Phase II study of shortened-duration immunosuppression following nonmyeloablative peripheral blood stem cell transplant with high-dose posttransplantation cyclophosphamide in malignancies that are challenging to engraft

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INDEX

Schema

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 (adjusted for renal function)
↓
Day –1 **TBI** 200 cGy
↓
Day 0 Infuse T-cell replete peripheral stem cells
Begin antibiotic prophylaxis
↓
Days 3, 4 **Cyclophosphamide** 50 mg/kg IV QD
Mesna 40 mg/kg IV QD in divided doses
↓
Day 5 Begin Sirolimus (preferred) or Tacrolimus , **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 mcg/kg/d SC or IV, continue until ANC ≥ 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 immunosuppression if eligible: up through Day 90 in cohort 1 OR up through Day 60 in cohort 2.
If ineligible: continue immunosuppression up through Day 180 [c]

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

1.0 INTRODUCTION

Recent advances in allogeneic blood or marrow transplant (HSCT) 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] Historically, we have used this approach for HSCTs which have used marrow as the graft source. We and the field note challenges to engraftment in diseases such as myelodysplastic syndrome (MDS), chronic lymphocytic leukemia (CLL), myeloproliferative diseases (MPD) and multiple myeloma (MM). More recently we have chosen to move towards peripheral blood stem cells (PBSC) as the graft source in these malignancies as there is data that there is lower graft failure with PBSC as well as overall survival (OS) and progressive free survival (PFS) improvements.[2] For nonmyeloablative, related donor, HLA haploidentical or matched HSCT, and nonmyeloablative, unrelated donor HSCT, 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. It is known that transplants using PBSCs have higher rates of GVHD than those from marrow sources but the GVHD may translate into better outcomes in terms of OS and PFS.[3,4] It stands to reason that some GVHD in these patients is a good thing and we believe there are several potential advantages to shorter-duration pharmacologic immunosuppression to be used in conjunction with PBSC grafts to improve outcomes in these patients. 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 in patients receiving PBSC transplants for these malignancies which are challenging to engraft.

1.1 Stem cell source

There has been a great deal of discussion on the importance of stem cell source on the risk of chronic graft-versus-host disease[5-9]. Several studies have addressed this issue in the related setting. Of the eight randomized trials published[10-18] only one reported astatistically significant increase in grades II-IV acute graft-versus-host disease with the use of peripheral blood stem cells when compared to bone marrow (52 vs. 39%)[16]. Regarding chronic graft-versus-host disease, the results are as follows: 3 studies have shown an increase of chronicgraft-versus-host disease with peripheral blood stem cells as opposed to bone marrow[12,16,19]. One study showed a trend towards increase in chronic graft-versus-host disease with the use of peripheral blood stem cells[19]. A meta-analysis by Cutler et al. confirmed that both, acute and chronic graft-versus-host disease are more common after peripheral blood stem cells than bone marrow[7]. Registry data showed in pediatric patients that chronic graft-versus-host disease was more frequent (as well as higher mortality) after peripheral blood stem cells than after bone marrow[8]. In adults, chronic graft-versus-host disease is also more prevalent[20]. Umbilical-cord stem cells have also been a source of grafts in children and young adults. As children tolerate mismatches better than adults, interpretation of risk in this group is difficult but it seems that the rate of chronic graft-versus-host disease is low for this stem cell sources, especially considering that almost all grafts are 1-3 antigen mismatches[21,22]. In the unrelated setting, a clinical trial by the BMT CTN comparing bone marrow versus peripheral blood did not detect significant survival. Peripheral-blood stem cells may reduce the risk of graft failure (the overall incidence of graft failure in the peripheral-blood group was 3% [95% CI, 1 to 5], versus 9% [95% CI, 6 to 13] in the bone marrow group [P=0.002]), whereas bone marrow may reduce the risk of chronic GVHD at 2 years (peripheral-blood group was 53% [95% CI, 45 to 61], as compared with 41% [95% CI, 34 to 48] in the bone marrow group [P=0.01].[17] The proportion of patients with extensive chronic GVHD was higher in the peripheral-blood group than in the bone marrow group (48% [95% CI, 42 to 54] vs. 32% [95% CI, 26 to 38], P<0.001). Among patients who were alive at 2 years, 57% of the patients in the peripheral-blood group were receiving immunosuppressive therapy, as compared with 37% of those in the bone marrow group (P=0.03). There were no significant between-group differences in the incidence of acute GVHD or relapse[17].

1.2 Rationale for shorter-duration immunosuppression

With the advent of posttransplantation high-dose Cy, our nonmyeloablative allogeneic HSCT 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.[23,24] 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. This may be very relevant in older MDS or CLL patients who suffer from nephrotoxicity and electrolyte disturbances from the calcineurin inhibitors.

Extensive published data demonstrate that allogeneic HSCT 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. Sirolimus is similarly used and applied. 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 HSCT.[18-20]

1.3 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.[25] 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.[25-30] As a form of drug-induced immunologic tolerance,[31] 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.[32] 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.[27] Additional studies demonstrated that posttransplantation Cy reduced the incidence and severity of GVHD in the setting of MHC-mismatched allogeneic HSCT after myeloablative conditioning.[26]

After allogeneic HSCT, 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 HSCT platform with high-dose posttransplantation Cy, MMF, and tacrolimus for GVHD and graft rejection prophylaxis has produced encouraging results[24]. This approach has been associated with rapid and stable engraftment in most patients.[24] Most importantly, this approach has carried acceptable rates of GVHD and NRM that parallel those seen with nonmyeloablative HLA-matched transplants (Figure 1).[23,24,33,34] Cy, when administered at high doses after myeloablative, HLA-matched, related or unrelated donor HSCT, notably has been found to be effective single-agent prophylaxis against GVHD, obviating the need for CNI's in this setting (Figure 1).[35]

