Reduced intensity, partially HLA mismatched allogeneic BMT for hematologic malignancies using donors other than first-degree relatives

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SCHEMA

Transplant regimens: overview

Regimen *	Preparative regimen	High-dose cyclophosphamide	MMF	Sirolimus	Tacrolimus
~~A~~	~~Flu-Cy-TBI~~	~~Day 3 and 4~~	~~None~~	~~Day 5 - 180~~	~~None~~
B	Flu-Cy-TBI	Day 3 and 4	Day 5 - 35	Day 5 - 180	None
B2	Flu-Cy-TBI	Day 3 and 4	Day 5 – 35	Day 5 – 180	None
B3	Flu-Cy-TBI	Day 3 and 4	Day 5 – 35	Day 5 – 180	None
C	Flu-Cy-TBI	Day 3 and 4	Day 5 - 35	None	Day 5 - 180
~~D~~	~~Flu-Bu~~	~~Day 3 and 4~~	~~Day 5 - 35~~	~~Day 5 - 180~~	~~None~~

***** See Section 5.2 for sequence of study.

SCHEMA [a]

REGIMEN B

Days –6, –5	**Fludarabine** 30 mg/m^2 IV QD, adjusted for CrCl **Cyclophosphamide** 14.5 mg/kg IV QD
	↓
Days –4, –3, –2	**Fludarabine** 30 mg/m^2 IV QD, adjusted for CrCl
	↓
Day –1	**TBI** 400 cGy
	↓
Day 0	Infuse T-cell replete bone marrow (at least 48 hours after last chemotherapy)
	Begin antibiotic prophylaxis (no voriconazole)
	↓
Days 3, 4	**Cyclophosphamide** 50 mg/kg IV QD **Mesna** 40 mg/kg IV QD in divided doses
	↓
Day 5	**Sirolimus** loading dose: 6 mg PO once (pts ≥ 18 y) [b]
	Begin **MMF** 15 mg/kg PO TID (maximum daily dose 3 g/day) (Immunosuppression must begin at least 24 hours after Cy completion)
	Begin **GCSF** 5 µg/kg SC or IV QD, until ANC ≥ 1000/mm^3 over 3 days
	↓
Day 6	Begin **sirolimus** 2 mg PO QD (pts ≥ 18 y) [b], target trough 5 – 12 ng/mL
	↓
Day 30 (+/- 3 d)	Assess chimerism in peripheral blood
	↓
Day 35	Discontinue MMF (optional if GVHD is active)
	↓
Day 60 (+/- 5 d)	Assess chimerism in peripheral blood Evaluate disease
	↓
Day 100 (+/- 5 d)	GVHD evaluation
	↓
Day 180	Discontinue sirolimus (optional if GVHD is active);
Day 180 (+/- 21 d)	Assess chimerism in peripheral blood Evaluate disease
	↓
1 yr (+/- 30 d)	Assess chimerism in peripheral blood Evaluate disease

a. See Sections 5.3 and 5.4 for complete dosing instructions. Up to 2 days of rest may be added after TBI, before BMT, per Section 5.34.
b. See Section 5.41 for sirolimus dosing in younger pts.

SCHEMA [a]

REGIMEN B2

Days –6, –5	**Fludarabine** 30 mg/m^2 IV QD, adjusted for CrCl **Cyclophosphamide** 14.5 mg/kg IV QD
	↓
Days –4, –3, –2	**Fludarabine** 30 mg/m^2 IV QD, adjusted for CrCl
	↓
Day –1	**TBI** 400 cGy
	↓
Day 0	Infuse T-cell replete peripheral stem cells (at least 48 hours after last chemotherapy)
	Begin antibiotic prophylaxis (no voriconazole)
	↓
Days 3, 4	**Cyclophosphamide** 50 mg/kg IV QD **Mesna** 40 mg/kg IV QD in divided doses
	↓
Day 5	**Sirolimus** loading dose: 6 mg PO once (pts ≥ 18 y) [b]
	MMF 15 mg/kg PO TID (maximum daily dose 3 g/day) (Immunosuppression must begin at least 24 hours after Cy completion)
	Begin **GCSF** 5 µg/kg SC or IV QD, continue until ANC ≥ 1000/mm^3 over 3 days
	↓
Day 6	Begin **sirolimus** 2 mg PO QD (pts ≥ 18 y) [b], target trough 5 – 12 ng/mL
Day 30 (+/- 3 d)	Assess chimerism
	↓
Day 35	Discontinue MMF (optional if GVHD is active)
	↓
Day 60 (+/- 5 d)	Assess chimerism Evaluate disease
	↓
Day 100 (+/- 5 d)	GVHD evaluation
	↓
Day 180	Discontinue sirolimus (optional if GVHD is active);
Day 180 (+/- 21 d)	Assess chimerism Evaluate disease
	↓
1 yr (+/- 30 d)	Assess chimerism Evaluate disease

a. See Section 5.3 and 5.4 for complete dosing instructions. Up to 2 days of rest may be added after TBI, before transplant, per Section 5.34.
b. See Section 5.41 for sirolimus dosing including in younger pts.

SCHEMA [a]

REGIMEN C

Days –6, –5	**Fludarabine** 30 mg/m^2 IV QD, adjusted for CrCl **Cyclophosphamide** 14.5 mg/kg IV QD
	↓
Days –4, –3, –2	**Fludarabine** 30 mg/m^2 IV QD, adjusted for CrCl
	↓
Day –1	**TBI** 400 cGy
	↓
Day 0	Infuse T-cell replete bone marrow (at least 48 hours after last chemotherapy)
	Begin antibiotic prophylaxis (no voriconazole)
	↓
Days 3, 4	**Cyclophosphamide** 50 mg/kg IV QD **Mesna** 40 mg/kg IV QD in divided doses
	↓
Day 5	Begin **tacrolimus** 1 mg IV QD (for pts ≥ 18 y) [b] and **MMF** 15 mg/kg PO TID (maximum daily dose 3 g/day) (Immunosuppression must begin at least 24 hours after Cy completion)
	Begin **GCSF** 5 µg/kg SC or IV QD, continue until ANC ≥ 1000/mm^3 over 3 days
	↓
Day 30 (+/- 3 d)	Assess chimerism
	↓
Day 35	Discontinue MMF (optional if GVHD is active)
	↓
Day 60 (+/- 5 d)	Assess chimerism Evaluate disease
	↓
Day 100 (+/- 5 d)	GVHD evaluation
	↓
Day 180	Discontinue tacrolimus (optional if GVHD is active);
Day 180 (+/- 21 d)	Assess chimerism Evaluate disease
	↓
1 yr (+/- 30 d)	Assess chimerism Evaluate disease

a. See Section 5.3 and 5.4 for complete dosing instructions. Up to 2 days of rest may be added after TBI, before BMT, per Section 5.34.
b. See Section 5.42 for tacrolimus dosing including in younger pts.

1.0 INTRODUCTION

Allogeneic blood or marrow transplantation (BMT) is a potentially curative therapy for a variety of hematologic malignancies, including the acute and chronic leukemias, myelodysplasia, and lymphomas. Recent advances in allogeneic BMT platforms have substantially lowered transplant-related morbidity both in the HLA-matched and partially-HLA-mismatched settings. One of these advances is the incorporation of high-dose post-transplantation cyclophosphamide (Cy) for prophylaxis of graft-versus-host-disease (GVHD) and graft rejection, as developed at Johns Hopkins. This agent, when administered at high doses after myeloablative, HLA matched, related or unrelated donor BMT, notably has been found to be effective single-agent prophylaxis against GVHD, obviating the need for calcineurin inhibitors (CNI's) in this setting.[1] In nonmyeloablative, HLA matched or mismatched, related-donor transplantation, the combination of high-dose post-transplantation Cy, mycophenolate mofetil (MMF), and tacrolimus has been associated with acceptable rates of engraftment and relatively low rates of acute and chronic GVHD.[2]

An advantage to nonmyeloablative, partially-HLA mismatched, related-donor BMT is that most individuals have multiple potential and readily available donors. However, in some cases patients lack a suitable first-degree related donor or an HLA-matched, unrelated donor. In patients infected by the human immunodeficiency virus (HIV) it appears that it may be possible to cure patients of HIV by selecting HIV-resistant donors as is elaborated below. Transplantation using mismatched, unrelated donors has historically been associated with increased rejection rates, severe acute GVHD, extensive chronic GVHD, and increased transplant-related mortality.[3,4] Due to increases in graft rejection risk and excessive toxicities with increasing degrees of mismatch, relatively few transplants using more than 1-locus mismatched, unrelated donors have been performed. Transplantation using partially-HLA mismatched donors who are second-degree as opposed to first-degree relatives is also investigational given the greater mismatching at minor histocompatibility antigens (mHAg). Thus, if transplantation using mismatched unrelated donors or non-first-degree relatives could be performed with an acceptable toxicity profile, an important unmet need would be served. Towards this goal, the current study extends our platform of nonmyeloablative, partially HLA-mismatched BMT to the use of such donors, investigating postgrafting immunosuppression regimens that incorporate high-dose Cy. Of central interest is the incorporation of sirolimus into this postgrafting immunosuppression regimen.

As an alternative to transplantation using bone marrow, there are circumstances in which only peripheral blood stem cells are available as a graft source for a variety of different reasons (e.g., the unrelated donor doesn't want to go to the OR) and there are some diseases such as myelodysplasia where peripheral blood stem cells may be more effective. While transplants with peripheral blood stem cells (PBSC) have higher rates of GVHD than transplants with bone marrow, there are data suggesting that PBSC transplants result in lower graft failure and improvements in overall survival (OS) and progressive free survival (PFS) [71,72,73]. Using the methods described above, the PBSC regimen investigates the ability of using mismatched unrelated donors or non-first-degree relatives with a PBSC transplant.

1.1 Post-transplantation high-dose cyclophosphamide

The immunologic rationale for administering 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.[5] 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.[5-10] As a form of drug-induced immunologic tolerance,[11] 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.[1] Pre-clinical studies demonstrated that engraftment of major histocompatibility complex (MHC)-mismatched bone marrow could be achieved by conditioning mice with pre-transplantation fludarabine and low dose (400 cGy) total body irradiation (TBI), with post-transplantation Cy.[7] Additional studies demonstrated that post-transplantation Cy reduced the incidence and severity of GVHD in the setting of MHC-mismatched allogeneic BMT after myeloablative conditioning.[6]

a) Efficacy of single agent post-transplantation cyclophosphamide in GVHD prevention

After allogeneic BMT, standard regimens of GVHD prophylaxis consist of a CNI (cyclosporine or tacrolimus) in combination with either methotrexate, MMF, or sirolimus. However, acute GVHD still occurs in 35-55% of BMT recipients from HLA-matched siblings, and more frequently in unrelated donor BMT recipients.[12-16] While CNI's inhibit acute GVHD, they are less effective in preventing chronic GVHD.[17] Moreover, they impair immune reconstitution by inhibiting T-cell development, potentially increasing the risk of disease relapse.[18-20] Thus, a platform that minimizes the use of CNI's, minimizes GVHD, and retains the donor graft antitumor efficacy would be desirable.

