A Pediatric Blood and Marrow Transplant Consortium (PBMTC) multi-center Phase II Pilot Trial of Myeloablative Conditioning and Transplantation of Partially HLA-mismatched T cell replete Bone Marrow with post-transplantation cyclophosphamide for Children and Young Adults with Hematologic Malignancies

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
ABW	Actual body weight
AE	Adverse event
aGVHD	Acute graft versus host disease
ALL	Acute lymphoblastic leukemia
alloHSCT	Allogeneic hematopoietic stem cell tranplant
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
AP	Anterior-posterior
AST	Aspartate transaminase
AUC	Area under the curve
BID	Twice a day
BM	Bone marrow
BMT	Bone marrow transplant
BMT CTN	Bone Marrow Transplant Clinical Trials Network
Bu	Busulfan
BUN	Blood urea nitrogen
CBC	Complete blood count
cGVHD	Chronic graft versus host disease
cGy	Centigray
CI	Confidence interval
CML	Chronic myeloid leukemia
CMV	Cytomegalovirus
CNI	Calcineurin inhibitors
CNS	Central nervous system
COG	Children's Oncology Group
CR	Complete remission
CrCl	Creatinine clearance
CRF	Case report forms
CSA	Cyclosporine
CSF	Cerebrospinal fluid
Css	Steady state concentration
CTCAE	Common Toxicity Criteria for Adverse Events
Cy	Cyclophosphamide
DLCO	Diffusing capacity for carbon monoxide
DFS	Disease free survival
DSMB	Data Safety Monitoring Board
dUCB	Double umbilical cord blood
ECHO	Echocardiogram
EFS	Event free survival
EKG	Echocardiogram
FACT	Foundation for the Accreditation of Hematopoietic Cell Therapy

FEV1	Forced expiratory volume
FISH	Fluorescence in situ hybridization
FMT	Fludarabine, melpahlan, thiotepa
FVC	Forced vital capacity
FWA	Federalwide Assurance
GCP	Good clinical practice
GFR	Glomerular filtration rate
GSCF	Granulocyte colony stimulating factor
GU	Genitourinary
GVHD	Graft versus host disease
IRB	Institutional Review Board
IT	Intrathecal therapy
haploHSCT	Haploidentical hematopoietic stem cell transplant
HCT	Hematopoietic cell transplantation
Hgb	hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HLA	Human leukocyte antigen
HSCT	Hematopoietic stem cell transplantation
HR	Hazard ratio
IBW	Ideal body weight
JHH	Johns Hopkins Hospital
JHM-IRB	Johns Hopkins Medicine Institutional Review Board
JHU	Johns Hopkins University
JMML	Juvenile myelomonocytic leukemia
LVEF	Left ventricular ejection fraction
MDS	Myelodysplastic syndrome
MFI	Mean fluorescence intensity
MMF	Mycophenolate mofetil
MRD	Minimal residual disease
MUGA	Multigated acquisition scan
NCI	National Cancer Institute
NK	Natural killer
NRM	Non relapse mortality
NS	Normal saline
OS	Overall survival
PA	Posterior-anterior
PB	Peripheral blood
PBMTC	Pediatric Blood and Marrow Transplant Consortium
PBSC	Peripheral blood stem cells
PCR	Polymerase chain reaction
PI	Principal investigator
PFS	Progression-free survival
PFT	Pulmonary function test
Ph+/-	Philadephia positive/negative
PK	Pharmacokinetics

PO	Oral
PT/Cy	Post transplantation cyclophosphamide
RA	Room air
RFLP	Restriction fragment length polymorphism
RSV	Respiratory syncytial virus
SAE	Serious adverse event
SIADH	Syndrome of inappropriate antidiuretic hormone secretion
SKCCC	Sidney Kimmel Comprehensive Cancer Center
TBI	Total body irradiation
TCD	T cell depleted
TCR	T cell replete
Tid	Three times a day
TKI	Tyrosine kinase inhibitor
TNC	Total nucleated cells
TRM	Transplant related mortality
TTP	Thrombotic thrombocytopenic purpura
URD	Unrelated donor

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Treatment Schema 1 (All patients <u>except</u> those with acute lymphoblastic leukemias)

Days -6 through -3	Busulfan q6h IV for four days (total 16 doses)* (Dose adjusted based on PKs) Seizure prophylaxis** ↓
Days -2 and -1	Cyclophosphamide 50mg/kg/day IV x 2 days+ Mesna 40 mg/kg/day IV+ (One day of rest may be added between Days-1 and bone marrow infusion on Day 0; see section 4.3) ↓
Day 0	Infuse unmanipulated bone marrow (at least 24 hrs after second pre-transplant Cy infusion) ↓
Day +3 and +4	Cyclophosphamide 50 mg/kg/day IV# (begin 60-72 hours after the start of bone marrow infusion) Mesna 40 mg/kg IBW/day IV# ↓
Day +5	Begin tacrolimus 0.015mg/kg/dose IBW dose IV over 4 hours q 12h and mycophenolate mofetil (MMF)15mg/kg/dose po/IV tid ↓
Day +30	Assess peripheral blood donor chimerism ↓
Day +35	Discontinue MMF ↓
Day +60	Assess chimerism and disease status in bone marrow ↓
Day 180	Discontinue tacrolimus Evaluate disease ↓
1 yr, 2 yrs	Evaluate disease

