Advised
Fluconazole	25%	Consider

8.2 Toxicity grading

Toxicities are graded using the NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)

8.3 Toxicity reporting

The agents being used in the study are used extensively in the BMT setting and have well-defined toxicity profiles. In addition, there are many expected toxicities of allogeneic BMT. The following are examples of toxicities that are serious but not unexpected: Grade 4 cytopenias; neutropenic fever and sepsis; bacterial, fungal, or viral (including CMV, BK virus) infection; severe mucositis; severe GVHD; hepatic veno-occlusive disease; pulmonary toxicities; hemorrhagic cystitis; bleeding without hemodynamic compromise.

For study purposes, the following will be recorded and reported in accordance with IRB requirements:

a. Any hospitalization and its reason in the first year of transplant, with the exception of hospitalizations related to relapsed disease or second BMTs.
b. Neutropenic fever is an expected, common complication; as such, hospitalizations for grade 4 neutropenic fever will be reported in real-time to the IRB with hospitalizations for lesser grade neutropenic fever routinely reported on a yearly basis.
c. Any death before Day 200, and any later death which is potentially transplant-related.
d. Any unexpected, serious events deemed significant by the PI.

In addition, the following toxicities will be tracked for study purposes and reported on a yearly basis to the IRB, or earlier if warranted:

a. Clinically significant infections during the first year of transplant, with the exception of uncomplicated, culture-negative neutropenic fever. This includes CMV disease, other clinically significant documented viral infections, bacterial infections, and proven or probable invasive fungal infections.
b. CMV reactivation (including asymptomatic reactivation)
c. Hepatic veno-occlusive disease
d. Grade 3 or greater pulmonary toxicity during the first year of transplant that is potentially transplant-related

Additional complications and toxicities may be tracked. This is in addition to evaluating hematologic parameters, GVHD, and disease and survival endpoints outlined in Section 6.0.

8.4 Monitoring plan

This is a Level I study under the SKCCC at Johns Hopkins Data and Safety Monitoring Plan. The protocol will be monitored internally by the PI. Data and safety monitoring oversight will be conducted by the SKCCC at Johns Hopkins Safety Monitoring Committee. An audit will be performed early in the study, and then audits will be performed periodically thereafter.

8.5 Risks and benefits

Allogeneic BMT carries risk for major morbidity and mortality. Major toxicities and risks of the transplant procedure include acute and chronic GVHD, severe infection, immunosuppression which may be prolonged, graft failure, end-organ damage, and death. High-dose posttransplantation Cy appears to significantly lower the risk of GVHD. Shorter-duration immunosuppression may be associated with increased risk of GVHD, increased severity of GVHD, and graft failure.

The potential benefits of this trial are palliation of disease-related symptoms and prolongation of overall or event-free survival, including the possibility of long-term disease-free survival and cure. Potential benefits also include fewer infectious and other complications and lower risk of relapse because of the shorter duration of immunosuppression.

9.0 STATISTICAL CONSIDERATIONS

9.1 Primary endpoint and design

The primary goal of this study is to determine whether one can shorten the duration of immunosuppression with tacrolimus following nonmyeloablative, related donor BMT that incorporates high-dose posttransplantation Cy.

To be relevant, an immunosuppression regimen must not only be safe, but be applicable to a sufficient number of patients. Therefore, both safety and feasibility are incorporated in the primary analysis. Feasibility is herein defined as the proportion of patients, measured from Day 0, who meet criteria for protocol-driven, early tacrolimus cessation (as defined in Section 5.283) and have tacrolimus stopped at the prespecified time point. Monitoring rules for feasibility (based on the proportion of patients who are able to stop tacrolimus at a prespecified time point) and safety (i.e. complications do not exceed cause-specific thresholds defined below) will be implemented to guide the choice of tacrolimus duration (tacrolimus until Day 60, 90, 120 or the historical standard, Day 180). The planned duration of tacrolimus will be assigned prior to the start of the preparative regimen.

It is possible that longer durations of immunosuppression are more safe (e.g., lower risk of GVHD), but less feasible (e.g., greater possibility of NRM or another event that would prevent protocol-driven early cessation of immunosuppression, as defined in Section 5.283). Conversely, shorter durations of immunosuppression may be more feasible but unsafe (e.g., excessive GVHD). Based on historical information, we expect that it will be feasible to reduce the duration of tacrolimus from the current standard (until Day 180) to until Day 90. We hypothesize that stopping tacrolimus on Day 90 will not carry excess risk of GVHD or graft failure (potential manifestations of inadequate immunosuppression).