Toward this end, high-dose Cy on Days 3 and 4 after myeloablative, HLA-matched related or unrelated donor BMT recently has been reported to be effective single-agent GVHD prophylaxis in patients with hematologic malignancies, obviating the need for CNI's in this setting.[1] Luznik et al studied 117 patients with advanced hematologic malignancies received HLA-matched related or HLA-matched unrelated donor allografts with a platform of conventional busulfan/cyclophosphamide conditioning, T-cell-replete bone marrow, followed by 50 mg/kg/day of Cy on Days 3 and 4 after transplantation as the only GVHD prophylaxis.[21] The non-relapse mortality (NRM) at Day 100 and 2 years were 9% and 17%, respectively. The 2-year event-free survival (EFS) was 39%. The incidences of acute grade II-IV and grade III-IV GVHD were only 43% and 10%, respectively, and the incidence of chronic GVHD was only 10%. In addition, this approach was marked by prompt immune reconstitution and a low incidence of opportunistic infections including CMV disease; the observed lymphocyte reconstitution compared favorably to the levels seen after T-cell-replete allogeneic transplantation with cyclosporine and methotrexate for GVHD prophylaxis.

b) <u>Nonmyeloablative, haploidentical BMT: role of postgrafting immunosuppression</u>

Independent clinical trials have evaluated a nonmyeloablative, partially HLA-mismatched (haploidentical), related-donor BMT platform with high-dose post-transplantation Cy, tacrolimus, and MMF for GVHD and graft rejection prophylaxis. This approach has been associated with rapid and stable engraftment in most patients. Most importantly, this approach has carried acceptable rates of acute GVHD, chronic GVHD, and NRM that parallel those seen with nonmyeloablative, HLA-matched transplants.[2,22-24]

The postgrafting immunosuppression regimen that underlies recent research efforts at Johns Hopkins has been published.[2,22,24] Conditioning in these studies has consisted of fludarabine, low-dose Cy, and 400 cGy TBI. A combined analysis of two independent clinical trials was reported in 2008 (40 patients at Johns Hopkins, 28 at Fred Hutchinson Cancer Research Center), evaluating the safety and efficacy of a high-dose post-transplantation Cy platform after outpatient nonmyeloablative conditioning and T-cell-replete BMT from partially HLA-mismatched related donors (Figure 1).[2] Eligible patients were 0.5-70 years of age with high-risk myeloid or lymphoid malignancies. Twenty-one patients (31%) had previously received autologous BMT. Conditioning consisted of Cy 14.5 mg/kg/day IV on Days –6 and –5, fludarabine 30 mg/m^2/day IV on Days –6 to –2, and 400 cGy of TBI on Day –1. On Day 0, patients received donor bone marrow, which was not T-cell depleted. Following transplantation, high-dose Cy (50 mg/kg) 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 post-transplantation Cy with tacrolimus and MMF. Filgrastim 5 μg/kg/day was administered until recovery of neutrophils to >1000/μL:

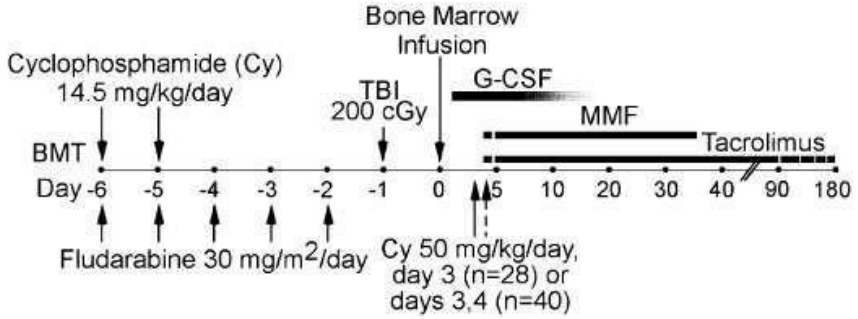

Figure 1: Treatment schema in previous studies

Engraftment and chimerism. 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.

Hospitalizations and infections. Patients received their initial treatment in the outpatient department. The median number of hospitalizations prior to Day 60 was 1 (range 0-4), with a median length of stay of 4 days and with neutropenic fever or nonneutropenic infection accounting for 80% of the admissions. Twenty-two patients (32%) did not require hospitalization within the first 60 days of transplantation.

Patients who are seropositive for cytomegalovirus (CMV) are known to be at high-risk for reactivating CMV after transplantation, regardless of the serologic status of the donor.[25] In this study, CMV reactivation occurred in 38% of high-risk patients, without CMV disease or CMV-associated mortality.

Graft-versus-host disease and survival outcomes. The cumulative incidences of grades II-IV and III-IV acute GVHD by Day 200 were <35% and <10%, respectively, on competing-risk analysis (Figure 2). The groups did not differ significantly in the incidence of grades II-IV or III-IV acute 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.

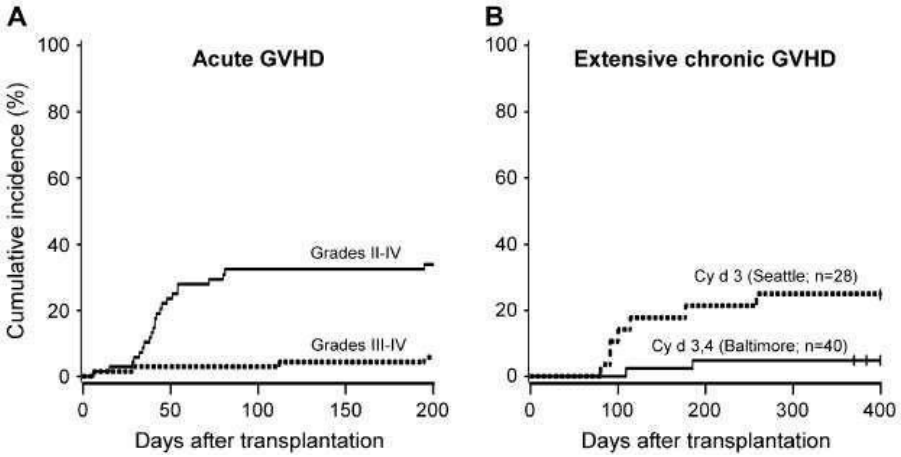

Figure 2: Low incidence of GVHD with post-transplantation Cy

The cumulative incidences of relapse and NRM at 1 year were 51% and 15% respectively, and the EFS probability at 1 year was 34%. Similar outcomes were seen in a recent analysis of 185 patients treated on these trials and a follow-up phase II trial (J0457).[24]

In summary, HLA-haploidentical BMT after nonmyeloablative conditioning and using 2 doses of post-transplantation Cy followed by tacrolimus and MMF is a well-tolerated procedure that can be administered largely in an outpatient setting. This postgrafting immunosuppression regimen for nonmyeloablative, HLA-haploidentical, related-donor BMT has been or is being investigated in several trials at Johns Hopkins, including a multicenter phase II trial through the BMT CTN (J0843).[26] The toxicity of the procedure compares favorably to the toxicity of nonmyeloablative 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.

1.2 Impact of HLA mismatching on outcome

Historically, HLA typing has been the most important predictor of outcome after allogeneic BMT.[4] Increasing degrees of HLA mismatch between patient and donor at either the antigen or allele level have been associated with worse outcomes in numerous series, with respect to GVHD, graft failure, and transplant-related mortality.[3,27-30] In the setting of myeloablative, unrelated donor transplantation, 1 or 2 allele mismatching has been associated with increased risk of these complications. Similarly in the setting of reduced-intensity transplants, high rates of transplant-related mortality have been observed with the use of 1 and 2 MHC Class I mismatched donors.[31,32] In the Fred Hutchinson experience with a nonmyeloablative strategy comprised of fludarabine and 200 cGy TBI followed by cyclosporine and MMF, transplantation using HLA Class I mismatched, mostly unrelated donors was associated with a NRM incidence of 22% at Day 100 and 36% at 1 year; a grade II-IV acute GVHD incidence of 69%; a grade III-IV acute GVHD incidence of 26%; and an incidence of extensive chronic GVHD of 41%.[33] On the other hand, supporting the possibility of safely performing nonmyeloablative, HLA mismatched transplants from unrelated donors is the recent UK experience.[34] In this study a regimen of fludarabine, melphalan, and alemtuzumab was followed by cyclosporine administration and transplantation from unrelated donors who were either 10/10 matches (n = 107) or HLA mismatched (n = 50, with only 3 donors mismatched at 3-4 loci). This approach was associated with high rates of durable engraftment and acceptable rates of grade II-IV acute GVHD (20% versus 22% respectively) and chronic extensive GVHD (23% versus 24% respectively).[34]

The reported effect of HLA disparity on relapse risk varies. However, a lower relapse risk has been reported in some series with increasing HLA disparity, suggesting a graft-versus-tumor effect. For example, in patients with poor-risk leukemia undergoing related-donor, myeloablative BMT, 2 and 3-locus mismatched transplants were associated with a significantly lower relapse than HLA-identical sibling transplants.[27] Likewise, in patients with high-risk leukemia or myelodysplastic syndrome undergoing myeloablative, T-cell replete BMT, significantly lower relapse ($p < 0.004$) was seen with using 1 antigen mismatched, versus no antigen mismatched, donors.[35] Following unrelated donor BMT, specific combinations of allele mismatches have been linked with lower relapse risk and improved overall survival, not necessarily those that lead to severe acute GVHD.[36]

However, it is possible that the type of GVHD prophylaxis could influence the balance between GVHD toxicity and relapse. A recent analysis of our nonmyeloablative haploidentical BMT data supports this hypothesis and suggests that HLA disparity need no longer be a barrier when selecting amongst potential donors.[24] We retrospectively analyzed the outcomes of 185 patients with poor-risk hematologic malignancies enrolled on three similar clinical trials of related-donor, haploidentical BMT utilizing post-transplantation high-dose Cy, MMF, and tacrolimus (J9966, J0457, and the Fred Hutchinson trial).[24] Notably, no adverse effect of HLA mismatching was found using this approach.[24] With increasing degrees of HLA mismatch, no deleterious effect was seen on EFS or on the incidence of NRM or acute GVHD (Figure 3). In fact, on multivariate analysis, more mismatches were associated with a possibly protective effect on EFS.

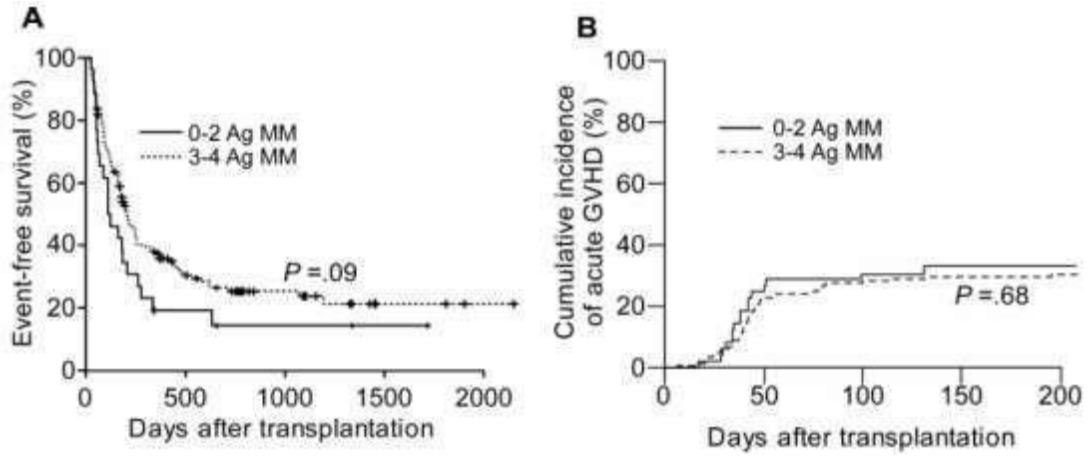

Figure 3: A, EFS according to number of antigen mismatches (HLA-A, -B, -Cw, and -DRB1 combined) in

either the GVH or HVG direction. B, Cumulative incidences of acute grade II-IV GVHD according to the number of antigen mismatches in the GVH direction.