*See section 4.3 for busulfan dosing
** See section 4.3 for guidelines
+ See section 4.3 for complete dosing instructions; use lesser of ideal body weight and actual body weight for Cy dosing
# See section 4.5 for complete dosing guidelines; use lesser of ideal body weight and actual body weight for Cy dosing

Treatment Schema 2 (Only patients with acute lymphocytic leukemias or patients who have had a prior non-TBI based transplant; can also consider for mixed phenotype leukemia)

Days -5 through -4	Cyclophosphamide 50mg/kg/day IV q24h x 2 days[+] Mesna 40 mg/kg/day IV[+]
	↓
Days -3 through -1	TBI 200 cGy twice a day for 3 days [+]
	(One day of rest may be added between Days-1 and bone marrow infusion on Day 0; see section 4.3)
	↓
Day 0	Infuse unmanipulated bone marrow (at least 24 hrs after second pre-transplant Cy infusion)
	↓
Day +3 and +4	Cyclophosphamide 50 mg/kg/day IV[**] (begin 60-72 hours after the start of bone marrow infusion) Mesna 40 mg/kg IBW/day IV[**]
	↓
Day +5	Begin tacrolimus 0.015mg/kg/dose IBW/dose IV over 4 hours q 12h and mycophenolate mofetil (MMF)15mg/kg/dose po/IV tid
	↓
Day +30	Assess chimerism in peripheral blood
↓	
Day +35	Discontinue MMF
	↓
Day +60	Assess chimerism and disease status in bone marrow
	↓
Day 180	Discontinue tacrolimus[#] Evaluate disease
	↓
1 yr, 2 yrs	Evaluate disease

+ See section 4.3 for complete dosing instructions; use lesser of ideal body weight and actual body weight for Cy dosing

****** See section 4.5 for complete dosing guidelines; use lesser of ideal body weight and actual body weight for Cy dosing

Tacrolimus may be discontinued and/or weaned as early as Day +90. See section 4.6 for complete guidelines.

1.0 OBJECTIVES

Primary Objective:

1.1 To estimate the incidence of non-relapse mortality at 180 days following myeloablative haploidentical BMT for children and young adults with high risk hematologic malignancies

Secondary Objectives:

1.2 To estimate the incidence of donor cell engraftment (donor chimerism) at Day 60 following myeloablative, haploidentical BMT

1.3 To estimate the time to neutrophil and platelet recovery following myeloablative haploidentical BMT

1.4 To estimate the incidence of primary and secondary graft failure following myeloablative haploidentical BMT

1.5 To estimate the cumulative incidence of acute graft versus host disease grades 2-4 and grades 3-4 using competing risk analysis following myeloablative haploidentical BMT

1.6 To estimate the cumulative incidence of chronic GVHD using competing risk analysis following myeloablative haploidentical BMT

1.7 Characterize the duration of use, number, and type of steroid and non-steroid immunosuppressants used to treat GVHD following myeloablative haploidentical BMT

1.8 To estimate overall survival (OS), relapse, progression-free survival (PFS), disease-free survival (DFS), event-free survival (EFS), and relapse-free GVHD-free survival in patients receiving myeloablative haploidentical BMT for patients with high risk hematologic malignancies at 1 year and 2 years

1.9 To assess additional hematologic and non-hematologic toxicities of myeloablative haploidentical BMT

1.10 Characterize immune reconstitution following myeloablative haploidentical BMT

2.0 BACKGROUND

Background

The need for alternative donor options in hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation (HSCT) is a curative treatment for a variety of malignant and non-malignant hematologic disorders[1-3]. HSCT from a human leukocyte antigen (HLA)-matched sibling has produced the best outcomes as measured by overall and progression-free survival[4]. Unfortunately, only about a third of candidates for HSCT have HLA-matched siblings[5;6]. For patients who lack HLA-matched siblings, there are two alternative sources of unrelated HLA-matched stem cells for HSCT: 1) volunteer matched unrelated donors and 2) umbilical cord blood[7]. However, outcomes following transplants using these sources are inferior compared to those performed using HLA-matched related donors, largely due to the increased incidence of post-transplant complications such as GVHD, infections and graft failure. Furthermore, the frequency with which an URD can be identified may be around 50% for Caucasians, but the likelihood falls to ≤10% for those of certain ethnic or mixed race backgrounds[6]. Worldwide, one million HSCT had been performed by the end of 2012, as treatment for over 70 different diseases[8]. Despite this success, over 1,000 patients are estimated to die each year in the U.S. because a suitable matched donor cannot be identified. Thus, the development of suitable approaches for alternative mismatched donors represents an important issue in the field of HSCT.