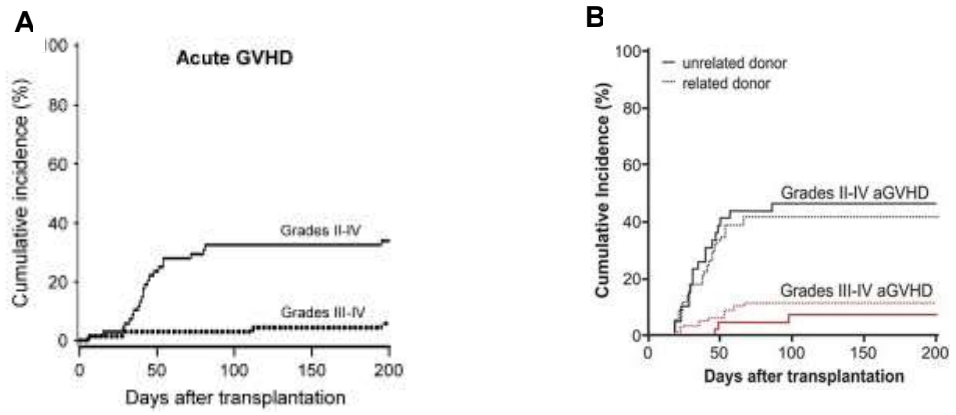

Figure 1. Acute GVHD after HSCT incorporating high-dose posttransplantation Cy. A) nonmyeloablative HSCT.[24] B) myeloablative, HLA-matched, related or unrelated donor HSCT, with Cy as single-agent prophylaxis.[35]

1.4 Nonmyeloablative HSCT with fludarabine, TBI, and posttransplantation cyclophosphamide

Independent clinical trials have evaluated or are evaluating a nonmyeloablative, partially HLA-mismatched (haploidentical), related donor marrow transplant 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.[24,34] 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 HSCT from partially HLA-mismatched, related donors (Figure 2).[24] 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:

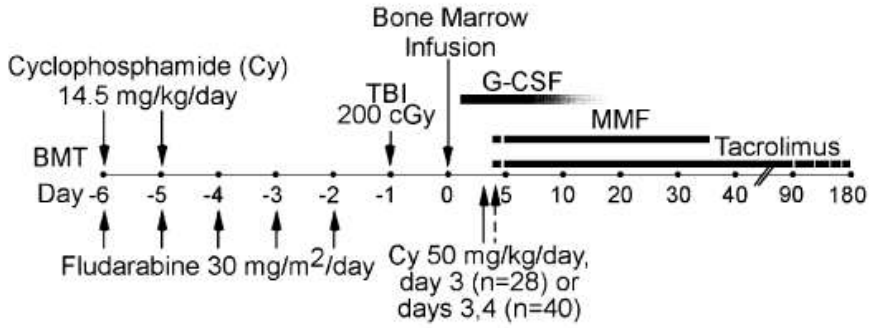

Figure 2. Treatment Schema in Previous Studies

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.[34] Interestingly, although increasing degrees of HLA mismatch between donor and recipient have historically been associated with greater GVHD and inferior survival after allogeneic HSCT, that analysis retrospectively found no such adverse effect of HLA mismatching using this approach in the haploidentical setting.[34]

On an updated analysis of 212 patients with advanced hematologic malignancies, uniformly treated at Johns Hopkins with related donor, partially HLA-mismatched HSCT 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 HSCT using partially HLA-mismatched, related donors has been adopted at Johns Hopkins for HLA-identical donor HSCT. In a phase I trial (J0169) of nonmyeloablative, matched sibling donor HSCT, 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 HSCT 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.[23] 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.

1.5 Shortened-course immunosuppression

MMF plus a CNI (i.e. tacrolimus or cyclosporine or sirolimus) 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 HSCT in general.

For example, in nonmyeloablative, HLA-matched HSCT 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.[36] 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 HSCT, 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 [37]. In another randomized trial of myeloablative, HLA-matched related or unrelated donor HSCT, 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.[38] Other nonrandomized studies of myeloablative, HLA-matched sibling transplant have suggested a benefit to longer duration cyclosporine in chronic GVHD prevention.[39] However, some studies have found an increased risk of relapse associated with higher doses of cyclosporine.[40,41]

The experiences with immunosuppression duration with other allogeneic HSCT 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 or other immunosuppression after nonmyeloablative HSCT 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.

Addendum: Cohort 1 with patients stopping IST on Day 90 was completed with favorable rates of GVHD and thus cohort 2 has been added to investigation cessation at Day 60. Sirolimus has been an institutional standard favored over tacrolimus for once daily dosing as well as less nephrotoxicity. This has now become first line in the majority of patients to start Day +5.

2.0 OBJECTIVES

2.1 Primary objective

In nonmyeloablative, related or unrelated donor, partially HLA-mismatched or HLA-matched PBSC transplant with post-grafting immunosuppression that includes high-dose cyclophosphamide and MMF, evaluate the safety and feasibility of reduced-duration immunosuppression (from Day 5 through Day 90 in cohort 1 and from Day 5 through Day 60 in cohort 2) compared to standard duration tacrolimus (From Day 5 through Day 180).

2.2 Secondary objectives

1. In patients eligible for reduced-duration immunosuppression, estimate the incidences of severe acute grade III or higher GVHD, chronic GVHD (overall and by extent) requiring additional immunosuppressive therapy (IST), graft failure, relapse, and NRM between Days 90-180 for cohort 1 or Days 60-180 for cohort 2, and beyond Day 180.
2. Estimate the cumulative incidence of severe acute grade III or higher GVHD, chronic GVHD (overall and by extent) requiring additional immunosuppressive therapy (IST), graft failure, relapse, 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 HSCT for the group overall

and for patients with shortened-duration immunosuppression; and describe the number and types of systemic immunosuppression used for GVHD treatment.
4. Estimate the progression-free survival, disease-free survival, overall survival, GVHD-free relapse-free survival, and chronic GVHD-free relapse-free survival after transplantation.
5. Evaluate MDS cohort 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 immunosuppression are designated in Section 5.283.

1. Presence of a suitable related, HLA-haploidentical or HLA-matched stem cell donor or unrelated matched donor.
    a. The donor and recipient must be identical 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 for related donors, and will be considered sufficient evidence that the donor and recipient share one HLA haplotype. Unrelated donors will be 10/10.
2. Eligible diagnoses:
    a. Myelodysplastic syndrome (MDS) including chronic myelomonocytic leukemia [CMML] with at least one of the following poor-risk features:
        i. Poor-risk cytogenetics
        ii. IPSS score of INT-2 of greater
        iii. Treatment-related or secondary MDS
        iv. MDS diagnosed before age 21
        v. Progression on or lack of response to standard DNA-methyltransferase inhibitor therapy
        vi. Life-threatening cytopenias, including those requiring frequent transfusions
    b. SLL or CLL with 17p deletion, or with progression < 6 months after second or greater treatment regimen. Must have the following to be an acceptable candidate as well:
        i. ≤ 20% of bone marrow cellularity involved by SLL/CLL (to lower risk of graft rejection)
        ii. No lymph nodes ≥ 5 cm in any dimension
        iii. No massive splenomegaly, defined as > 6 cm below the left costal margin
    c. T-cell PLL in PR or better prior to transplantation. Must also have ≤ 20% of bone marrow cellularity involved by PLL (to lower risk of graft rejection).
    d. Interferon- or tyrosine kinase-refractory CML in first chronic phase, TKI-intolerant CML in first chronic phase, or CML in second or subsequent chronic phase
    e. Philadelphia chromosome negative myeloproliferative disease (including myelofibrosis)
        i. Intermediate-2 or High risk score by DIPSS Plus is required for a diagnosis of myelofibrosis
    f. Multiple myeloma or plasma cell leukemia with a PR or better to the last treatment regimen, based on the International Myeloma Working Group (IMWG) criteria.[49]
    g. Hematologic malignancy in complete remission with minimal residual disease (MRD) detectable by conventional cytogenetics, FISH, flow cytometry, or molecular testing
3. No active extramedullary leukemia or known active CNS involvement by malignancy. Such disease treated into remission is permitted.
4. Any previous autologous HSCT must have occurred at least 3 months prior to start of conditioning
5. No previous allogeneic HSCT
6. 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