These results suggest an anti-tumor effect of partially HLA-mismatched BMT that is irrespective of clinically significant GVHD. Consistent with these observations, a retrospective study of nonmyeloablative BMT with post-transplantation high-dose Cy for relapsed/refractory Hodgkin lymphoma (data from Johns Hopkins, Seattle, and collaborating sites) found similar overall outcomes with HLA-matched related and HLA-haploidentical related donors. Incidences of acute grade III-IV and extensive chronic GVHD were similar (11%/35% for HLA-haploidentical, and 16%/50% for HLA matched related transplants, respectively).[23] The haploidentical transplants actually had significantly lower NRM, a significantly decreased risk of relapse, and a significantly improved progression-free survival than HLA-matched related transplants.

Because of the recent progress in prevention of GVHD and graft rejection with high-dose post-transplantation Cy, a Johns Hopkins study has been able to examine haploidentical, related-donor BMT for poor-risk hematologic malignancies following myeloablative conditioning. There have not been prohibitive rates of toxicity or graft rejection on preliminary experience (H. Symons, personal communication).

1.3 Reduced-intensity conditioning with fludarabine-busulfan

With reduced-intensity conditioning (RIC), theoretically dose-equivalent regimens have been associated with significant differences in outcome, including differences in relapse, toxicities, engraftment kinetics, and survival.[37] In a CIBMTR retrospective analysis of conditioning intensity, flu-200TBI was associated with higher treatment failure rates than flu-bu or flu-melphalan RIC (M. Pasquini, unpublished data). Although variability in patient risk and transplant procedure may account for some of these differences, based on such concerns flu-200TBI has been omitted from a BMT CTN trial (0901) comparing RIC and myeloablative conditioning for these diseases. Accordingly, and consistent with our programmatic interest at Johns Hopkins to extend the experience of nonmyeloablative partially HLA-mismatched BMT with fludarabine and TBI (flu-200TBI) to a platform based on fludarabine and busulfan (flu-bu), the current protocol uses the latter conditioning strategy. The flu-bu regimen is typically considered to be reduced-intensity or nonmyeloablative if it has $\leq$ 8 mg/kg PO busulfan or IV equivalent, with busulfan dosing in representative series ranging from one-quarter to one-half of that used in myeloablative conditioning.[37,38] In a Dana Farber analysis of RIC transplantation using HLA-matched related or unrelated donors, fludarabine 120 mg/m^2 IV + busulfan 6.4 mg/kg IV, as compared with fludarabine 120 mg/m^2 IV + busulfan 3.2 mg/kg IV, was associated with greater progression-free survival (HR 0.6, p = 0.04) without difference in overall survival (V. Ho, EBMT 2010 annual meeting). In the context of nonmyeloablative regimens, one must weigh the potential risks of more intensive conditioning against the potentially greater risks of graft rejection and relapse with less intensive conditioning. The cumulative doses of fludarabine (150 mg/m^2) and busulfan (8 mg/kg PO or 6.4 mg/kg IV) selected for the current study are standard.[37]

With the reduced morbidity of transplantation regimens incorporating high-dose post-transplantation Cy for graft rejection and GVHD prophylaxis, relapse has remained the major problem particularly with nonmyeloablative transplants. The combination of flu-bu with post-transplantation Cy in the nonmyeloablative setting is new. Our group has studied the combination of fludarabine, busulfan, and post-transplantation Cy for hematologic malignancies patients undergoing myeloablative, HLA-matched BMT (J0844). There has not been an excessive incidence of toxicity on that study to date (L. Luznik, personal communication). The toxicities of a reduced intensity, flu-bu conditioning regimen are not expected to differ substantially from the flu-low dose Cy-200TBI platform incorporating post-transplantation Cy. This is expected to be a more immunosuppressive regimen, however, and the engraftment kinetics and toxicities may differ. Given the advances in GVHD prophylaxis with post-transplantation Cy, RIC with flu-bu combined with postgrafting immunosuppression that includes high-dose post-transplantation Cy was the initial platform for the current study in patients with poor-risk hematologic malignancies. However, based on subsequent preliminary engraftment data from a study involving reduced-intensity flu-bu conditioning with BMT from first-degree related donors (reduced-intensity flu-bu, followed by high-dose Cy on Days 3 and 4, MMF on Days 5-35, and tacrolimus on Days 5-180), it was questioned whether this conditioning regimen is

sufficiently immunosuppressive for graft failure prophylaxis. Given the potentially higher risk of graft failure with the use of unrelated and multiply HLA-mismatched donors, the current study has been amended (5/18/2011 version date) to change the conditioning regimen to our Johns Hopkins historical standard of flu-low dose Cy-200TBI.

1.4 Sirolimus and post-transplantation cyclophosphamide: rationale for study

Mechanistically, immunosuppressive drugs given to control GVHD suppress alloimmunity by nonspecific inhibition of alloreactive T-cell activation, proliferation, and differentiation. This is in clear contrast to the essential requirement for the induction of stable tolerance, which entails the apoptosis of alloreactive T-cells.[39] Thus, global immunosuppression by blocking T-cell activation and apoptosis precludes and delays the induction of transplantation tolerance after allografting. Immunosuppressive drugs can be classified according to their action on induction of apoptosis and inhibition of T-cell proliferation.[40] Of all the commonly used immunosuppressants (steroids, tacrolimus, cyclosporine, MMF, sirolimus, Cy, methotrexate), only methotrexate and Cy induce the apoptosis of alloantigen-activated human T-cells, whereas other immunosuppressants mainly inhibit their proliferation.[40] By promoting tolerance induction, high dose Cy has facilitated the use of alternative donor sources, such as HLA-mismatched grafts. Our underlying hypothesis is that high-dose Cy prevents acute GVHD by reducing the frequency of alloreactive T effector cells while sparing donor-specific immunity and without critically depleting the T regulatory (Treg) cell pool. If the precursor frequency of alloreactive T effectors remains high or the Treg pool declines below a critical threshold, then increased differentiation toward the pathogenic T effector cells ensues and acute GVHD develops.

Sirolimus is an immunosuppressive agent that inhibits the mammalian target of rapamycin (mTOR), downregulating T-cell proliferation and activation.[41] Since it does not inhibit T-cell receptor induced signaling, it does not block T-cell receptor-induced tolerance.[39] This agent has been used widely to prevent graft rejection in solid organ and hematopoietic transplantation, and has been used both to prevent and treat acute and chronic GVHD.[42]

This study investigates regimens for transplantation that may inhibit graft rejection and GVHD by promoting T-cell tolerance. As previously outlined, past regimens have relied heavily on immunosuppression with CNI's.[43] However, these agents also inhibit T-cell receptor induced signaling required for the generation of T-cell tolerance. On the other hand, activation of Th1 effector cells in the setting of mTOR signaling blockade with sirolimus has been shown to induce anergy.[44] Therefore, of central interest is a postgrafting immunosuppressive approach with mTOR inhibition in combination with other agents that promote tolerance induction, such as high-dose post-transplantation Cy.

a) mTOR inhibition promotes anergy and generation of regulatory CD4+ T-cells

It is thought that cyclosporine and tacrolimus inhibit tolerance induction in vivo by limiting IL-2 production and Treg function, while sirolimus does not inhibit tolerance induction biochemically and promotes Treg expansion.[45,46] In murine models of hematopoietic transplantation, rejection is mediated in part by activation of alloreactive CD4+ Th1 cells.[47] Activation of Th1 cells in the presence of sirolimus results in anergy upon subsequent rechallenge with antigen.[44] Critically, this effect of sirolimus depends on the presence of normal T-cell receptor signaling during the exposure to sirolimus; thus simultaneous exposure of T-cells to sirolimus and a CNI will block the induction of anergy.[44] In contrast to committed Th1 cells, activation of naïve T-cells in the presence of sirolimus blocks CD4+ T-cell effector differentiation and promotes generation of FoxP3+, CD4+ T-cells (Tregs) that can inhibit effector T-cell responses in vitro.[48] Laboratory work at Johns Hopkins confirmed these findings using a genetic approach based on conditional deletion of mTOR and other components of the TORC1 and TORC2 mTOR signaling complexes in murine T-cells.[49] These experiments have demonstrated that CD4+ T-cell effector differentiation is possible in the absence of either TORC1 or TORC2 (Delgoffe and Powell, unpublished observation) but that the absence of both TORC1 and TORC2 following deletion of mTOR causes naïve T-cells to differentiate into functional Tregs upon activation.[49]

b) Potential synergy of sirolimus and post-transplantation Cy

Sirolimus has the ability to promote T-cell tolerance even in the presence of T-cell costimulation,[44] and in murine models of haploidentical BMT conditioned with low-dose TBI, can not only prevent graft rejection but induce tolerance in the absence of long-term immunosuppression.[50] Preclinical data further demonstrate that the anti-proliferative effects of sirolimus do not inhibit the effectiveness of post-transplant Cy, and that sirolimus and post-transplantation Cy are potentially synergistic in preventing graft rejection and facilitating stable mixed chimerism.[51] This synergic effect appears to be mediated independently from expression of CD25+ Tregs. In murine models of nonmyeloablative, haploidentical BMT involving post-transplantation Cy on Day 2, initiation of sirolimus on Day -1 did not block Cy-induced tolerance (Figure 4). Additionally, sirolimus administration on either Day -1 through Day 30, or Day +4 through Day 30, in the context of post-transplantation Cy was effective in preventing rejection and inducing stable mixed chimerism (Figure 4), whereas there was no sustained donor chimerism with either agent alone.

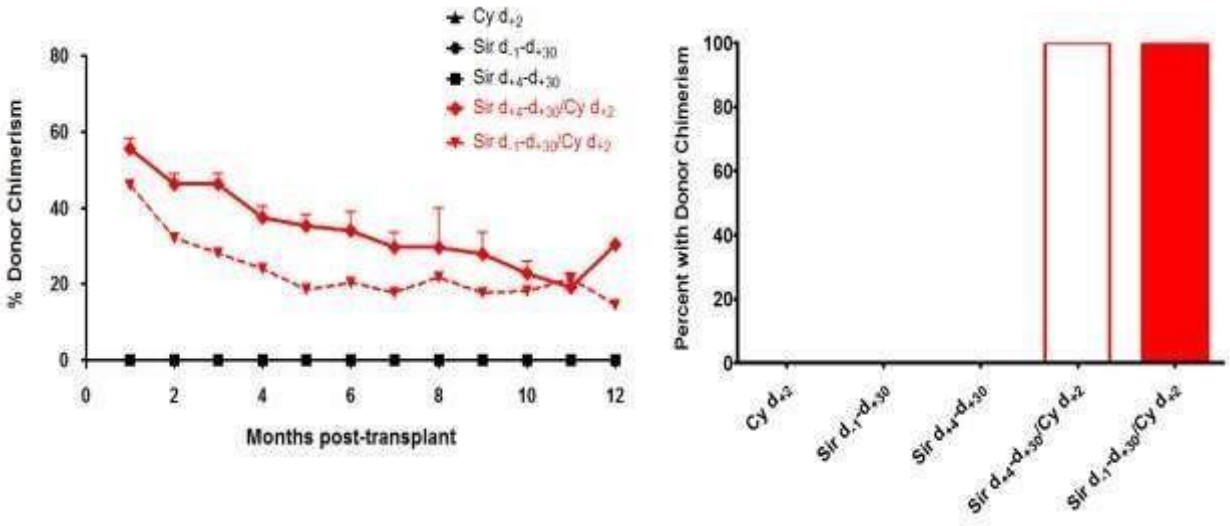

Figure 4: Synergism of sirolimus and Cy in preventing rejection and inducing stable mixed chimerism in preclinical models of haploidentical BMT.