9.11 <u>Sequence of study</u>

The duration of tacrolimus will be evaluated in the following order, with monitoring rules (specified in Section 9.12) based on feasibility and safety. A maximum of two reduced durations of tacrolimus will be evaluated, and based on this, a decision made in favor of a particular tacrolimus duration (until Day 60, 90, 120, or 180).

a. Begin with tacrolimus until Day 90.

b. If tacrolimus until Day 90 appears feasible but unsafe, evaluate tacrolimus until Day 120 with analogous monitoring rules.
    - If tacrolimus until Day 120 appears both feasible and safe, we will decide in favor of this regimen. Otherwise, we will decide in favor of tacrolimus until Day 180.
    - If accrual to the tacrolimus until Day 90 arm is complete, subsequent patients can be assigned to tacrolimus until Day 120 while evaluation of the Day 90 arm is pending.

c. If tacrolimus until Day 90 appears both safe and feasible, evaluate tacrolimus until Day 60 with analogous monitoring rules.
    - If tacrolimus until Day 60 appears both safe and feasible, we will decide in favor of this regimen. Otherwise, we will decide in favor of tacrolimus until Day 90.

d. If tacrolimus until Day 90 does not appear feasible (such that less than the minimum target proportion of patients are able to discontinue tacrolimus), and this is due to high rates of GVHD or graft failure (or low donor T cell chimerism) or both, tacrolimus until Day 60 will not be evaluated and we will decide in favor of tacrolimus until Day 180. Otherwise, tacrolimus until Day 60 will be studied as this may be more feasible than tacrolimus until Day 90 (i.e., less time to develop an event that would preclude early cessation of immunosuppression), and may be associated with frequencies of GVHD, graft failure, NRM, and relapse/progression that do not exceed the cause-specific thresholds defined below (Section 9.12).

9.12 Criteria for feasibility and safety

This study considers it "feasible" to stop tacrolimus early (e.g., Day 90) if at least one-third (33%) of all evaluable transplanted patients have not had any of the events (defined in Section 5.283) that would render them ineligible to stop at the prespecified time point and who have tacrolimus stopped accordingly. A Bayesian stopping rule will be implemented to declare a particular shortened duration of tacrolimus not feasible, if there is 80% or more certainty that fewer than 33% of patients could have tacrolimus stopped early. The Bayesian rule begins with a Beta (3, 3) prior probability distribution that tacrolimus until Day 90 is feasible. This prior distribution is based on historical information below and has mean 50% and 90% probability that the fraction of patients who will be available to have tacrolimus stopped is between 32% and 68%. For a particular planned duration of tacrolimus, up to 55 patients will be transplanted in order to identify at least 15 who are evaluable for the safety of early tacrolimus cessation. Up to 5 additional patients may be transplanted per regimen to replace unevaluable patients (per Sections 4.1 and 5.286).

Historical estimates were derived from 212 uniformly treated patients with hematologic malignancies (fludarabine/Cy/TBI conditioning, with partially HLA-mismatched BMT, posttransplantation high-dose Cy, MMF on Days 5-35, and tacrolimus on Days 5-180). Event-specific risks were calculated within 4 intervals: Days 1-60, 61-90, 91-120, and 121-180. (Note that these probabilities are specific to the time interval indicated for each row and conditional on reaching the interval.)

Days	Graft Failure	NRM	Relapse	GVHD[a]	No Event
1-60	0.1133	0.0191	0.0803	0.2217	0.5656
61-90	0.0254	0.0337	0.0337	0.0586	0.8486
91-120	0.0005	0.0005	0.0983	0.0494	0.8513
121-180	0.0006	0.0120	0.1610	0.0235	0.8029

[a] acute grade II or higher or chronic GVHD

Separate stopping rules will be implemented for each of 4 events occurring between the date of tacrolimus discontinuation and the Day 180 evaluation (Day 180 evaluations are +/- 7 days for logistical reasons): acute, clinical grade II or higher GVHD or severe chronic GVHD; graft failure; NRM; and disease relapse or progression. The latter is incorporated so as to have a sufficient number of patients evaluable for safety. For the stopping rules, the prior precision of these event-specific risks within each interval corresponds to a prior sample size of 53 patients, one-fourth of the 212 historical sample size. We discounted the historical data to avoid having the prior information dominate the inference from the current study's data.