7. ECOG performance status ≤ 2 or Karnofsky or Lansky score ≥ 60.
8. Not pregnant or breast-feeding.
9. 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 either:
   a. HLA-haploidentical or HLA-identical relatives of the patient based on allele or allele group level typing as defined in Section 3.1.
   b. Unrelated donor who is a 10/10 match to the recipient as defined in section 3.1.
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 prioritized over unrelated
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.
5. Avoidance of female donor for male recipient

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 60 patients with 90 day duration of immunosuppression in cohort 1 (per Section 9.0). Sixty patients will be transplanted in order to identify at least 20 who are evaluable for the safety of day 90 immunosuppression cessation. Up to 5 additional patients may be transplanted to replace unevaluable patients (per Sections 4.1 and 5.286).

The goal is to transplant 60 patients with 60 day duration of immunosuppression in cohort 2 (per Section 9.0). Sixty patients will be transplanted in order to identify at least 20 who are evaluable for the safety of day 60 immunosuppression cessation. Up to 5 additional patients may be transplanted to replace unevaluable patients (per Sections 4.1 and 5.286).

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. Tacrolimus may be replaced by sirolimus for clinical reasons (e.g. nephrotoxicity or PRES).

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 or by institutional standard:

CrCl 40-69 mL/min, fludarabine = 24 mg/m^2
CrCl 20-39 mL/min, fludarabine = 20 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.

Fludarabine dosing is based on the last CrCl prior to the start of conditioning. The estimated CrCl on the day preceding start of conditioning may be used. The fludarabine dose should be the same on Days -6 to -2, even if the creatinine changes. However, adjustment in fludarabine dose due to creatinine changes during conditioning is permitted.

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 peripheral blood stem cell 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 Peripheral blood stem cell transplantation

On Day 0, peripheral blood stem cells are infused. The target stem cell dose is between 2 x 10^6/kg and 10 x 10^6/kg (actual body weight) CD34+ cells. 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 maximum CD34+ cell dose is 10 x 10^6/kg. If more than 10 x 10^6/kg CD34+ stem cells are collected, the excess will be discarded and not administered to the patient. Up to two leukapheresis procedures may be performed to obtain the minimum CD34+ cell target. If, after two leukapheresis procedures, fewer than 2 x 10^6/kg CD34+ cells have been collected, a bone marrow harvest will be recommended and the patient will be taken off trial.

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

5.26 Posttransplantation cyclophosphamide

Hydration prior to and following Cy, management of volume status, and monitoring for hemorrhagic cystitis will follow institutional standards. Mesna will be used with posttransplantation Cy as per institutional standards.

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 Sirolimus or Tacrolimus

5.281 Immunosuppression initiation and dosing

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

For patients > 18 years old, the sirolimus starting dose is 6mg loading dose followed by 2mg daily or as per institutional standards. The tacrolimus starting dose is 2 mg PO BID or as per institutional standard. Patients who cannot tolerate PO may be started IV and changed to PO as per institutional standard. 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 or as per institutional standard and is 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 Duration of immunosuppression

The duration of immunosuppression is assigned prospectively in cohorts of patients before initiation of the preparative regimen. Immunosuppressionto Day 90 or Day 60 will be compared to Day 180. Immunosuppression is stopped without taper. The same applies to sirolimus/ tacrolimus if the patient was switched. Patients who are switched will be

evaluated the same regardless of timing of switch as long as full criteria are met to stop immunosuppression on Day 90 in cohort 1 or Day 60 in cohort 2.

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

In patients ineligible for protocol-specified early immunosuppression cessation, immunosuppression is discontinued after the last dose on Day 180 without taper; however in these patients, immunosuppression 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 immunosuppression 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 or sirolimus

In order to be eligible for protocol-specified shortened-course immunosuppression, patients must meet all of the following criteria by the scheduled date of immunosuppression cessation by cohort 1 or 2:

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 immunosuppression discontinuation will stop agent 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) or of unplanned therapy for persistent disease

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

5.284 Early immunosuppression cessation in the context of pending evaluations

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

For logistical reasons, stopping immunosuppression up to 5 days after the scheduled stop date is permissible in patients for whom early, protocol-specified immunosuppression cessation is planned.

Stopping immunosuppression more than 5 days later than the scheduled stop date constitutes a protocol deviation and is further discussed in Section 5.286.

5.285 Changes in planned immunosuppression duration

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

5.286 Evaluability for the primary endpoint

For protocol-driven, early cessation of immunosuppression, stopping immunosuppression 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 immunosuppression cessation.

Patients who should stop immunosuppression 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 immunosuppression 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 pre-specified date of immunosuppression cessation. Cases in which eligible patients do not stop immunosuppression early because of physician discretion will count *against* feasibility.

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 ≥ 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 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, 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., hypomethylating agent for MDS, tyrosine kinase inhibitor for Philadelphia chromosome-positive malignancy) is permitted after transplantation. 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 peripheral blood stem cells 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 ≥ 5% donor chimerism, followed by sustained <5% donor chimerism in blood and/or bone marrow.

< 5% donor T cell chimerism, but with ≥ 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).[42] 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 initial diagnosis of GVHD (even if non-severe) and grade III-IV GVHD will be recorded.