The optimal timing of sirolimus initiation in the context of BMT with high-dose post-transplantation Cy is not defined. In our clinical trials incorporating high-dose post-transplantation Cy, tacrolimus has been initiated on day 5 based on preclinical data. Given the above data, and the efficacy in patients of administering tacrolimus on day + 5 though day 180 (together with MMF on Day 5 through Day 35), this window has been selected for sirolimus in this study. Following post-transplantation Cy, sirolimus will be studied in combination with MMF. Based on the known mechanisms of MMF and sirolimus, MMF is not expected to interfere with sirolimus-induced tolerance.

c) <u>Effect of mTOR inhibition on antiviral and antitumor responses</u>

While these immunosuppressive effects of sirolimus on T-cells would be expected to contribute favorably to post-transplantation tolerance, they might also be expected to inhibit desired immune responses against pathogens such as CMV and influenza. Despite the theoretically increased risk of such infections while on treatment with sirolimus, epidemiologic data from both solid organ transplantation [52] and BMT [53] do not seem to bear this concern out. To the contrary, those clinical data suggest a possible anti-CMV effect of sirolimus, and data from animal models of LCMV suggest that CD8+ T-cell responses are augmented by low-dose sirolimus in vivo.[54] Furthermore, sirolimus does not interfere with in vitro function (recognition and killing) of anti-mHAg-specific CD8+ T-cell clones, while its delayed in vivo administration does not block the graft-versus-tumor effect in murine model of allogeneic BMT. Thus, mTOR inhibition appears to be permissive for sustained antiviral and antitumor activity.[55,56]

Another rationale for the incorporation of sirolimus into allogeneic transplant regimens is its potential anti-lymphoma activity. mTOR inhibitors (temsirolimus, everolimus) have established clinical

activity in relapsed or refractory mantle cell lymphoma.[57] Preclinical activity has been demonstrated against Hodgkin lymphoma and a variety of non-Hodgkin lymphomas, and phase II trials have suggested single agent activity in Hodgkin lymphoma,[58] diffuse large B-cell lymphoma,[59] and other lymphoid neoplasms.

In a retrospective analysis of allogeneic transplantation at the Dana Farber Cancer Institute, lymphoma patients who received sirolimus following RIC transplantation (mostly with flu-bu) had a similar incidence of NRM, but a statistically significantly lower incidence of disease progression, than patients who did not receive sirolimus.[60] The benefit appeared to be restricted to patients receiving RIC regimens and to patients with lymphoma. This effect persisted after adjusting for GVHD and was associated with a statistically significant improvement in overall survival. Thus this class of agents may have dual activity against GVHD and against selected tumor types.

1.5 Special considerations in patients with HIV

A patient with HIV and acute myelocytic leukemia was cured of HIV infection by unrelated allogeneic transplantation using a donor who was homozygous for the CCR5delta32 polymorphism that confers HIV resistance. Another patient received a cord blood transplant with a CCR5delta32 homozygous donor and also appeared to eliminate HIV but the patient relapsed with lymphoma and died. These experiences are consistent with our understanding of CCR5 as an HIV coreceptor and suggest that selection of appropriate CCR5delta32 homozygous donors may allow additional patients to be cured of HIV.

1.6 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 disease5-9. Several studies have addressed this issue in the related setting. Of the eight randomized trials published10-18 only one reported a statistically 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 chronic graft-versus-host disease with peripheral blood stem cells as opposed to bone marrow12,16,19. One study showed a trend towards increase in chronic graft-versus-host disease with the use of peripheral blood stem cells19. 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 marrow7. 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 marrow8. In adults, chronic graft-versus-host disease is also more prevalent20. 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 mismatches21,22. 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 relapse17.

2.0 OBJECTIVES

2.1 Primary objectives

1. Phase 1 portion: In reduced-intensity, partially HLA mismatched allogeneic BMT from unrelated or non-first-degree related donors, identify a transplant regimen associated with acceptable rates of severe acute GVHD (≤ 25%) and transplant-related NRM (≤ 20%) by Day 100.
2. Phase 2 portion: With the selected transplant regimen, as a measure of immunologic efficacy, estimate the 6-month probability of survival without having had acute grade III-IV GVHD or evidence of graft failure.

2.2 Secondary objectives

1. Estimate the progression-free survival, disease-free survival, overall survival, cumulative incidence of progression or relapse, and cumulative incidence of NRM.
2. Estimate the cumulative incidence of acute grade II-IV GVHD, acute grade III-IV GVHD, and chronic GVHD.
3. Determine the need for systemic immunosuppressive treatment for GVHD beyond the originally planned prophylaxis regimen; estimate the cumulative incidence of systemic steroid initiation for GVHD, cumulative incidence of non-steroid immunosuppressant use, and cumulative incidence of discontinuation of systemic immunosuppression for GVHD treatment; describe the types of immunosuppression used for GVHD treatment; and evaluate GVHD composite endpoints (GVHD-free relapse-free survival, chronic GVHD-free relapse-free survival).
4. Describe graft failure frequency, kinetics of T-cell donor chimerism and total leukocyte donor chimerism in peripheral blood, and kinetics of neutrophil and platelet recovery.
5. Characterize immune reconstitution and the immunobiology of sirolimus and post-transplantation Cy by analyzing peripheral blood mononuclear cells collected prospectively at defined time points.

3.0 SELECTION OF PATIENTS AND DONORS

3.1 Patient eligibility

1. Patient age 0.5-75 years old.
2. Absence of a suitable related or unrelated bone marrow or peripheral stem cell donor who is molecularly matched at HLA-A, -B, -Cw, -DRB1, and -DQB1.
3. Absence of a suitable partially HLA-mismatched (haploidentical), first-degree related donor.
   Note: Determination of matching is based on allele or allele group level typing. To be considered haploidentical, the donor and recipient must be identical at at least one allele of each of the following genetic loci: HLA-A, -B, -Cw, -DRB1, and -DQB1. A minimum match of 5/10 is therefore required, and will be considered sufficient evidence that the donor and recipient share one HLA haplotype. Donors who are homozygous for the CCR5delta32 polymorphism are given preference.
4. Eligible diagnoses:
   a. Relapsed or refractory acute leukemia (acute myeloid leukemia or acute lymphoblastic leukemia or lymphoma) in second or subsequent remission, with remission defined as <5% bone marrow blasts morphologically
   b. Poor-risk acute leukemia in first remission, with remission defined as <5% bone marrow blasts morphologically:
      - AML with at least one of the following:
        - AML arising from MDS or a myeloproliferative disorder, or secondary AML
        - Presence of Flt3 internal tandem duplications
        - Poor-risk cytogenetics: Complex karyotype [≥ 3 abnormalities], inv(3), t(3;3), t(6;9), MLL rearrangement with the exception of t(9;11), or abnormalities of chromosome 5 or 7
        - Primary refractory disease

- ALL (leukemia and/or lymphoma) with at least one of the following:
  - Adverse cytogenetics such as t(9;22), t(1;19), t(4;11), or MLL rearrangement
  - Clear evidence of hypodiploidy
  - Primary refractory disease
- Biphenotypic leukemia

c. MDS with at least one of the following poor-risk features:
- Poor-risk cytogenetics (7/7q minus or complex cytogenetics)
- IPSS score of INT-2 or greater
- Treatment-related MDS
- MDS diagnosed before age 21 years
- Progression on or lack of response to standard DNA-methyltransferase inhibitor therapy
- Life-threatening cytopenias, including those generally requiring greater than weekly transfusions

d. Interferon- or imatinib-refractory CML in first chronic phase, or non-blast crisis CML beyond first chronic phase.
e. Philadelphia chromosome negative myeloproliferative disease.
f. Chronic myelomonocytic leukemia.
g. Juvenile myelomonocytic leukemia.
h. Low-grade non-Hodgkin lymphoma (including SLL and CLL) or plasma cell neoplasm that has progressed after at least two prior therapies (excluding single agent rituximab and single agent steroids), or in the case of lymphoma undergone histologic conversion; patients with transformed lymphomas must have stable disease or better.
i. Poor-risk CLL or SLL as follows: 11q deletion disease that has progressed after a combination chemotherapy regimen, 17p deletion disease, or histologic conversion; patients with transformed lymphomas must have stable disease or better.
j. Aggressive non-Hodgkin lymphoma as follows, provided there is stable disease or better to last therapy:
- NK or NK-T-cell lymphoma, peripheral T-cell lymphoma (including angioimmunoblastic T-cell lymphoma, hepatosplenic T-cell lymphoma, subcutaneous panniculitic T-cell lymphoma, and other variants), T-cell prolymphocytic leukemia, or blastic/blastoid variant of mantle cell lymphoma; or
- Hodgkin or aggressive non Hodgkin lymphoma that has failed at least one multiagent regimen, and the patient is either ineligible for autologous BMT or autologous BMT is not recommended.

  Eligible subtypes of aggressive non-Hodgkin lymphoma include:
  - mantle cell lymphoma
  - follicular grade 3 lymphoma
  - diffuse large B-cell lymphoma or its subtypes, excluding primary CNS lymphoma
  - primary mediastinal large B-cell lymphoma
  - large B-cell lymphoma, unspecified
  - anaplastic large cell lymphoma, excluding skin-only disease
  - Burkitt's lymphoma or atypical Burkitt's lymphoma (high-grade B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt's), in complete remission

5. Patients with CLL, SLL, or prolymphocytic leukemia must have < 20% bone marrow involvement by malignancy (to lower risk of graft rejection).
6. One of the following, in order to lower risk of graft rejection:

a. Cytotoxic chemotherapy, an adequate course of 5-azacitidine or decitabine, or alemtuzumab within 3 months prior to start of conditioning; or
b. Previous BMT within 6 months prior to start of conditioning.

Note: Patients who have received treatment outside of these windows may be eligible if it is deemed sufficient to reduce graft rejection risk; this will be decided on a case-by-case basis by the PI or co-PI.

7. Any previous BMT must have occurred at least 3 months prior to start of conditioning.
8. No active extramedullary leukemia or known active CNS involvement by malignancy. Such disease treated into remission is permitted.
9. Adequate end-organ function as measured by:
   a. Left ventricular ejection fraction ≥ 35%, or shortening fraction > 25%, unless cleared by a cardiologist
   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 in pediatric patients, if unable to perform pulmonary function tests due to young age, oxygen saturation >92% on room air
10. ECOG performance status ≤ 2 or Karnofsky or Lansky score ≥ 60.
11. Not pregnant or breast-feeding.
12. No uncontrolled bacterial, viral, or fungal infection (infection is permitted if there is evidence of response to medication).

    Note: HIV-infected patients are potentially eligible. Eligibility of HIV-infected patients will be determined on a case-by-case basis.