The table above shows risks for acute grade II-IV and chronic GVHD combined. Of the 61 GVHD events, 3 were chronic. We combined these three events with the 58 acute GVHD events to be more conservative in our stopping rules. Given the discounting of the prior data, these three events contribute very little influence on the performance of the stopping rules, relative to the acute GVHD events in the historical data. The simulation results below reveal that these stopping rules perform well and provide reasonable protection of patients against unexpectedly elevated risks.

The estimated historical cause-specific (cumulative) risks between Day 91 and 180 are 7% (GVHD), <1% (graft failure), 1.3% (NRM), and 26% (relapse). For monitoring, we will compute the posterior probability of each of four events, given the data and a historical prior distribution, and evaluate whether to stop throughout the study. The stopping rule declares a particular duration of tacrolimus unsafe if the posterior probability is 67% or higher that the risk of that adverse event between the day tacrolimus stops and ~ Day 180 overly exceeds the following cause-specific probabilities: stopping for ≥ 20% combined of acute, clinical grade II or higher GVHD and severe chronic GVHD, ≥ 10% NRM, or ≥ 5% graft failure in this window, or stopping for a ≥ 50% incidence of disease relapse or progression in this window.

Although chronic GVHD usually manifests later than Day 180, the window chosen for the primary monitoring rule is justifiable for several reasons: a) In our haploidentical BMT experience, the majority of patients who develop chronic GVHD will have had acute GVHD; [3] b) A more extended observation window for stopping rule purposes would make timely completion of the trial difficult; and c) In the setting of myeloablative, matched, related or unrelated donor BMT that utilizes high-dose posttransplantation Cy as the sole agent for GVHD prophylaxis (i.e., no mycophenolate mofetil or CNI's), the rate of chronic GVHD has been very low.[22]

Patients who develop both acute and chronic GVHD will be regarded as having one adverse GVHD event for stopping rule purposes. Patients who develop GVHD after treatment for relapse/progression will be regarded as having a competing risk for GVHD. However, patients who have relapse/progression then develop either acute grade II-IV GVHD or severe chronic GVHD by ~Day 180, before treatment of that relapse/progression, will be regarded as having both relapse/progression and an adverse GVHD event for stopping rule purposes.

Monitoring will occur in groups of 5 assessable patients, beginning after 18 assessable patients have been transplanted.

In deciding upon the safety of a particular duration of tacrolimus, the stopping rule(s) met will be considered. Excess GVHD or graft failure is a potential manifestation of inadequate immunosuppression, thus would lead one to favor a longer course of tacrolimus. NRM may or may not be due to less immunosuppression; thus if only the NRM rate exceeds the predefined threshold, the recommended duration of tacrolimus will be contingent on the causes of NRM (whether they are unrelated or possibly related to reduced immunosuppression). On the other hand, excess relapse/progression is not an expected manifestation of inadequate immunosuppression, since shorter-course or lower-dose CNI's may lower the relapse risk.[27,28] If only this stopping criterion is met, the length of time that the relapsed patients are evaluable for safety endpoints will be assessed and a decision rendered whether to increase the accrual goal.

It is possible that with the combined analysis of HLA-matched and HLA-mismatched transplants, an increased risk of graft failure, GVHD, or NRM with shortened-course immunosuppression, as compared to the historical outcomes with HLA-mismatched BMT, may be less readily identified. However, most of the transplants will be HLA-mismatched, and the potential benefits to shortened-duration immunosuppression are not specific to the type of transplant. Inclusion of HLA matched transplants is justified because a) there will be continual monitoring for safety as described above; b) with postgrafting immunosuppression that includes high-dose Cy, our rates of graft failure and GVHD appear to be similar in the HLA-matched and -mismatched settings (Burroughs LM et al, BBMT 2008;14(11):1279-87),[2] and c) with such postgrafting immunosuppression, increasing degrees of HLA mismatch have not been found to be associated with inferior outcomes in haploidentical BMT (Kasamon YL et al, BBMT16(4):482-9).[21]

9.13 <u>Operating characteristics of design</u>

We carried out simulations of possible risks of each of four events among simulated groups of patients enrolling in the study. The four events of interest are a) graft failure, b) relapse/progression, c) NRM, and d) GVHD. We used historical information to derive a prior distribution for the risks of each of these four events within each of the following time intervals (days) following the transplant:

1-60, 61-90, 91-120, and 121-180. Monitoring in groups of 5 patients started after 18 patients entered the study. The following table shows results for the first scenario when tacrolimus is to stop at Day 90 and the simulated risks are the same as those seen historically. Other scenarios are given in the appendix. Each scenario is shown with operating characteristics based on 1000 simulations.