The cumulative incidences of acute grade III-IV and grade III-IV GVHD will be determined through competing risk analysis. Treatment of disease relapse/progression/persistence (with the exception of planned maintenance or consolidative therapy), 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[43] and Seattle criteria.[44] 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 (PFS)**: Interval from Day 0 to date of first objective disease progression or relapse, unplanned treatment for disease persistence, or death from any cause. Patients without these failures will be censored at the last date they were assessed

and deemed failure-free. Disease persistence in the absence of progression is not considered a PFS failure unless it leads to treatment.

6.32 **Disease-free survival (DFS)**: Interval from Day 0 to date of first objective detection of disease persistence, progression or relapse, or death from any cause. Patients without such failures will be censored at the last date they were assessed and deemed failure-free. Disease persistence posttransplantation, followed by disappearance of detectable disease in the absence of treatment, is not considered a DFS failure.

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

6.34 **Nonrelapse mortality (NRM)**: 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. CLL: 2008 International Workshop criteria [45]
b. MDS: 2006 IWG criteria [46]

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

6.36 **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. In Ph+ disease, PCR-only detectable disease posttransplantation is not considered a DFS or PFS failure for study purposes.

6.37 **GVHD-related survival endpoints**

6.371 GVHD-free relapse-free survival (GFRFS): Interval from Day 0 to acute grade III-IV GVHD, systemic treatment of chronic GVHD, or PFS failure (per Section 6.31), whichever occurs first. Patients without these failures will be censored at the last date they were assessed and deemed failure-free.

6.372 Chronic GVHD-free relapse-free survival (cGFRFS): Interval from Day 0 to a chronic GVHD event (variably defined as either moderate or severe chronic GVHD, or systemic treatment of any chronic GVHD) or PFS failure, whichever occurs first. Patients without these failures will be censored at the last date they were assessed and deemed failure-free.

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/posttransplant evaluations [a, b]								
History and physical exam	X		X	X[l]	X[l]	X[l]	X[l]	X
Performance status	X							
CBC / differential [d]	X	X	X	X[l]	X[l]	X[l]	X[l]	X[l]
Comprehensive metabolic panel (CMP) [e]	X		X	X[k]	X[k]	X[k]	X[k]	X[k]
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 (CLL only)	X		X			X[l]		X[l]
Response assessment to last therapy [i]	X							
HLA typing	X							
Lymphocytotoxic antibody screen	X							
Donor marrow or blood for VNTR or RFLP analysis	X							
Patient blood for baseline VNTR or RFLP analysis	X							
Peripheral blood chimerism, both total leukocyte (unsorted) and T-cell sorted		X	X	X[k]	X[k]	X[k]	X[k]	X[k]
Peripheral blood plasma collections	X	X	X	X[k]	X[k]	X[k]	X[k]	X[k]
GVHD and other morbidity assessments [j]			X	X[k]	X[k]	X[k]	X[k]	X[k]

[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] 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] May be omitted if there is documented disease persistence, progression or relapse before scheduled assessment.

[i] Include comparison of pre- and post-treatment scans with bidimensional measurements where relevant.

[j] 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.

[k] 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.

[l] May be omitted in patients who receive treatment for disease persistence, progression, relapse. The dates of treatment initiation and DLI will be recorded.

8.0 LABORATORY CORRELATES

8.1 Plasma-derived cell free DNA (cfDNA): cfDNA released from malignant cells can be reliably detected in patients' plasma and has been widely used in diagnosis, early detection and disease burden monitoring in several solid malignancies. Most ctDNA is primarily derived from hematopoietic tissue and plasma-derived ctDNA appears to be the most reliable marker of the disease burden. Thus, we will assess the patient-specific mutations burden (minimal residual disease – MRD) at baseline and longitudinally post-HSCT. We will then correlate the ctDNA-based MRD Adetection with clinical outcomes. Somatic mutations will be assessed using the SureSelect Target Enrichment System (Agilent). The captured samples will be then sequenced on a HiSeq25000 (Illumina) using a 2x150 bp protocol. Data will be analyzed using our custom variant calling pipeline.

9.0 RISKS AND REPORTING REQUIREMENTS

9.1 Drug information

9.11 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.

9.12 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.

9.13 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).

9.14 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.

9.15 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.

9.16 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, carbamazepine), 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 section 8.17. Dose adjustments for therapy with other azoles may be indicated. However, the initial tacrolimus dose (on Day 5) remains fixed.

9.17 Sirolimus

Sirolimus is an immunosuppressant that inhibits cytokine-stimulated T-cell activation and proliferation, and also inhibits antibody formation.

Drug formulations: The mean bioavailability of sirolimus after administration of the tablet is ~27% higher than the oral solution. Sirolimus oral tablets are not bioequivalent to the oral solution. Clinical equivalence has been demonstrated at the 2-mg dose level; however, it is not known if higher doses are clinically equivalent on a mg to mg basis.

a) Sirolimus oral solution: Sirolimus oral solution (1 mg/mL) should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). For dilution, the appropriate dose should be measured using an amber oral syringe, then added to a glass or plastic container that holds at least 60 mL. Before taking the dose, it should be diluted with water or orange juice then taken immediately; it should not be diluted with grapefruit juice. The syringe should be discarded after one use. Sirolimus oral solution provided in bottles may develop a slight haze when refrigerated, which does not affect product quality; allow the product to stand at room temperature and shake gently until the haze disappears.

b) Sirolimus tablets: Sirolimus tablets are available in 1 mg and 2 mg tablets that cannot be crushed or broken. Sirolimus tablets should be stored at 20° to 25° C (68°–77°F), protected from light.

Toxicities: The most common adverse reactions of sirolimus are: peripheral edema, hypertriglyceridemia, hypercholesterolemia, hypertension, increased creatinine, constipation, abdominal pain, nausea, diarrhea, headache, fever, urinary tract infection, anemia, thrombocytopenia, arthralgia, pain. Adverse reactions that have resulted in rates of sirolimus discontinuation >5% were increased creatinine, hypertriglyceridemia, and thrombotic thrombocytopenic purpura (TTP) / thrombotic microangiopathy (TMA). Sirolimus toxicities are summarized in the table below:

Table: Sirolimus toxicities

	Common (>20%)	**Occasional** (5-20%)	Rare (<5%)
Immediate (within 1-2 days)	Headache (L), hypertension (L), immunosuppression (L), fever, nausea, diarrhea, constipation	Chest pain, insomnia, dyspepsia, vomiting, ***dyspnea***	Hypotension, asthma, cough, flu-like syndrome, tachycardia, anorexia, ***hypersensitivity reactions***
Prompt (within 2-3 weeks)	Tremor (L), renal dysfunction, pain (abdominal, back, arthralgias), ***hyperlipidemia*** [c] *(hypercholesterolemia, hypertriglyceridemia),* hyperglycemia, edema including ***peripheral edema*****,** anemia	Elevated LFT's (with elevated sirolimus levels) [a], stomatitis, infections (including UTI, URI), mild ***thrombocytopenia***, ***leukopenia***, electrolyte disturbances (hyper/hypokalemia [L], hypophosphatemia, hypomagnesemia [L]), rash, hives, pruritus, ***delayed wound healing or dehiscence (L), proteinuria, TTP/HUS/TMA*** [b] especially with concurrent CNI	Pleural and pericardial effusions, ***pulmonary toxicity*** (non-infectious pneumonitis, BOOP, pulmonary fibrosis), thombosis, myalgias
Delayed (any time later during therapy, excluding above conditions)	Acne		Kidney disease, CHF, ascites, arthrosis, bone necrosis, osteoporosis
Late (any time after completion of treatment)			Lymphoproliferative disorders, skin malignancies
Unknown frequency and timing	Embryo/fetotoxic; unknown whether excreted in human milk		

(L): Toxicity may also occur later.

[a] Significant transaminitis, generally without sequelae, may occur. Sirolimus has been associated with higher rates of venoocclusive disease after myeloablative conditioning.

[b] Incidence 3% to < 20% in a trial of kidney transplantation. In allogeneic BMT, increase in TMA from 4.2% with tacrolimus or cyclosporine alone, versus 10.8% with tacrolimus/sirolimus combination was noted.[68]

[c] Lipid-lowering agent may be required; consider if fasting serum triglycerides are > 2.5 x ULN, and recommend starting if > 800 mg/dL.

Drug interactions: Sirolimus is known to be a substrate for both cytochrome CYP3A4 and P-glycoprotein. Agents that may increase sirolimus levels include tri-azole drugs (especially voriconazole and posaconazole*), amiodarone, calcium channel blockers, macrolide antibiotics (but not azithromycin), micafungin, gastrointestinal prokinetic agents (cisapride, metoclopramide), cimetidine, cyclosporine, grapefruit juice, and HIV protease inhibitors. Agents that may decrease

sirolimus levels include anticonvulsants (carbamezepine, phenobarbital, phenytoin), rifamycins, St. John's Wort.

Dose adjustments: The sirolimus dose is adjusted to maintain a serum trough level of 5-12 ng/mL. Changes in levels due to altered bioavailability should be apparent within 24-48 hours. For sirolimus without CNI as in this study, a 20-25% reduction of sirolimus dose is recommended for trough levels >12 – 18 ng/mL, and a 20-25% increase is recommended for trough levels < 5 ng/mL.

Renal failure does not affect the excretion of sirolimus. Excretion is reduced in liver failure; impaired hepatic function should prompt consideration of reduction in sirolimus maintenance doses but no dose adjustment of the loading dose is necessary.

Due to extreme interactions with voriconazole and posaconazole, these drugs are relatively contraindicated during sirolimus therapy. Sirolimus dose is to be reduced by 90% when voriconazole is initiated and should also be significantly reduced with posaconazole. Dosing guidelines are provided in the table below.

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 and sirolimus are not advised when azoles are stopped. Rather, 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 or sirolimus

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

9.2 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)

9.3 Toxicity reporting

The agents being used in the study are used extensively in the HSCT setting and have well-defined toxicity profiles. In addition, there are many expected toxicities of allogeneic HSCT. 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 HSCTs.

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.

9.4 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.

9.5 Risks and benefits

Allogeneic HSCT 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.

10.0 STATISTICAL CONSIDERATIONS

10.1 Primary endpoint and design

Amendment 2/19/2017
The interim analysis of the 90 day immunosuppression arm indicated that the shortened duration of tacrolimus/sirolimus was feasible and safe. This amendment will further shorten the duration of immunosuppression to 60 days to determine if this is likewise feasible and safe. The criteria for feasibility (threshold 33%) and safety (thresholds: 5% graft failure, 5% NRM, 50% relapse, and 10% combined grade III-IV acute GVHD and severe chronic GVHD) will be the same as those for the 90 day arm. We evaluated the operating characteristics under similar scenarios that were used for the day 90 arm of the study. Historically, the cumulative risks of events between day 60 and 180 were: 2.7% for graft failure, 5.2% for NRM, 26.9% for relapse, and 5.0% for combined grade III-IV acute GVHD and severe chronic GVHD. The prior for the day 60 arm will be based on the posterior for the 90 day arm, discounted by one half.

Results of interim analysis:
At the time of the interim analysis, the posterior probabilities of feasibility and the four event types being greater than their respective stopping criterion are given in the table below. The stopping rule declares the shortened duration of immunosuppression not feasible if there is 80% or more certainty that fewer than 33% of patients could have stopped tacrolimus/sirolimus early. The stopping rule declares the duration of tacrolimus/sirolimus unsafe if the posterior probability is 67% or higher that the risk of that adverse event between the day tacrolimus/sirolimus stops and ~ Day 180 overly exceeds the following cause-specific probabilities: ≥ 5% graft failure, ≥ 5% NRM, ≥ 50% relapse, and ≥ 10% combined grade III-IV acute GVHD and severe chronic GVHD.

Table of interim analysis results for 90 days of immunosuppression:

Safety Event	Stopping Criterion	Posterior Probability Greater than Stopping Criterion
Feasibility	0.33	0.001
Graft failure	0.05	0.002
NRM	0.05	0.024
Relapse	0.50	0.000
GVHD	0.10	0.054

Sample size: Up to 60 patients will be transplanted in order to identify at least 20 who are evaluable for the safety of day 60 immunosuppression cessation. Up to 5 additional patients may be transplanted to replace unevaluable patients.

The primary goal of cohort 1 of this study is to determine whether one can shorten the duration of immunosuppression with tacrolimus or sirolimus to 90 days following nonmyeloablative, related or unrelated donor peripheral blood HSCT that incorporates high-dose posttransplantation Cy in patients with diagnoses that are difficult to engraft.

This has been amended as above to include cohort 2 with the same goals for cessation of immunosuppression at Day 60.

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 immunosuppression cessation (as defined in Section 5.283) and have immunosuppression stopped at 90 days. Monitoring rules for feasibility (based on the proportion of patients who are able to stop immunosuppression at day 90 and safety (i.e. complications do not exceed cause-specific thresholds defined below) will be implemented.

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 immunosuppression from the current standard (until Day 180) to until Day 90 or Day 60. We hypothesize that stopping immunosuppression on Day 90 will not carry excess risk of GVHD or graft failure (potential manifestations of inadequate immunosuppression).