3.2 Donor eligibility

1. Potential donors consist of:
   b. Unrelated donors
   c. Second-degree relatives
   d. First cousins
2. Donor must not be HLA identical to the recipient.
3. The donor and recipient must be identical at at least 5 HLA alleles based on high resolution typing of HLA-A, -B, -Cw, -DRB1, and -DQB1, with at least one allele matched for a HLA class I gene (HLA-A, -B, or -Cw) and at least one allele matched for a class II gene (HLA-DRB1 or -DQB1).
4. Meets institutional selection criteria and medically fit to donate.
5. 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 result.
6. Has not donated blood products to recipient.

Donor prioritization criteria are designated in Section 3.3.

3.3 Donor prioritization

Eligible donors will be prioritized in the following order:

1. Donor matched with the recipient for at least one allele each at HLA-A, -B, -Cw, -DRB1, and -DQB1.
2. Fewest number of HLA-mismatched loci between donor and recipient. Preference will be given for donors who are matched for at least one allele each of HLA-A, -B, and -DRB1.

3. If multiple donors are available and the total number of mismatches is the same, donors who are mismatched at the allele level are prioritized over those who have antigen level mismatches; e.g., a 1 allele mismatch has priority over a 1 antigen mismatch.
4. Major ABO compatibility.
5. CMV serostatus: CMV negative donor preferred, if the recipient is CMV negative; CMV positive donor preferred, if the recipient is CMV positive.
6. ABO compatibility preferred over minor incompatibility.

Other considerations, such as donor age, health history and anti-donor HLA antibody status, may be prioritized over the above criteria. For patients with HIV infection, donors who are CCR5delta32 homozygous may be prioritized over other donors so long as they meet the donor eligibility criteria in Section 3.2.

4.0 REGISTRATION PROCEDURES

4.1 Registration requirements

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

- Signed and dated informed consent
- Patient eligibility checklist

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

4.2 Accrual goal

The accrual goal is to transplant up to 99 patients such that at least 20 patients will have mismatched unrelated peripheral blood donors using Regimen B2, per Sections 5.2 and 9.0. Up to 10 HIV+ patients with partially matched unrelated CCRd32 homozygous donors will have transplants using Regimen B2 as part of Cohort B3. Up to 3 additional patients per regimen may be transplanted to replace inevaluable patients (maximum number of transplants, 114). Additional patients may be screened and registered, in order to identify the target number of patients who meet all eligibility criteria and receive the transplant.

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 Overview of study design

Up to 3 regimens involving flu-Cy-200TBI conditioning (Regimens A though C) were initially planned (2 containing sirolimus and 1 containing tacrolimus), summarized in Table 1 below. Criteria for moving from one regimen to another are based upon the number of patients developing severe acute GVHD, transplant-related NRM, or graft failure, as detailed in Section 9.0 (Statistical Considerations). Whether a regimen is deemed prohibitive or not prohibitive is based specifically on the safety and stopping criteria in Section 9.0.

The first cohort of patients enrolled prior to the 5/18/11 version date (receiving flu-bu followed by post-transplantation Cy, MMF, and sirolimus) is now designated as having received Regimen D. Study of this regimen was stopped, per the Background section. The protocol was amended 12-21-2011 to remove Regimen A containing sirolimus without MMF. The other regimens will be studied in the following order:

- Begin with Regimen B (MMF + sirolimus), and expand if appropriate.
- If Regimen B is prohibitive at any point, move to Regimen C (MMF + tacrolimus) and expand if appropriate.
- If Regimen C is prohibitive, accrual will stop pending external review.

Table 1: Transplant regimens

Regimen	Preparative regimen	High-dose cyclophosphamide	MMF	Sirolimus	Tacrolimus
A	Flu-Cy-TBI	Day 3 and 4	None	Day 5 - 180	None
B	Flu-Cy-TBI	Day 3 and 4	Day 5 - 35	Day 5 - 180	None
B2	Flu-Cy-TBI	Day 3 and 4	Day 5 – 35	Day 5 – 180	None
B3	Flu-Cy-TBI	Day 3 and 4	Day 5 – 35	Day 5 – 180	None
C	Flu-Cy-TBI	Day 3 and 4	Day 5 - 35	None	Day 5 - 180
D	Flu-Bu	Day 3 and 4	Day 5 - 35	Day 5 - 180	None

Additional cohorts "B2" and "B3" will be added using peripheral blood donor cells according to the "B" Prep regimen.

Accrual need not pause while the first 5 patients on a given regimen complete monitoring to Day 100 (specified in Section 9.0). In the event that more than 5 patients are enrolled during this time, accrual to that regimen will continue. This is justifiable based on preliminary data with MMF + sirolimus and our historical data in nonmyeloablative, related-donor BMT with MMF + tacrolimus.

5.3 Conditioning, transplantation, and post-transplantation cyclophosphamide: Regimens B, B2, B3 and C

The preparative regimen for Regimens B and C consists of fludarabine, Cy, and TBI, with post grafting immunosuppression consisting of high-dose Cy with two other immunosuppressants (MMF and sirolimus, or MMF and tacrolimus respectively). Post grafting immunosuppression other than high-dose Cy is specified in Section 5.4.

5.31 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), if no days of rest before transplantation is planned.

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

For decreased creatinine clearance (CrCl), fludarabine dosage is reduced as follows:
CrCl ≥ 70 ml/min – fludarabine 30 mg/m2
CrCl 40-69 ml/min - fludarabine 24 mg/m2
CrCl 20-39 ml/min – fludarabine 20 mg/m2
CrCl < 20 ml/min – fludarabine 15 mg/m2

Alternatively, dose-adjustments of fludarabine for decreased CrCl can follow current institutional standard.

For patients ≥ 18 years old, CrCl will be estimated by the Cockcroft Formula, based on body weight:

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

When calculating CrCl: if Actual Body Weight is less than Ideal Body Weight, Actual Body Weight will be used; if Actual Body Weight is between 100-120% of Ideal Body Weight, Ideal Body Weight

will be used; and if Actual Body Weight is > 120% of Ideal Body Weight, 25% Adjusted Body Weight

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

CrCl may change during the days fludarabine is given. Adjustment in fludarabine dose due to creatinine changes during conditioning is permitted per institutional standard.

5.32 Pre-transplantation 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 these drugs according to actual weight.

5.33 Total body irradiation

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

5.34 Day of rest

A day of rest, i.e. after preparative regimen completion and prior to bone marrow infusion, is not routinely scheduled for Regimens B and C. 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. Should logistical issues preclude one of these schedules, TBI may be given on Day 0, prior to bone marrow infusion, with PI or co-PI permission.

5.35 Hematopoietic cell transplantation

On Day 0, the harvested bone marrow or peripheral blood stem cells are infused.

The graft will not be manipulated to deplete T-cells. Processing for ABO incompatibility follows institutional practices. Guidelines for cellular infusion are established and outlined in the allogeneic BMT standing orders.

5.37 Post-transplantation cyclophosphamide

Hydration with Cy, management of volume status, and monitoring for hemorrhagic cystitis will follow institutional standards.

Mesna is given in divided doses IV 30 minutes pre- and at 3, 6, and 8 hours post-Cy, unless patients are treated in the Children's Center in which case mesna is dosed per pediatric oncology standard

(e.g., divided doses IV 30 minutes pre- and at 3, 6, and 9 hours post-Cy). The total daily dose of mesna is equal to 80% of the total daily dose of Cy.

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

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

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

5.4 Additional post-transplantation therapies

5.41 Regimen B and B2 and B3: Sirolimus and Mycophenolate Mofetil

5.411 Sirolimus

For patients ≥ 18 years old: A one-time sirolimus loading dose, 6 mg PO, is given on Day 5, at least 24 hours after Cy completion. Sirolimus is then continued at a maintenance dose (start 2 mg PO QD), with dose adjustments to maintain a trough of **5 – 12 ng/mL** as measured by HPLC or immunoassay. There is no planned taper. Sirolimus prophylaxis is discontinued after the last dose on Day 180, or may be continued if there is GVHD. Sirolimus troughs should be checked at minimum weekly.

For patients < 18 years old: Sirolimus dosing is based on actual body weight; however an adjusted body weight may be used if the actual weight is > 50% greater than IBW. A one-time sirolimus loading dose, 3 mg/m^2 PO with the dose not to exceed 6 mg, is given on Day 5, at least 24 hours after Cy completion. Sirolimus is then continued at a maintenance dose (start 1 mg/m^2 PO QD, maximum 2 mg PO QD), with dose adjustments to maintain a trough of **5 – 12 ng/mL** as measured by HPLC or immunoassay**.** There is no planned taper. Sirolimus prophylaxis is discontinued after the last dose on Day 180, or may be continued if there is GVHD. Sirolimus troughs should be checked at minimum weekly.

Sirolimus may be discontinued earlier than Day 180 in the context of relapse, progression, graft failure, or prohibitive toxicity. It is suggested that patients with suspected graft failure remain on sirolimus until at least the ~Day 60 chimerism assessment. Decisions regarding early discontinuation of immunosuppression will be made on a case-by-case basis in consultation with the PI or co-PI.

5.412 Mycophenolate Mofetil

MMF begins on Day 5, at least 24 hours after completion of post-transplantation 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). An equivalent intravenous dose (1:1 conversion) may instead be given. Dose modification guidelines 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.

5.42 Regimen C: Tacrolimus and Mycophenolate Mofetil

5.421 Tacrolimus

Tacrolimus begins on Day 5, at least 24 hours after completion of post-transplantation Cy.

For patients ≥ 18 years old, the tacrolimus starting dose is 1 mg IV QD. The starting dose of tacrolimus may be increased with PI or co-PI permission should institutional practice guidelines change. 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**.

For patients < 18 years old, the starting dose of FK-506 is 0.015mg/kg IV Q12 hours, based on ideal body weight, unless 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**.

Tacrolimus is discontinued after the last dose on Day 180, or may be continued if GVHD is present. At PI or co-PI discretion, cyclosporine (target concentration 200-400 ng/mL) may be substituted for tacrolimus if the patient is significantly intolerant of tacrolimus.

Tacrolimus may be discontinued earlier than Day 180 in the context of relapse, progression, graft failure, or prohibitive toxicity. It is suggested that patients with suspected graft failure remain on tacrolimus until at least the ~Day 60 chimerism assessment. Decisions regarding early discontinuation of immunosuppression will be made on a case-by-case basis in consultation with the PI or co-PI.

5.422 Mycophenolate Mofetil

MMF begins on Day 5, at least 24 hours after completion of post-transplantation 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). An equivalent intravenous dose (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.

5.43 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.

5.44 Post-transplantation donor lymphocyte infusion (DLI)

Prophylactic post-transplantation DLI (for persistent detectable malignancy, prophylaxis in the absence of detectable malignancy, or mixed donor chimerism) is not permitted before Day 100, 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.

5.45 Other post-transplantation therapy

Preemptive systemic cancer therapy is permitted post-transplantation (e.g., DNA-methyltransferase inhibitor, tyrosine kinase inhibitor, rituximab for CD20+ malignancy). Intrathecal chemotherapy and consolidative radiation therapy are permitted. The use of such posttransplantation therapies other than intrathecal chemotherapy will be tracked.

5.5 Supportive care

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

5.51 Anti-ovulatory treatment

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

5.52 Indwelling central venous catheter

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

5.53 Infection prophylaxis

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

Because of the extreme interaction between sirolimus and voriconazole or posaconazole, prophylactic voriconazole or posaconazole is not permitted while on sirolimus. All azole antifungals with the exception of fluconazole should be discontinued at least 1 week prior to sirolimus initiation.