Scenario #1: Historical risks of events within each interval

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.1133	0.0191	0.0803	0.2217	0.5656
61-90	0.0254	0.0337	0.0337	0.0586	0.8486
91-120	0.0005	0.0005	0.0983	0.0494	0.8513
121-180	0.0006	0.0120	0.1610	0.0235	0.8029

After 1000 simulations with the historical probabilities of events and a stopping criterion for each event's risk between days 91 and 180 of: Graft Failure = 0.05, NRM = 0.10, relapse/progression = 0.50, GVHD = 0.20:

Stopped for lack of feasibility 8 (0.8%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety or relapse problem declared 0 (0%) times.
Safety or relapse problem by reason (% of times):

GraftFail	NRM	Relapse	GVHD	No Event
0	0	0	0	0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.00	28.00	33.00	32.74	38.00	53.00

9.2 Secondary endpoints

a. In patients eligible for reduced-duration tacrolimus, estimate the incidences of acute grade II-IV GVHD, acute grade III or higher GVHD, chronic GVHD, graft failure, relapse, and NRM between the date of early tacrolimus cessation and Day 180, and beyond Day 180.

Cumulative incidences of GVHD, relapse and NRM will be computed in these time frames using Fine and Gray's method for competing risks.[38,39] Treatment of relapse/progression, graft failure, and death are considered competing risks for GVHD; relapse/progression is a competing risk for NRM; and death before relapse/progression is a competing risk for relapse/progression. In addition, we plan to report GVHD incidences with only graft failure and death regarded as competing risks.

The graft failure frequency in evaluable patients will be reported with 90% confidence intervals.

Although historically, most cases acute GVHD develop before Day 90 and graft failure after Day 60 is rare, the time course for these events in patients treated with less than 6 months of immunosuppression is not well defined. The observation time for the primary safety monitoring rule varies according to tacrolimus duration, and it is possible though not expected that excess GVHD (acute or chronic), graft failure, or NRM will develop after Day 180. Although these events are not incorporated in the primary monitoring rule, their incidences will be continually monitored, and the trial will pause pending IRB review should these incidences appear prohibitive.

These outcomes and the outcomes in points b through f below will be described with HLA-mismatched and HLA-matched transplants combined, and individually where appropriate.

b. Estimate the cumulative incidences of acute grade II-IV GVHD, acute grade III-IV GVHD, chronic GVHD, relapse and NRM for the group overall.

The cumulative incidence of each of these events will be calculated from Day 0 using Fine and Gray's method for competing risks.

c. Estimate the cumulative incidence of systemic steroid initiation, the cumulative incidence of non-steroid immunosuppression use, and the cumulative incidence of discontinuation of systemic immunosuppression for GVHD treatment by 1 year and 2 years after BMT for the group overall and for patients with shortened-duration tacrolimus; and describe the number and types of systemic immunosuppression used for GVHD treatment.

These cumulative incidences will be similarly estimated using competing-risk analyses, wherein graft failure and death, or graft failure, death and treatment of relapse/progression, are considered competing risks. The number and types of systemic immunosuppression used for GVHD treatment will be reported descriptively.

d. Estimate event-free survival, progression-free survival, and overall survival after transplantation.

Using the Kaplan-Meier method, the probabilities of 1-year and longer-term event-free survival, progression-free survival, and overall survival will be estimated and reported with 90% confidence intervals. The proportions of patients who are event-free at 1 year and who are alive at 1 year will also be estimated with a 90% exact binomial confidence interval.

e. Describe the graft failure frequency, kinetics of neutrophil and platelet recovery, and kinetics of donor chimerism in unsorted and $CD3^+$ sorted peripheral blood.