10.11 Criteria for feasibility and safety

This study considers it "feasible" to stop immunosuppression 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 day 90 or day 60 and who have immunosuppression stopped accordingly. A Bayesian stopping rule will be implemented to declare a particular shortened duration of immunosuppression not feasible, if there is 80% or more certainty that fewer than 33% of patients could have immunosuppression stopped early. The Bayesian rule begins with a Beta (3, 3) prior probability distribution that immunosuppression 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 immunosuppression stopped is between 32% and 68%. Up to 65 patients will be transplanted in order to identify at least 20 who are evaluable for the safety of day 90 immunosuppression cessation. Up to 5 additional patients may be transplanted to replace inevaluable patients (per Sections 4.1 and 5.286). Patients who switch to sirolimus will be evaluated in the same fashion.

Historical estimates were derived from 55 uniformly treated patients with diagnoses of hematologic malignancies that are hard to engraft (PLL, CLL, MDS, MPN, CML and CMML) treated with fludarabine/Cy/TBI conditioning, partially HLA-mismatched HSCT, posttransplantation high-dose Cy, MMF on Days 5-35, and tacrolimus on Days 5-180. Event-specific risks for graft failure, non-relapse mortality (NRM), relapse, and severe acute graft versus host disease (aGVHD) are shown in Table 1 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.) The cumulative incidences of these events between day 90 and day 180 are <1%, 3.3%, 23.0%, and 5.9% respectively.

Days	Graft Failure	NRM	Relapse	GVHD [a]	No Event
1-60	0.0914	0.0371	0.0733	0.0190	0.7792
61-90	0.0243	0.0243	0.0474	0.0001	0.9029
91-120	0.0013	0.0013	0.1031	0.0522	0.8420
121-180	0.0015	0.0316	0.1519	0.0015	0.8135

[a] acute grade III-IV or chronic GVHD

Separate stopping rules will be implemented for each of 4 events occurring between the date of immunosuppression discontinuation and the Day 180 evaluation (Day 180 evaluations are +/- 7 days for logistical reasons): a GVHD event specified below; graft failure; NRM; and disease relapse or progression. For the stopping rules, the prior precision of these event-specific risks within each interval corresponds to a prior sample size of 15 patients, approximately one-fourth of the historical sample size of 55. We discounted the historical data to avoid having the prior information dominate the inference from the current study's data.

The estimated historical cause-specific (cumulative) risks between Day 90 and 180 are 5.9% (GVHD), <1% (graft failure), 3.3% (NRM), and 23% (relapse). For monitoring, we will compute the posterior probability of each of the four events, given the data and historical prior distribution, and evaluate whether to hold accrual after fixed cohorts of patients (per below).

The stopping rule will declare a particular duration of immunosuppression unsafe if the posterior probability is 67% or higher that the risk of that adverse event between the day immunosuppression stops and ~ Day 180 exceeds the following cause-specific probabilities: stopping for $\geq$ 10% combined incidence of acute, clinical grade III-IV (severe) GVHD and severe chronic GVHD, $\geq$ 5% NRM, or $\geq$ 5% graft failure in this window, or stopping for a $\geq$ 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 HSCT experience, the majority of patients who develop chronic GVHD will have had acute GVHD;[24] 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 HSCT 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.[35]

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/disease persistence (with the exception of maintenance or consolidative therapy) will be regarded as having a competing risk for GVHD. The number of cases of acute grade III-IV occurring after treatment initiation for residual or relapsed/progressive disease will be reported.

Monitoring will occur in groups of 5 assessable patients, beginning after the first 20 assessable patients have been transplanted and their immunosuppression stopped early at the prespecified time.

In deciding upon the safety of a 90 day duration of immunosuppression, the nature of 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 immunosuppression. NRM may or may not be due to less immunosuppression; thus if only the NRM rate exceeds the predefined threshold, the recommended duration of immunosuppression 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.[40,41] 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 HSCT, 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, BHSCT 2008;14(11):1279-87),[23] and c) with such postgrafting immunosuppression, increasing degrees of HLA mismatch have not been found to be associated with inferior outcomes in haploidentical HSCT (Kasamon YL et al, BHSCT16(4):482-9).[34]

Amendment 2/19/2017 calculation of the prior for the 60 day immunosuppression arm:
Using the stopping criteria for the 90 day arm of the study, events observed during the study were combined with the historical prior to calculate the posterior probability of adverse events in the chosen time intervals when stopping tacrolimus/sirolimus on day 90. This posterior, discounted by one-half, will be used as the prior for monitoring the current Day 60 data. Event-specific risks for graft failure, NRM, relapse, and severe acute or chronic GVHD based on this prior are shown in the amended table within 4 intervals: Days 1-60, 61-90, 91-120, and 121-180. (These probabilities are specific to the time interval indicated for each row and conditional on reaching the interval.) The cumulative incidences of these events between day 60 and day 180 are <1%, 3 .8%, 16.5%, and 4.0% respectively.

Amendment prior event specific risks for 60 day immunosuppression arm:

Days	Graft Failure	NRM	Relapse	GVHD [a]	No Event
1-60	0.044025	0.06487	0.074933	0.00593	0.810243
61-90	0.007286	0.029366	0.057629	0.001104	0.904615
91-120	0.001214	0.001214	0.052708	0.014817	0.930046
121-180	0.001299	0.008574	0.063653	0.02728	0.899195

10.12 Operating characteristics of design

Simulation results reveal that these stopping rules perform well and provide reasonable protection of patients against unexpectedly elevated risks. 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 120-180. Monitoring in groups of 5 patients started after 20 patients entered the study. Table 1. summarizes simulations of this design for historical risks, Table 1a, increased risks of each monitored event type, Table 1b, and increased risks and lower feasibility, Table 1c. The risks in columns 2 through 5 are for the events being monitored in the day 90 to 180 interval during which immunosuppression is discontinued. Columns 7 and 8 give the percentage of time, out of 1,000 simulations, that the monitoring plan called for early stopping, either for safety, column 7, or feasibility, column 8. Details of these simulations are provided in the Appendix.

Table 1a. Summary of 1,000 simulations using historical risks within each tie interval.

Scenario	Graft failure	NRM	Relapse	aGVHD	Cum Feas Rsk prior to day 90	Pct Stop	Pct Stop Feas
Historical risks	**0.0089**	**0.0334**	**0.2318**	**0.059**	**0.2268**	**22.2**	**0**

Table 1b. Summary of 1,000 simulations with increased risks within the day 90-180 intervals.