5.54 Antiemetics

Note that dexamethasone should not be used as an anti-emetic agent after the graft is infused, in the absence of relapsed/progressive disease. Such use will not constitute a protocol deviation.

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

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

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

Donor chimerism from T-cells (CD3+ sorted) and whole blood (total nucleated cells) from the peripheral blood will be serially determined 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 30 or after and on all subsequent measurements, in the absence of documented bone marrow involvement by malignancy. .

- Primary graft failure: < 5% donor chimerism in blood and/or bone marrow by ~ Day 60
- Secondary graft failure: Achievement of ≥ 5% donor chimerism, followed by sustained < 5% donor chimerism in blood and/or bone marrow.

Less than 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 clinical criteria (Appendix).[61] All suspected cases of acute GVHD must be confirmed histologically by biopsy of an affected organ (skin, liver, or gastrointestinal tract). Date of symptom onset, date of biopsy confirmation of GVHD, maximum clinical grade, and dates and types of treatment will be recorded. Dates of symptom onset of grade II or higher GVHD and grade III-IV GVHD will be recorded.

The cumulative incidence of grade II-IV and grade III-IV acute GVHD will be determined through competing risk analysis. Relapse/progression, graft failure, and death are considered competing risks for GVHD for study purposes, including stopping rules. 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 NIH consensus criteria[62] and Seattle criteria.[63] Date of onset, date of biopsy proof (if any), dates and types of treatment, and extent will be recorded. The cumulative incidence of chronic GVHD (overall, and according to extent) will be determined through competing risk analysis.

6.3 Disease and survival endpoints

6.31 **Progression-free survival**: Interval from Day 0 to date of first objective disease progression or relapse, death from any cause, unplanned treatment of disease persistence, or last patient evaluation. Patients without such failures will be censored at the last date they were assessed and deemed free of relapse or progression. Disease persistence in the absence of progression is not considered a PFS failure unless it leads to treatment.

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

6.33 **Failure-free survival**: In the phase 2 expansion, failure-free survival, as it pertains to

"immunologic success" of the transplant regimen, is the interval from Day 0 to the date of severe acute (grade III-IV) GVHD, graft failure, non-relapse mortality, or last patient evaluation. Patients without these events will be censored at the last date they were assessed and deemed failure-free. Patients who relapse, progress, or receive unplanned treatment for disease persistence will be censored on that date of failure.

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

6.35 **Non-relapse mortality**: Death without evidence of disease progression or relapse. Relapse/progression and unplanned treatment of disease persistence are competing risks for non-relapse mortality.

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

- Lymphoma: 2007 International Working Group (IWG) criteria for lymphoma[64]
- Acute leukemia: 2010 European LeukemiaNet criteria,[65] based on 2003 IWG criteria[66]
- MDS: 2006 IWG criteria [67]

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

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

6.38 **GVHD-related survival endpoints**

- **GVHD-free relapse-free survival (GFRFS):** Interval from Day 0 to acute grade III-IV GVHD, systemic treatment of 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.

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

7.1 Core evaluations

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. Telemedicine visits are an acceptable alternative if deemed appropriate by the investigator, and will be conducted in accordance with established Johns Hopkins policies.

Table 2: Core evaluations

	Baseline [a,b]	D30 +/- 10d	D60 +/- 15 d	If question of graft failure	D100 +/- 15 d	D180 +/- 30 d	D365 +/- 45 d [c]
Standard pre/post transplant evaluations [a, b]							
History and physical exam	X	X	X		X	X	X
ECOG performance status	X						
Karnofsky or Lansky score	X						
CBC / differential [d]	X	X	X	X	X	X	X
Comprehensive metabolic panel [e]	X	X	X		X	X	X
Infectious disease titers [f]	X						
Fasting cholesterol and serum triglycerides (sirolimus arms only)	X		X		X[o]		
Serum HCG (if applicable)	X						
EKG	X						
LV ejection fraction or shortening fraction	X						
Pulmonary function tests [m]	X						
Bone marrow biopsy and aspirate with flow cytometry and relevant cytogenetic and molecular studies [g]	X		X, with chimerism studies [h, n]	X, with chimerism studies		X [h]	X [h]
CT of sinuses	X						
CT, PET/CT, or MRI of chest, abdomen, and pelvis (lymphoma and CLL only)	X		X				X
Response assessment to last therapy [i]	X						
HLA typing	X						
Lymphocytotoxic antibody screen	X						
Donor marrow or blood for VNTR or RFLP analysis [j]	X						
Patient blood for baseline VNTR or RFLP analysis [j]	X						
Peripheral blood chimerism, both total leukocyte (unsorted) and T-cell sorted [j]		X	X	X		X	X
GVHD and other morbidity assessments [k]		X	X		X	X	X

[a] Baseline evaluations should occur $\leq$ 1 month before initiation of conditioning therapy, with the exception of the following: cardiac and pulmonary evaluations may occur $\leq$ 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 autologous transplantation, donor age, donor relationship to patient, donor gender, CMV serostatus of patient and donor, ABO compatibility.

[c] All patients will be considered off study with regard to monitoring for adverse effects at 1 year after transplantation. Patients should continue to follow-up at Johns Hopkins at least yearly on study. Follow-up data may be captured more frequently for study purposes. Data that will continue to be recorded beyond 1 year include disease status, vital status, and GVHD. Patients who relapse or progress will also be considered off study. .

[d] At minimum, CBC/differential should also be performed twice a week from start of preparative regimen, until ANC is $\geq$1000/μL over course of 3 days, then weekly until 12 weeks post-transplantation, and periodically thereafter; those need not be captured in the CRF.

[e] Chemistries include: BUN, creatinine, sodium, potassium, chloride, AST, ALT, total bilirubin, alkaline phosphatase. At minimum, these should be performed weekly until 12 weeks post-transplantation, then periodically until off immunosuppression; those need not be captured in the CRF.

[f] Standard infectious disease evaluations include: CMV IgG, HSV IgG, VZV IgG, Hepatitis panel (Hep B surface Ag, Hep B core antibody, Hep C antibody), RPR, HIV antibody, and HTLV I/II antibody.

[g] Flow cytometry in diseases other than Hodgkin's lymphoma. Follow-up studies should include relevant cytogenetics and molecular markers to detect residual disease, i.e. repeat of studies found to be positive at baseline.

[h] Bone marrow aspiration and biopsy will be done at the discretion of the physician caring for the patient

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

[j] Collect 10 cc lavender top.

[k] GVHD and other morbidity assessments are also standardly performed weekly until Day 100 and may be collected via telemedicine. Results of these and subsequent assessments may be collected for research purposes. Patients may be asked to complete GVHD questionnaires.

[l m] For pediatric patients unable to perform PFT's, document oxygen saturation on room air.

[n] If an adequate bone marrow biopsy is performed for suspected graft failure before but in close proximity to Day 60 evaluations, the Day 60 bone marrow biopsy may be omitted at PI or co-PI discretion.

[o] Fasting cholesterol and serum triglycerides (sirolimus arms only) day 100 assessments to be done between day 90 – day 130.

8.0 RISKS AND REPORTING REQUIREMENTS

8.1 Drug information

8.11 Fludarabine (Fludara®)

Fludarabine is a fluorinated nucleoside analog. After phosphorylation to fluoro-ara-ATP the drug appears to incorporate into DNA and inhibit DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. Excretion of fludarabine is impaired in patients with impaired renal function.

Fludarabine toxicities 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 if the estimated CrCl is $\leq$ 60 mL/min (per Sections 5.31 and 5.41). Fludarabine is dosed in this study according to actual body weight.

8.12 **Cyclophosphamide** (Cytoxan®)

Cyclophosphamide is an alkylating agent. It is activated by the liver cytochrome P450 system to cytotoxic metabolites, which form cross-links with DNA. It is cell cycle-nonspecific.

Toxicities 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. Cyclophosphamide is dosed in this study according to IBW, unless actual body weight is less.

8.13 **Mesna**

Mesna (sodium-2-mercaptoethanesulphonate) 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.

Toxicities: 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.

Dose adjustments for mesna will not be made. The total daily dose of mesna is equal to 80% of the total daily dose of cyclophosphamide in this study. Mesna is dosed in this study according to IBW, unless actual body weight is less.

8.14 **Mycophenolate Mofetil** (MMF; Cellcept®)

MMF is an ester prodrug of the active immunosuppressant mycophenolic acid (MPA).

Toxicities include:
Pancytopenia, infection, nausea, vomiting, diarrhea, allergic reactions, hypertension, headache, dizziness, insomnia, hyperglycemia, electrolyte imbalances, rash, and leg cramps/bone pain.

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

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

8.15 Sirolimus (rapamycin, Rapamune®)

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 Table 4 below:

Table 4: 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***
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 [a], stomatitis, infections (including UTI, URI), mild ***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 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 sequellae, 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 Section 8.18.

8.16 **Tacrolimus** (FK-506, Prograf®)

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

Drug formulation: Tacrolimus injection must diluted with 0.9% Sodium Chloride or 5% Dextrose to a concentration between 0.004 mg/mL and 0.02 mg/mL prior to use. Diluted infusion solution should be stored in glass or polyethylene containers and discarded after 24 hours. PVC-free tubing is preferable for more dilute solutions. Due to chemical instability in alkaline media, tacrolimus injection should not be mixed or co-infused with solutions of pH 9 or greater (e.g., ganciclovir or acyclovir). Supplied as a 5 mg/mL solution, to be stored between 5° - 25°C, and as capsules (0.5 mg, 1 and 5 mg) to be stored at room temperature, 15°- 30° C.

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

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

Dose adjustments: The tacrolimus dose is adjusted to maintain a serum trough level of 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. Dose adjustments for therapy with other azoles may be indicated (see Section 8.18). The tacrolimus loading dose in this study takes fluconazole prophylaxis into account.

8.16 **Concurrent azole therapy**

Triazole antifungal medications are expected to increase serum CNI and sirolimus levels; therefore dosages of CNIs and sirolimus should be adjusted accordingly. Guidelines are provided in Table 5 below. In the event of suspected or documented fungal infection, alternative antifungal therapy should be considered.

Table 5: Suggested pre-emptive dose reduction of tacrolimus or sirolimus when azoles are initiated at steady state levels of tacrolimus or sirolimus

Antifungal	Tacrolimus		Sirolimus	
	Dose ↓	Comment	Dose ↓	Comment
Voriconazole	67%	Strongly advised	90%	Essential
Posaconazole	67%	Advised	67%	Advised
Itraconazole	50%	Advised	No data	No data
Fluconazole	25%	Consider	50%	If > 400 mg qd

Notes:

a. If voriconazole is given IV or if voriconazole and sirolimus are not given together, the effect on sirolimus bioavailability will be weaker. Empiric dose reduction is advised; however, guidelines are not well established.
b. Regarding dose increases of CNI's or sirolimus when azoles are stopped: Reversal of azole-mediated inhibition of cytochrome CYP3A4 (and others) and P-glycoprotein is gradual. Therefore, immediate significant dose increases are not advised. Rather, tacrolimus and sirolimus dose increases should be cautious and based on more frequent monitoring of levels as appropriate.

8.17 Total Body Irradiation (TBI)

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

8.2 Toxicity grading

Toxicities are graded using NCI's Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0.