The graft failure frequency in evaluable patients (those having chimerism results at least at ~Day 28) will be described, with 90% confidence intervals. Times to neutrophil and platelet recovery will be described with medians and ranges, and with cumulative incidence functions with death before count recovery as a competing risk. The degree of donor chimerism in unsorted and $CD3^+$ sorted peripheral blood at predefined time points (per Section 7.0) will be summarized with medians and ranges, and the proportion reaching full donor chimerism (total leukocyte, T cell) by ~ Day 28 and ~ Day 56 will be estimated with 90% confidence intervals. The proportion achieving >50% T cell donor chimerism at ~ Day 28 and ~ Day 56 will similarly be estimated.

The association between the amount of donor T cell chimerism at ~ Day 28 and patient/graft characteristics (e.g., prior therapies, graft cell dose) and transplantation outcomes (sustained engraftment, relapse or progression, GVHD) will be investigated. Because of the limited sample size these investigations will be exploratory.

f. Characterize immune reconstitution after transplantation and its relationship to duration of pharmacologic immunosuppression and clinical outcomes.

Patterns of immune reconstitution at ~ 2 months and ~ 6 months after transplantation, including peripheral blood concentrations of T cells, B cells, and natural killer cells, in relation to tacrolimus duration will be described using summary statistics. Descriptive comparisons of immune reconstitution in patients treated with shortened-duration tacrolimus will be made to that of historical patients who did not experience GVHD, graft failure, or relapse and were treated with tacrolimus until Day 180. In addition, the association of cellular immune reconstitution, including frequencies of T regulatory cell subpopulations and early changes in these populations, with the development of GVHD will be described with boxplots and logistic or GEE (generalized estimating equations) regression models.

g. Describe major toxicities and complications associated with the transplantation procedure. Selected toxicities will be reported descriptively.

h. Evaluate selected patient and transplant characteristics in relation to transplantation outcomes. The association between selected baseline patient and transplant characteristics and event-free survival, overall survival, relapse, and GVHD will be investigated using Cox proportional hazard models or proportional hazard models for competing risk. Planned variables include donor-recipient HLA mismatch status and models of NK alloreactivity; however because of limited sample size, it is anticipated that outcomes in this regard will be analyzed with those of other studies.

10.0 PATHOLOGY REVIEW

Specimens diagnostic of the malignancy (from the original diagnosis and/or relapse) must be reviewed by the Johns Hopkins department of pathology prior to starting protocol therapy. In cases diagnosed solely by peripheral blood flow cytometry, the diagnostic flow cytometry report must be reviewed.

11.0 RECORDS TO BE KEPT

Records to be filed include the following:

a. Patient consent form
b. Registration form
c. Eligibility checklist(s)
d. Case report forms
e. Adverse event report form(s)
f. Follow-up assessments

The principal investigator will review case report forms on a regular basis. Case report forms will be supported by primary source documents.

12.0 PATIENT CONSENT AND PEER JUDGMENT

Current federal, NCI, state, and institutional regulations regarding informed consent will be followed.

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APPENDIX A Acute GVHD Grading

Clinical Staging

Stage	Skin	Liver: Total Bilirubin	Intestinal Tract: Diarrhea
0	No rash	<2.0 mg/dL	≤500 ml/day
1	<25% of skin surface	2.0-3.0	500-1000 ml/day
2	25-50%	3.1-6.0	1001-1500 ml/day
3	Erythroderma	6.1-15.0	>1500 ml/day
4	Erythroderma with bullae and desquamation	>15.0	Severe abdominal pain with or without ileus

Clinical Grading

Grade	Skin*	Liver	GI
I	1-2	0	0
II	3	1	1
III	-	2-3	2-4
IV	4	4	-

*Each column identifies minimum stage for organ grade

From Przepiorka D et al. 1994 Consensus Conference on Acute GVHD Grading. BMT 1995; 15: 825-828.

APPENDIX B **Statistical Supplement**

The following are operating characteristics of the statistical design, with varying simulated risks, and planned cessation of tacrolimus on Day 90. In each scenario, the stopping criteria between Days 91 and 180 are as follows: Graft Failure = 0.05, nonrelapse mortality (NRM) = 0.10, relapse/progression = 0.50, GVHD (acute grade II-IV or severe chronic GVHD) = 0.20. Each scenario is shown with operating characteristics based on 1000 simulations.