Scenario	Graft failure	NRM	Relapse	aGVHD	Cum Feas Rsk prior to day 90	Pct Stop	Pct Stop Feas
Increase GF	**0.082**	0.0317	0.2242	0.0587	0.2268	58.4	0
Increase GF	**0.1449**	0.0299	0.2165	0.0585	0.2268	87.4	0
Increase GVHD	0.0083	0.0299	0.2165	**0.1951**	0.2268	82.8	0
Increased GVHD	0.0081	0.0282	0.2089	**0.2423**	0.2268	93.3	0
Increased NRM	0.0086	**0.1051**	0.2242	0.0587	0.2268	73.4	0
Increased NRM	0.0083	**0.1665**	0.2165	0.0585	0.2268	92.7	0

Table 1c. Increased risks within the day 90-180 intervals and decreased feasibility prior to day 90.

Scenario	Graft failure	NRM	Relapse	aGVHD	Cum Feas Rsk: prior to day 90	Pct Stop	Pct Stop Feas
Increase GF	**0.082**	0.0317	0.2242	0.0587	**0.4733**	42.5	4.9
Increase GF	**0.1449**	0.0299	0.2165	0.0585	**0.5426**	38.8	48.6
Increase GVHD	0.0083	0.0299	0.2165	**0.1951**	**0.4733**	61.9	4.3
Increased GVHD	0.0081	0.0282	0.2089	**0.2423**	**0.5426**	46	41.2
Increased NRM	0.0086	**0.1051**	0.2242	0.0587	**0.4733**	54.1	5.2
Increased NRM	0.0083	**0.1665**	0.2165	0.0585	**0.5426**	40.5	48.4

Operating characteristics Amendment 2/19/2017

Simulation results under the amended protocol are given in amended Tables 1a – 1c. Monitoring occurred in groups of 5 patients starting after 10 patients entered the study. Detailed results of these simulations are shown in the amended Appendix.

Amendment Table 1a. Summary of 1,000 simulations using historical risks within the day 60-180 interval.

Scenario	**Graft failure**	**NRM**	**Relapse**	**aGVHD**	**Cum Feas Rsk prior to day 60**	**Pct Stop**	**Pct Stop Feas**
1 Historical risks	0.0267	0.052	0.2685	0.0496	0.2208	18.3	0

Amendment Table 1b. Summary of 1,000 simulations with increased risks within the day 60-180 intervals.

Scenario	**Graft failure**	**NRM**	**Relapse**	**aGVHD**	**Cum Feas Rsk prior to day 60**	**Pct Stop**	**Pct Stop Feas**
2 Increased GF	0.0981	0.0512	0.2621	0.0482	0.2208	43.5	0
3 Increased GF	0.1588	0.0504	0.2557	0.0469	0.2208	68	0
4 Increased GVHD	0.0266	0.0504	0.2557	0.1791	0.2208	47	0
5 Increased GVHD	0.0265	0.0496	0.2493	0.221	0.2208	61.2	0
6 Increased NRM	0.0266	0.1227	0.2621	0.0482	0.2208	60.9	0
7 Increased NRM	0.0266	0.1826	0.2557	0.0469	0.2208	83.4	0
8 Increased relapse	0.026	0.0438	0.7659	0.0361	0.2208	14.2	0
9 Increased relapse	0.0259	0.0422	0.8354	0.0335	0.2208	12.8	0

Amendment Table 1c.
Increased risks within the day 60-180 intervals and decreased feasibility prior to day 60.

Scenario	Graft failure	NRM	Relapse	aGVHD	Cum Feas Rsk prior to day 60	Pct Stop	Pct Stop Feas
10 Increased GF	0.143	0.0504	0.2557	0.0469	0.6208	58.1	8.5
11 Increased GF	0.1989	0.0488	0.2481	0.0468	0.7008	54.2	34.1
12 Increased GVHD	0.0265	0.0488	0.2481	0.2192	0.6208	55.3	8.8
13 Increased GVHD	0.0264	0.0472	0.2405	0.2593	0.7008	46.3	37.1
14 Increased NRM	0.0266	0.1668	0.2557	0.0469	0.6208	71.9	8.9
15 Increased NRM	0.0265	0.2212	0.2481	0.0468	0.7008	62.8	30.2
16 Increased relapse	0.026	0.0438	0.7659	0.0361	0.6208	14.1	10.8
17 Increased relapse	0.0259	0.0422	0.8354	0.0335	0.7008	8.9	43.5

10.2 Secondary endpoints

a. In patients eligible for reduced-duration immunosuppression, 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 immunosuppression 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.[47,48] Treatment of disease relapse/progression/persistence, 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 of 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 rules varies according to immunosuppression 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 later-occurring 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 HSCT for the group overall and for patients with shortened-duration immunosuppression (i.e., for whom reduced duration immunosuppression was stopped at the assigned duration); 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 progression-free survival, disease-free survival, overall survival, and GVHD-related survival endpoints (GFRFS, cGFRFS) after transplantation.

   Using the Kaplan-Meier method, the probabilities of these survival endpoints at 1-year and longer-term will be estimated and reported with 90% confidence intervals.

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. Describe major toxicities and complications associated with the transplantation procedure.

   Selected toxicities will be reported descriptively.

g. Evaluate selected MDS patients and transplant characteristics in relation to transplantation outcomes.

The association between selected baseline patient and transplant characteristics and progression-free survival, overall survival, relapse, and GVHD will be investigated using Cox proportional hazard models or proportional hazard models for competing risks

11.0 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.

12.0 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.

13.0 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. HSCT 1995; 15: 825-828.