8.3 Toxicity reporting

The agents being used in the study are used extensively in the BMT setting, have well-defined toxicity profiles, and are FDA approved. In addition, there are many expected toxicities of allogeneic BMT. The following are examples of toxicities that are serious but not unexpected: Grade 4 cytopenias; neutropenic fever and sepsis; bacterial, fungal, or viral (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:

- Any hospitalization and its reason in the first year of transplant.
  - 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.
- Any death before Day 200, and any later death which is potentially transplant-related.
- 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:

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

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

All patients will be considered off study with regard to monitoring for adverse effects at 1 year after transplantation. Further, all patients who relapse will be considered off study.

8.4 Monitoring plan

The SKCCC Compliance Monitoring Program will provide external monitoring for JHU affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring. All trial monitoring and reporting will also be reviewed annually by the SKCCC Safety Monitoring Committee. The PI will review data to assure the validity of data, as well as the safety of the subjects. The PI will also monitor the progress of the trial. The PI will review safety reports and clinical trial efficacy endpoints and to confirm that the safety outcomes favor continuation of the study.

8.5 Risks and benefits

Allogeneic BMT carries risk for major morbidity and mortality, the risk of which is expected to increase, potentially substantially, with the use of HLA-mismatched unrelated donors or second-degree relatives. 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, thrombotic microangiopathy, and death. High-dose post-transplantation cyclophosphamide appears to significantly lower the risk of GVHD in other settings.

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.

9.0 STATISTICAL CONSIDERATIONS

9.1 Primary statistical plan: phase 1 study

The primary objective is to identify a reduced-intensity transplant regimen incorporating high-dose post-transplantation Cy that carries acceptable rates of severe (grade III-IV) acute GVHD and transplant-related NRM by Day 100. Up to two immunosuppressive regimens with flu-Cy-TBI conditioning (sirolimus + MMF, or tacrolimus + MMF, all with post-transplantation cyclophosphamide) will be studied. As described in the Background, sirolimus has been prioritized over tacrolimus for initial study, and study of a regimen with flu-bu conditioning was stopped.

A Bayesian monitoring rule for two adverse events, grade III-IV acute GVHD by Day 100 and transplant-related NRM by Day 100, will be used to examine the safety of each regimen along a decision tree. The study will be continuously monitored (for each occurrence of any such adverse event) for safety. A regimen will be considered prohibitive if the probability of severe acute GVHD is convincingly >25% or the probability of transplant-related NRM is >20% by this time point. This design is based on the Bayesian posterior probability derived for the bivariate case.[69] The likelihood assumes that occurrence of one adverse event precludes that of the other. Although GVHD and NRM are not mutually exclusive, the Day 100 mortality from acute GVHD is expected to be low. Therefore, a patient who experiences grade III-IV acute GVHD then NRM will be counted once as

having the GVHD adverse event. In the absence of previous experience with these types of

transplant regimens, a bivariate uniform prior distribution is assumed. The posterior distribution is a product of piecewise beta densities and the calculation has been programmed using Mathematica.[70]

Each time a new regimen is tested, it will first be tested in a cohort of 5 patients. If a patient begins the preparative regimen but does not receive the transplant (e.g., because of a donor issue), that patient will be replaced. When a final regimen has been selected, phase 1 study of this regimen will be expanded to a planned total of 20 patients, including the initial 5 patients. Per Section 5.2, phase 1 expansion may begin while evaluation of the first cohort of 5 patients is in progress. If a regimen is tested with an expanded sample size and a stopping boundary is met, the study of that regimen will stop. The stopping rule will hold enrollment to the expansion cohort if, by Day 100, the posterior probability is 80% or higher that the severe acute GVHD risk exceeds 25% or transplant-related NRM risk exceeds 20%. A regimen will additionally be considered prohibitive if, during its initial or expanded phase 1 testing, the frequency of graft failure by ~Day 60 convincingly exceeds 35%, per Section 9.14.

The sequence of regimens is designated in Section 5.2.

The B2 cohort will include up to 20 patients with safety monitoring as outlined below.

Cohort B3: HIV patients with CCRd32 homozygous donors (N=10):
We would like to accrue additional HIV patients with CCRd32 homozygous donors (who are resistant to HIV infection). You may be aware of the "Berlin patient" reported in 2009 and the "London patient" reported 10 years later in 2019 who had fully matched unrelated marrow transplants from HIV resistant donors and were cured of HIV[74-75]. There have been so few because it is difficult to find HLA-matched HIV-resistant donors[76].

The J1055 protocol has demonstrated that unrelated bone marrow donors needn't be HLA matched[77], that related donors who are haploidentical need not be first degree relatives[78], and most recently that the approach is successful with a peripheral blood stem cell graft as well as bone marrow (in preparation). This work has led to a national trial based on ours that confirmed the bone marrow results, and a follow-up national trial not yet activated to confirm our peripheral blood stem cell results. The J1055 has allowed enrollment of HIV patients. Because of the more relaxed requirements for HLA matching, it has been possible to identify unrelated partially mismatched HIV resistant donors in most instances. We have transplanted two in a little over a year on J1055. The first died in the second month after transplant. He had been hospitalized with severe CMV and adenovirus infections (not uncommon after allo transplant). A second patient is early after transplant and is alive and fully engrafted and being followed closely in our outpatient clinic. With this amendment, we add an additional cohort of 10 patients with HIV needing transplant for standard indications but having partially matched unrelated HIV-resistant donors identified. The stopping rule and operating characteristics of the rule for this 10 patient cohort are given in the sections 9.12 and 9.13.

Upon completion of the above, a phase 2 expansion cohort is planned for this study.

9.11 First test of a regimen

Table 6 below shows the stopping rule for the initial test of a regimen after the first 5 patients, when the allowed probabilities of severe acute GVHD and transplant-related NRM are 25% and 20%, respectively, by Day 100. The bolded cells are combinations of severe acute GVHD and NRM events by this time point that would prevent further study of the regimen, with a threshold for the Bayesian posterior probability of 80%. As an example, if we were to observe $k_1 = 2$ patients with severe acute GVHD and $k_2 = 2$ patients with transplant-related NRM by Day 100, the posterior probability that the frequency of either event exceeds that allowed would be 92% and study of the regimen should be stopped. Similar tables have been calculated for sample sizes of 6 to 20, and stopping boundaries for continuous monitoring are summarized in Table 7.

Table 6: Posterior probabilities that the severe acute GVHD risk exceeds 25% or NRM risk exceeds 20% by Day 100, with a sample size of 5

	NRM (k_2)					
	0	1	2	3	4	5
aGVHD (k_1)						
0	0.03	0.12	0.37	0.79	**0.98**	1
1	0.10	0.27	0.65	**0.94**	1	1
2	0.32	0.62	**0.92**	0.99	1	1
3	0.74	**0.93**	0.99	1	1	1
4	**0.97**	1	1	1	1	1
5	1	1	1	1	1	1

9.12 Continuous safety monitoring

As long as a stopping boundary is not met, we expect to accrue a total of 20 patients in the phase 1 portion using the chosen regimen. Tables similar to Table 6 have been calculated to monitor the study continuously as each patient in the cohort reaches the Day 100 benchmark. The stopping boundaries from these tables, using a posterior probability threshold of 80%, have been consolidated in Table 7. The combinations of severe acute GVHD and transplant-related NRM events by Day 100 that lead to stopping are shown as row and column headers with the range of sample sizes in the body of the table. As an example, when 7 patients have completed the 100 day observation period, the combinations of adverse events (severe acute GVHD, transplant-related NRM) by that time point that would stop the trial are: (5,0), (4,1), (3,2), (2,3), (1,4) and (0,4).

Table 7: Stopping boundaries based on the number of severe acute GVHD and NRM events by Day 100 using a posterior probability threshold of 80%. The table entries are the sample sizes for which the row and column numbers of severe acute GVHD cases and transplant-related NRM cases, respectively, constitute the stopping rule.

	NRM										
	0	1	2	3	4	5	6	7	8	9	10
aGVHD											
0					5-7	8-10	11-13	14-16	17-19	20-23	24-
1				5-6	7-8	9-11	12-14	15-17	18-20	21-24	25
2			5	6-7	9-10	11-12	13-15	16-18	19-20	22-25	
3		5-6	6-7	8-9	10-11	12-14	15-17	18-20	21-23	24-25	
4	5-6	7-8	8-9	10-11	12-13	14-16	17-18	19-20	22-24	25	
5	7-9	9-10	10-11	12-13	14-16	17	18-20	21-22	23-25		
6	10-11	11-12	12-13	14-15	16-17	18-19	20-22	23-24	25		
7	12-13	13-14	14-16	16-17	18-19	20-21	22-23	24-25			
8	14-16	15-17	17-18	18-19	20-21	22-23	24-25				
9	17-19	18-19	19-20	20-22	22-23	24-25					
10	20-21	20-22	21-23	23-24	24-25						
11	22-24	23-25	24-25	25							
12	25										

Continuous safety monitoring for HIV cohort (N=10):

Because there was an event in one of the two HIV patients with a resistant donor in the previous cohort, the

rule has been modified to start after the second patient. The thresholds for allowed probabilities of severe acute GVHD and transplant-related NRM remain the same, 25% and 20% by Day 100, respectively, as well as the 80% threshold for the posterior probability,

Table showing when to stop based on the number of acute GVHD (aGVHD) cases and non-relapse mortalities (NRMs). Table entries are the sample sizes for which the row and column numbers of acute GVHD cases and non-relapse mortalities constitute the stopping rule. The rule is based on allowed probabilities of severe acute GVHD and transplant-related NRM of 25% and 20%, respectively, by Day 100.

	NRM					
	0	1	2	3	4	5
aGVHD						
0			2	3-4	5-7	8-10
1		2	3-4	5-6	7-8	9-10
2	2	3-4	5	6-7	9-10	
3	3-4	5-6	6-7	8-9	10	
4	5-6	7-8	8-9	10		
5	7-9	9-10	10			
6	10					

9.13 Operating characteristics of design

The probability of stopping the phase 1 study early under different scenarios is shown in Table 8. By Day 100 the allowed probability of severe acute GVHD (A_1) is 25% and the allowed probability of transplant-related NRM (A_2) is 20%, while the simulated adverse events (k_1 or k_2) were either equal to or greater than the allowed amount. The probability of stopping early was calculated from 500 simulated trials for a minimum sample size of 5 or maximum sample sizes of 20 per a given regimen with 3 different posterior probability thresholds: 0.60, 0.70 and 0.80. The stopping boundaries in Table 7 use a posterior probability threshold of 80%.

Table 8: Probability of stopping early under three adverse event scenarios with continuous monitoring and a minimum sample size of 5 or a maximum sample size of 20 per given regimen

Posterior Threshold	Cohort size	Equivalence: **A_1=.25, A_2=.20** k_1=.25, k_2=.20	Increase both: **A_1=.25, A_2=.20** k_1=.35, k_2=.35	More NRM: **A_1=.25, A_2=.20** k_1=.25, k_2=.45
0.60	5	31.0	64.4	61.6
0.70	5	17.4	44.0	39.4
0.80	5	11	30.6	31.8
0.60	20	51.8	89.8	92.6
0.70	20	37.8	84.4	90.0
0.80	20	29.8	76.8	82.8

Operating characteristics HIV cohort (N=10):
Probability of stopping early under three adverse event scenarios with continuous monitoring starting after the second patient, and posterior threshold of 80%.