Scenario #2: Increased risk of graft failure in first 60 days:
After 1000 simulations with probabilities:

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.50	0.05	0.05	0.10	0.30
61-90	0.01	0.05	0.05	0.10	0.79
91-120	0.01	0.05	0.05	0.10	0.79
121-180	0.05	0.05	0.05	0.10	0.75

Stopped for lack of feasibility 768 (76.8%) times.
Stopped for lack of feasibility at first look 441 (44.1%) times.
Safety or relapse problem declared 15 (1.5%) times.
Safety or relapse problem by reason (% of times):

GraftFail	NRM	Relapse	GVHD	No Event
1.1	0.4	0.0	0.0	0.0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.00	18.00	28.00	32.64	48.00	58.00

Scenario #3: Increased risk of graft failure in first 60 days and of GVHD between Days 91 and 120:
After 1000 simulations with probabilities:

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.50	0.05	0.05	0.10	0.30
61-90	0.01	0.05	0.05	0.10	0.79
91-120	0.01	0.05	0.05	0.30	0.59
121-180	0.05	0.05	0.05	0.10	0.75

Stopped for lack of feasibility 774 (77.4%) times.
Stopped for lack of feasibility at first look 421 (42.1%) times.
Safety or relapse problem declared 41 (4.1%) times.
Percent of times safety or relapse problem by reason (%):

GraftFail	NRM	Relapse	GVHD	No Event
0.3	0.0	0.0	3.8	0.0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.00	18.00	28.00	31.49	43.00	58.00

Scenario #4: Increased risk of graft failure in first 90 days

After 1000 simulations with probabilities:

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.50	0.05	0.05	0.10	0.30
61-90	0.20	0.05	0.05	0.10	0.60
91-120	0.01	0.05	0.05	0.10	0.79
121-180	0.05	0.05	0.05	0.10	0.75

Stopped for lack of feasibility 958 (95.8%) times.
Stopped for lack of feasibility at first look 369 (36.9%) times.
Safety or relapse problem declared 8 (0.8%) times.
Percent of times safety or relapse problem by reason (%):

GraftFail	NRM	Relapse	GVHD	No Event
0.6	0.2	0.0	0.0	0.0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.00	18.00	18.00	24.61	28.00	58.00

Scenario #5: Increased risk of graft failure and GVHD in first 60 days and after Day 90

After 1000 simulations with probabilities:

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.15	0.02	0.12	0.25	0.46
61-90	0.01	0.02	0.05	0.10	0.82
91-120	0.01	0.01	0.12	0.35	0.51
121-180	0.05	0.05	0.16	0.35	0.39

Stopped for lack of feasibility 113 (11.3%) times.
Stopped for lack of feasibility at first look 54 (5.4%) times.
Safety or relapse problem declared 578 (57.8%) times.
Percent of times safety or relapse problem by reason (%):

GraftFail	NRM	Relapse	GVHD	No Event
1.1	0.0	0.0	56.7	0.0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.00	28.00	33.00	33.51	43.00	58.00

Scenario #6: Increased risk of GVHD
After 1000 simulations with probabilities:

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.15	0.02	0.12	0.25	0.46
61-90	0.01	0.02	0.05	0.10	0.82
91-120	0.01	0.01	0.20	0.30	0.48
121-180	0.01	0.05	0.25	0.35	0.34

Stopped for lack of feasibility 118 (11.8%) times.
Stopped for lack of feasibility at first look 63 (6.3%) times.
Safety or relapse problem declared 395 (39.5%) times.
Percent of times safety or relapse problem by reason (%):

GraftFail	NRM	Relapse	GVHD	No Event
0.3	0.0	0.0	39.2	0.0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.00	28.00	38.00	35.96	43.00	58.00

Scenario #7: Increased risk of GVHD and relapse
After 1000 simulations with probabilities:

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.15	0.02	0.12	0.35	0.36
61-90	0.01	0.02	0.25	0.20	0.52
91-120	0.01	0.01	0.50	0.40	0.08
121-180	0.01	0.05	0.25	0.30	0.39

Stopped for lack of feasibility 940 (94%) times.
Stopped for lack of feasibility at first look 384 (38.4%) times.
Safety or relapse problem declared 28 (2.8%) times.
Percent of times safety or relapse problem by reason (%):