APPENDIX B **Statistical Supplement**

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

Scenario #1 Historical risks:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.093	0.039	0.075	0.021	0.773
61-90	0.027	0.027	0.05	0.004	0.893
91-120	0.004	0.004	0.105	0.055	0.832
121-180	0.005	0.035	0.153	0.005	0.802

Stopped for lack of feasibility 0 (0%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety problem declared 222 (22.2%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
0.8	13.8	0.0	7.6	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
20.00	45.00	45.00	40.95	45.00	45.00

Scenario #2 Same as historical risks, but more GF, feasibility unchanged:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.093	0.039	0.075	0.021	0.773
61-90	0.027	0.027	0.05	0.004	0.893
91-120	0.054	0.004	0.105	0.055	0.782
121-180	0.035	0.035	0.153	0.005	0.772

Stopped for lack of feasibility 0 (0%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety problem declared 584 (58.4%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
44.0	11.1	0.0	7.0	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
20.00	20.00	40.00	34.13	45.00	45.00

Scenario #3 Same as historical risks, but more GF, feasibility unchanged:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.093	0.039	0.075	0.021	0.773
61-90	0.027	0.027	0.05	0.004	0.893
91-120	0.104	0.004	0.105	0.055	0.732
121-180	0.055	0.035	0.153	0.005	0.752

Stopped for lack of feasibility 0 (0%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety problem declared 874 (87.4%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
80.7	8.0	0.0	2.9	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
20.00	20.00	20.00	27.26	35.00	45.00

Scenario #4 Same as historical risks, but more GVHD, feasibility unchanged:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.093	0.039	0.075	0.021	0.773
61-90	0.027	0.027	0.05	0.004	0.893
91-120	0.004	0.004	0.105	0.155	0.732
121-180	0.005	0.035	0.153	0.055	0.752

Stopped for lack of feasibility 0 (0%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety problem declared 828 (82.8%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
0.7	7.3	0.0	77.0	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
20.00	20.00	20.00	27.93	35.00	45.00

Scenario #5 Same as historical risks, but more GVHD, feasibility unchanged:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.093	0.039	0.075	0.021	0.773
61-90	0.027	0.027	0.05	0.004	0.893
91-120	0.004	0.004	0.105	0.205	0.682
121-180	0.005	0.035	0.153	0.055	0.752

Stopped for lack of feasibility 0 (0%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety problem declared 933 (93.3%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
0.6	7.9	0.0	89.0	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
20.00	20.00	20.00	24.88	25.00	45.00

Scenario #6 Same as historical risks, but more NRM, feasibility unchanged:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.093	0.039	0.075	0.021	0.773
61-90	0.027	0.027	0.05	0.004	0.893
91-120	0.004	0.054	0.105	0.055	0.782
121-180	0.005	0.065	0.153	0.005	0.772

Stopped for lack of feasibility 0 (0%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety problem declared 734 (73.4%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
0.3	68.5	0.1	6.0	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
20.00	20.00	25.00	30.72	45.00	45.00

Scenario #7 Same as historical risks, but more NRM, feasibility unchanged:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.093	0.039	0.075	0.021	0.773
61-90	0.027	0.027	0.05	0.004	0.893
91-120	0.004	0.104	0.105	0.055	0.732
121-180	0.005	0.085	0.153	0.005	0.752

Stopped for lack of feasibility 0 (0%) times.
Stopped for lack of feasibility at first look 0 (0%) times.
Safety problem declared 927 (92.7%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
0.3	91.4	0.0	3.8	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
20.00	20.00	20.00	24.66	25.00	45.00

Scenario #8 Same as historical risks, but more GF and lower feasibility:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.143	0.089	0.125	0.071	0.573
61-90	0.077	0.077	0.1	0.054	0.693
91-120	0.054	0.004	0.105	0.055	0.782
121-180	0.035	0.035	0.153	0.005	0.772

Stopped for lack of feasibility 49 (4.9%) times.
Stopped for lack of feasibility at first look 32 (3.2%) times.
Safety problem declared 425 (42.5%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
31.1	7.6	0.0	5.0	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
20.0	25.0	45.0	36.6	45.0	45.0

Scenario #9 Same as historical risks, but more GF and lower feasibility:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.193	0.139	0.175	0.121	0.373
61-90	0.077	0.077	0.1	0.054	0.693
91-120	0.104	0.004	0.105	0.055	0.732
121-180	0.055	0.035	0.153	0.005	0.752

Stopped for lack of feasibility 486 (48.6%) times.
Stopped for lack of feasibility at first look 274 (27.4%) times.
Safety problem declared 388 (38.8%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
33.3	2.1	0.0	4.0	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
20.00	20.00	25.00	28.68	35.00	45.00

Scenario #10 Same as historical risks, but more GVHD and lower feasibility:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.143	0.089	0.125	0.071	0.573
61-90	0.077	0.077	0.1	0.054	0.693
91-120	0.004	0.004	0.105	0.155	0.732
121-180	0.005	0.035	0.153	0.055	0.752

Stopped for lack of feasibility 43 (4.3%) times.
Stopped for lack of feasibility at first look 25 (2.5%) times.
Safety problem declared 619 (61.9%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
0.5	6.5	0.0	55.8	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
20.00	20.00	35.00	33.14	45.00	45.00

Scenario #11 Same as historical risks, but more GVHD and lower feasibility:
[1] Cumulative Risks days 90 - 180:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.193	0.139	0.175	0.121	0.373
61-90	0.077	0.077	0.1	0.054	0.693
91-120	0.004	0.004	0.105	0.205	0.682
121-180	0.005	0.035	0.153	0.055	0.752

Stopped for lack of feasibility 412 (41.2%) times.
Stopped for lack of feasibility at first look 226 (22.6%) times.
Safety problem declared 460 (46%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
0.2	2.5	0.0	43.3	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
20.00	20.00	25.00	27.99	35.00	45.00

Scenario #12 Same as historical risks, but more NRM and lower feasibility:
[1] Cumulative Risks days 90 - 180:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.143	0.089	0.125	0.071	0.573
61-90	0.077	0.077	0.1	0.054	0.693
91-120	0.004	0.054	0.105	0.055	0.782
121-180	0.005	0.065	0.153	0.005	0.772

Stopped for lack of feasibility 412 (41.2%) times.
Stopped for lack of feasibility at first look 226 (22.6%) times.
Safety problem declared 460 (46%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
0.2	2.5	0.0	43.3	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.

20.00 20.00 25.00 27.99 35.00 45.00

Scenario #13 Same as historical risks, but more NRM and lower feasibility:
[1] Cumulative Risks days 90 - 180:
After 1000 simulations with probabilities:

Days	Graft Failure	Nonrelapse mortality	Relapse	GVHD	No Event
1-60	0.193	0.139	0.175	0.121	0.373
61-90	0.077	0.077	0.1	0.054	0.693
91-120	0.004	0.104	0.105	0.055	0.732
121-180	0.005	0.085	0.153	0.005	0.752

Stopped for lack of feasibility 484 (48.4%) times.
Stopped for lack of feasibility at first look 273 (27.3%) times.
Safety problem declared 405 (40.5%) times.
Percent of times safety problem by reason (%):

GraftFail	NRM	Relapse	aGVHD	NoEvent
0.4	37.6	0.0	3.2	0.0

Number of patients entered into the study:

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
20.00	20.00	25.00	28.47	35.00	45.00