Posterior Threshold	Cohort size	Equivalence: **A1=.25, A2=.20** k1=.25, k2=.20	Increase both: **A1=.25, A2=.20** k1=.35, k2=.35	More NRM: **A1=.25, A2=.20** k1=.25, k2=.45
0.80	10	29.4	61.8	70.4

9.14 Additional stopping guidelines

Early stopping guideline for graft failure: Phase 1 study of a given regimen will stop if the frequency of graft failure by Day 60 evaluations convincingly exceeds 35% in evaluable patients. For this stopping guideline, each regimen will be separately assessed using a one-sided exact binomial 90% confidence bound. We will stop study of a given regimen if 4 out of the first 5, 6 out of first 10, or 9 out of first 15 evaluable patients experience graft failure by this time point (corresponding lower one-sided 90% confidence limits: 0.416, 0.354, 0.404, and 0.385). Patients who die before Day 60 evaluations without evidence or documentation of $\geq$5% donor chimerism, who have < 5% donor chimerism in the context of any bone marrow involvement by persistent or progressive malignancy, or who have < 5% donor chimerism without assessment of bone marrow disease status if the bone marrow was involved pretransplantation may be considered inevaluable for this stopping guideline. However, chimerism data in these and the other patients may be considered in the overall evaluation of engraftment, and a go/no-go decision rendered on this basis.

9.2 Primary statistical plan: phase 2 expansion

The study will accrue an additional 45 patients to a phase 2 expansion cohort, using the regimen chosen in the initial phase (effective with protocol version 1/30/2017). The primary objective of this portion of the study will be to evaluate the "immunologic efficacy" of the chosen regimen.
Immunologic efficacy is herein defined as surviving to 6 months posttransplant without having had severe acute GVHD or evidence of graft failure. Accrual to the phase 2 portion may begin while full evaluation of the phase 1 portion is in progress. The 20 patients in the safety/ regimen finding portion of the study will be included in the analysis of this expansion cohort.

9.21 Futility monitoring

The non-parametric Kaplan-Meier estimate will be used to monitor the failure-free survival (FFS) function at 6 months wherein, from an immunologic standpoint, failure is defined as transplant- related non-relapse mortality, severe acute GVHD, or graft failure. Patients will be censored at the time of relapse. Futility monitoring will start after the 5th patient has been enrolled in the expansion cohort. There will be two interim analyses for futility, after the 5th and 15th patient have been enrolled in the expansion cohort. The study is designed to stop for futility if there is 80% certainty that the 6-month FFS is below 40%.

The study design operating characteristics assume a total sample size of 45 patients, a 5-year recruitment period, and additional follow-up of 6 months. The following table summarizes the operating characteristics of the futility stopping rule under various scenarios for the underlying exponential FFS, based on 1000 simulations. For futility monitoring we optimistically characterize the uncertainty of the 6 month FFS estimate with the prior: beta(3,2). This implies that our prior guess at the 6 month FFS in this study is 60% and there is 90% certainty that it is between 25% and 90%. Because patients will be censored at the time of relapse for the primary endpoint, the simulations assume an exponential hazard rate (per month) for relapse of 0.02. The resulting overall proportion of censored observations in the simulations, Table 9, is close to what we might see if the true 6-month FFS is similar to that for nonmyeloablative haplo transplants from first-degree related donors, i.e. approximately 23% censoring when the true 6-month FFS is 0.60.

Table 9. Sample size of 45 with a null 6-month FFS of 0.40, prior beta(3,2) and an alternative of 0.60

6-month FFS	Prob Stop for Futility	Avg N	Estimated 6-month FFS	lo 95% Post Int'l	hi 95% Post Int'l	Overall % censored
0.30	1.00	25.2	0.30	0.13	0.50	9.6
0.35	0.99	25.5	0.34	0.16	0.52	11.2
0.40	0.97	26.2	0.39	0.21	0.55	12.9
0.45	**0.91**	**27.9**	**0.43**	**0.23**	**0.59**	**14.8**
0.50	0.77	31.1	0.48	0.27	0.64	17.0
0.55	0.56	35.2	0.53	0.35	0.70	20.0
0.60	**0.33**	**39.3**	**0.60**	**0.40**	**0.75**	**23.1**
0.65	0.17	42.0	0.64	0.46	0.79	26.9
0.70	0.06	44.0	0.69	0.50	0.83	31.9

9.22 Continuous safety monitoring

Table 10 provides expanded stopping boundaries to monitor the study continuously for safety as patients 21 through 45 in the phase 2 cohort reach the Day 100 benchmark. As in the phase 1 portion, the allowed probabilities of severe acute GVHD and transplant-related NRM are 25% and 20% respectively by Day 100. The stopping boundaries use a posterior probability threshold of 80%. The combinations of severe acute GVHD and transplant-related NRM events by Day 100 that lead to stopping are shown as row and column headers, with the range of sample sizes in the body of the table. As an example, when 26 patients have completed the 100-day observation period, the combinations of adverse events (severe acute GVHD, transplant-related NRM) by that time point that would stop the trial are: (12,2), (12,1), (10,5), (10,4), (9,6), (8,7), (5,9), (3,9), (2,10) and (0,10).

If Regimen B (with post-transplant Cy, MMF, and sirolimus) is selected for phase 2 expansion, but its study then stops due to futility or safety, Regimen C (with post-transplant Cy, MMF, and tacrolimus) will be evaluated and expanded to phase 2 study if appropriate.

Table 10: Phase 2 expansion stopping boundaries, based on the number of severe acute GVHD and transplant-related NRM events by Day 100, using a posterior probability threshold of 80%. The table entries are the sample sizes for which the row and column numbers of severe acute GVHD cases and transplant-related NRM cases, respectively, constitute the stopping rule.

	NRM																
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
aGVHD																	
0					5 -7	8 - 10	11- 13	14- 16	17- 19	20- 23	24- 26	27- 30	31- 33	34- 37	38- 41	42- 44	45
1				5 -6	7 -8	9 - 11	12 - 14	15- 17	18- 20	21- 24	25- 27	28- 31	32- 34	35- 38	39- 42	43- 45	
2			5	6 -7	9 - 10	11 - 12	13- 15	16- 18	19- 20	22- 25	26- 28	29- 32	34- 35	36- 39	40- 43	44- 45	
3		5 -6	6 -7	8 -9	10 - 11	12 - 14	15- 17	18- 20	21- 23	24- 26	28- 29	30- 33	34- 36	37- 40	41- 44	45	
4	5 -6	7 -8	8 -9	10 - 11	12- 13	14- 16	17- 18	19- 20	22- 24	25- 27	28- 30	31- 34	35- 37	38- 41	42- 45		
5	7 -9	9- 10	10 - 11	12 - 13	14- 16	17	18- 20	21- 22	23- 25	26- 28	29- 32	33- 35	36- 38	39- 42	43- 45		
6	10-11	11- 12	12- 13	14- 15	16- 17	18- 19	20- 22	23- 24	25- 27	28- 30	31- 33	34- 36	37- 40	41- 43	44- 45		
7	12-13	13- 14	14- 16	16- 17	18- 19	20- 21	22- 23	24- 27	27- 29	30	31- 34	35- 38	39- 41	42- 44	45		
8	14-16	15- 17	17- 18	18- 19	20- 21	22- 23	24- 25	26- 28	29- 30	31- 33	34- 36	37- 39	40- 42	43- 45			
9	17-19	18- 19	19- 20	20- 22	22- 23	24- 25	26- 27	28- 30	31- 32	33- 35	36- 37	38- 40	41- 44	45			
10	20-21	20- 22	21- 23	23- 24	24- 26	26- 27	28- 29	30- 32	33- 34	35- 36	37- 39	40- 42	43- 45				
11	22-24	23- 25	24- 25	25- 27	27- 28	28- 30	30- 32	33- 34	35- 36	37- 38	39- 41	42- 44	45				
12	25-27	26- 27	26- 28	28- 29	29- 30	31- 32	33- 34	35- 36	37- 38	39- 40	41- 43	44- 45					
13	28-30	28- 30	29- 31	30- 32	31- 33	33- 34	35- 36	37- 38	39- 40	41- 42	43- 45						
14	31-33	31- 33	32- 34	33- 34	34- 35	35- 37	37- 38	39- 40	41- 42	43- 44	45						
15	34-36	34- 36	35- 36	35- 37	36- 38	38- 39	39- 41	41- 43	43- 44	45							
16	37-39	37- 39	37- 39	38- 40	39- 41	40- 42	42- 43	44- 45	45								
17	40-42	40- 42	40- 42	41- 43	42- 44	43- 45	44- 45										
18	43-45	43- 45	43- 45	44- 45	45												

9.3 Secondary endpoints

9.31 Disease and survival endpoints

The probabilities of 1-year and longer-term progression-free survival, disease-free survival, overall survival, GFRFS, and cGFRFS after transplantation will be estimated and reported with 90% confidence intervals using the Kaplan-Meier method. In addition, the proportion of patients who are progression-free and who are alive will be reported at 1 year with 90% exact binomial confidence intervals, in patients who have been followed for that minimum time.

Cumulative incidences of progression/relapse and NRM will be estimated with competing risk analyses using Gray's method. The disease and survival endpoints will be described for the

group as a whole, for the final selected regimen, and if numbers permit, for myeloid versus lymphoid histologies without formal statistical comparison.

9.32 Toxicities

The cumulative incidence of acute GVHD (grade II-IV, grade III-IV) and chronic GVHD (overall, and according to extent) will be estimated through competing-risk analysis, wherein relapse/progression, graft failure, and death are competing risks for GVHD. We also plan to report the cumulative incidence of GVHD with only graft failure and death regarded as competing risks.

The cumulative incidences of systemic steroid initiation for GVHD treatment, of non-steroid immunosuppression use, and of discontinuation of immunosuppression for GVHD treatment will be similarly estimated through competing-risk analysis, wherein graft failure and death, or graft failure, death and relapse/progression, are considered competing risks. The frequency of steroid use for GVHD will be reported. The number and types of systemic immunosuppression used for GVHD treatment will be reported descriptively.

Other selected toxicities will be reported descriptively.

9.33 Graft failure and engraftment kinetics

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 amount of donor chimerism in T cells and total leukocytes at ~Day 30, ~Day 60, and beyond will be described. The graft failure frequency will be described with exact 90% binomial confidence intervals.

9.4 Clinical trial reporting

Up to two reports of preliminary outcomes are planned, including publication of the phase 1 results, in order to describe the experience with feasibility, toxicity, overall clinical outcomes, and laboratory correlative studies. These analyses will be descriptive in nature rather than formal interim analyses.

10.0 PATHOLOGY REVIEW

In accordance with standard institutional practice, at least one specimen diagnostic of the malignancy (from the original diagnosis and/or relapse) must be reviewed by the Johns Hopkins department of pathology prior to starting protocol therapy. In cases diagnosed solely by peripheral blood flow cytometry, the diagnostic flow cytometry report must be reviewed.

11.0 RECORDS TO BE KEPT

Records to be filed include the following:

1. Patient consent form
2. Registration form
3. Case report forms, including patient-donor HLA reports
4. Adverse event report form(s)
5. Follow-up assessments

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

12.0 PATIENT CONSENT AND PEER JUDGMENT

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

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APPENDIX

Clinical grading of acute GVHD

Clinical Staging

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

Clinical Grading

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

*Each column identifies minimum stage for organ grade

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