GraftFail	NRM	Relapse	GVHD	No Event
0.0	0.0	0.0	2.8	0.0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.00	18.00	18.00	25.04	28.00	58.00

Scenario #8: Increased risk of GVHD and relapse with very few graft failures

After 1000 simulations with probabilities:

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.01	0.02	0.12	0.35	0.50
61-90	0.01	0.02	0.25	0.20	0.52
91-120	0.01	0.01	0.50	0.40	0.08
121-180	0.01	0.05	0.25	0.30	0.39

Stopped for lack of feasibility 632 (63.2%) times.
Stopped for lack of feasibility at first look 354 (35.4%) times.
Safety or relapse problem declared 126 (12.6%) times.
Percent of times safety or relapse problem by reason (%):

GraftFail	NRM	Relapse	GVHD	No Event
0.0	0.0	0.0	12.6	0.0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.00	18.00	33.00	33.58	43.00	58.00

Scenario #9: High probability of relapse after Day 90

After 1000 simulations with probabilities:

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.01	0.02	0.05	0.05	0.87
61-90	0.01	0.02	0.10	0.05	0.82
91-120	0.01	0.01	0.60	0.05	0.33
121-180	0.01	0.05	0.85	0.05	0.04

Stopped for lack of feasibility 0 (0%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety or relapse problem declared 55 (5.5%) times.
Percent of times safety or relapse problem by reason (%):

GraftFail	NRM	Relapse	GVHD	No Event
0.0	0.0	5.5	0.0	0.0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.00	23.00	23.00	23.07	23.00	33.00

Scenario #10: High probability of GVHD after Day 90
After 1000 simulations with probabilities:

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.01	0.02	0.05	0.05	0.87
61-90	0.01	0.02	0.05	0.10	0.82
91-120	0.01	0.01	0.05	0.40	0.53
121-180	0.01	0.05	0.05	0.60	0.29

Stopped for lack of feasibility 0 (0%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety or relapse problem declared 983 (98.3%) times.
Percent of times safety or relapse problem by reason (%):

GraftFail	NRM	Relapse	GVHD	No Event
0.3	0.0	0.0	98.2	0.0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.0	18.0	18.0	18.7	18.0	33.0

Scenario #11: High probability of graft failure after Day 90
After 1000 simulations with probabilities:

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.01	0.02	0.05	0.05	0.87
61-90	0.01	0.02	0.05	0.10	0.82
91-120	0.10	0.01	0.05	0.10	0.74
121-180	0.15	0.05	0.05	0.10	0.65

Stopped for lack of feasibility 0 (0%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety or relapse problem declared 635 (63.5%) times.
Percent of times safety or relapse problem by reason (%):

GraftFail	NRM	Relapse	GVHD	No Event
63.3	0.0	0.0	0.2	0.0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.00	18.00	18.00	20.54	23.00	38.00

Scenario #12: High probability of NRM after Day 90
After 1000 simulations with probabilities:

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.01	0.02	0.05	0.05	0.87
61-90	0.01	0.02	0.05	0.10	0.82
91-120	0.01	0.20	0.05	0.10	0.64
121-180	0.01	0.30	0.05	0.10	0.54

Stopped for lack of feasibility 0 (0%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety or relapse problem declared 781 (78.1%) times.
Percent of times safety or relapse problem by reason (%):

GraftFail	NRM	Relapse	GVHD	No Event
0.2	77.9	0.0	0.2	0.0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.0	18.0	18.0	20.3	23.0	38.0

Scenario #13: Higher probability of relapse after Day 90
After 1000 simulations with probabilities:

Days	Graft Failure	NRM	Relapse	GVHD	No Event
1-60	0.01	0.02	0.05	0.05	0.87
61-90	0.01	0.02	0.10	0.10	0.77
91-120	0.01	0.01	0.85	0.10	0.03
121-180	0.01	0.01	0.90	0.05	0.03

Stopped for lack of feasibility 0 (0%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety or relapse problem declared 40 (4%) times.
Percent of times safety or relapse problem by reason (%):

GraftFail	NRM	Relapse	GVHD	No Event
0.1	0.0	3.9	0.0	0.0

Number of patients transplanted to reach 15:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
18.00	23.00	23.00	24.37	28.00	38.00