Cost is also a consideration for alternative donor strategies, as overall transplant costs, duration of hospitalization, and donor acquisition fees vary by graft source and are greatly increased in unrelated transplants. A recently published single-institution report of pediatric allogeneic HSCT demonstrated the increased costs of unrelated donor and umbilical cord blood transplants compared to matched related donor transplants. For 2004-2006, the mean costs per day survived were $3,446 for matched related donors (median duration of hospitalization 36 days), $4,050 for matched unrelated donors (median duration of hospitalization 47 days), and $4,522 for umbilical cord blood recipients (median duration of hospitalization 57 days). Additionally, costs of graft acquisition varied by donor source. The mean costs of graft acquisition were $8,891 for matched related donors, $57,134 for matched unrelated donors, and $58,910 for umbilical cord blood[9]. Thus, an unrelated donor source adds approximately $100,000 to the cost of the first 3 months of HSCT medical care.

Historical Haploidentical HSCT

Since individuals share exactly one HLA haplotype with each biological parent or child and with half of one's siblings, haploidentical relatives can be identified quickly as a readily available source of stem cells for virtually all patients. Historically, haploHSCT has been associated with significant risks of graft rejection and severe GVHD[10-12] , which are manifestations of excessive alloreactivity by host[13] and donor T cells[14], respectively. The risk of severe GVHD may be reduced in intensively conditioned recipients of grafts that have been rigorously depleted of mature T cells or selectively depleted of alloreactive T cells, so called T cell depleted (TCD) grafts, but the risks of serious infection and death from prolonged immune compromise in these patients remains high[15-20]. Mortality from CMV disease alone was 14% in a

recent study of nonmyeloablative transplantation using HLA-haploidentical donors[21] and as high as 35% in other reports from all causes[16-18]

TCD haploidentical grafts with "megadoses" of $CD34^+$ cells have achieved acceptable engraftment rates[22]. This approach was tested in a phase II clinical trial at MD Anderson Cancer Center (MDACC) using a reduced-intensity conditioning regimen with fludarabine, melphalan and thiotepa (FMT) for patients with hematologic malignancies[23]. A high rate of disease relapse and serious infectious complications occurred, similar to previous reports[19-21;24-27]. Patients with AML/MDS and high disease burden at the time of transplant (>10% blasts) had a particularly poor outcome (**Figure 1**) [23]. These results were confirmed in a large retrospective study summarizing the experience of haploHSCT with T-cell depletion in Europe[28]. Thus, the use of a T-cell depleted (TCD) graft, even with increased $CD34^+$ stem cells, decreases the rate of GVHD at the expense of a higher risk of rejection, reduced graft-versus-leukemia effect, and severe infections[29]. Thus, to reduce toxicity and improve the efficacy of haploidentical HSCT, methods to selectively inhibit alloreactivity while preserving immunity to infection and the malignancy are needed[30].

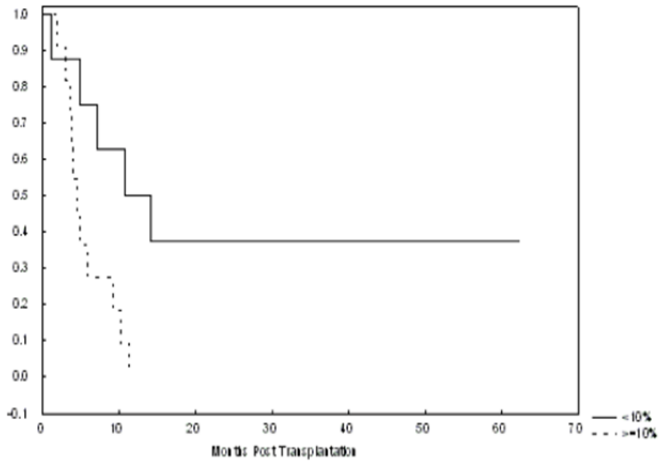


Figure 1. Cumulative survival in patients with AML/MDS receiving a T-cell depleted haploidentical stem cell transplant with FMT conditioning regimen, in remission (solid line) or not (dotted line) at the time of transplant, based on percent bone marrow blasts.

The use of cyclophosphamide as a post-transplant immunosuppressant

Cyclophosphamide (Cy) is a highly immunosuppressive antineoplastic agent that has an established role in conditioning for HSCT. Typically, the drug is administered prior to transplantation to prevent graft rejection by suppressing the host immune system. However, *pre*-transplantation conditioning with Cy also *increases* the risk of GVHD following allogeneic T cell infusion in mouse models[31]. In contrast, administration of a properly timed, high dose of Cy *after* HSCT inhibits both graft rejection *and* GVHD[32-35] .

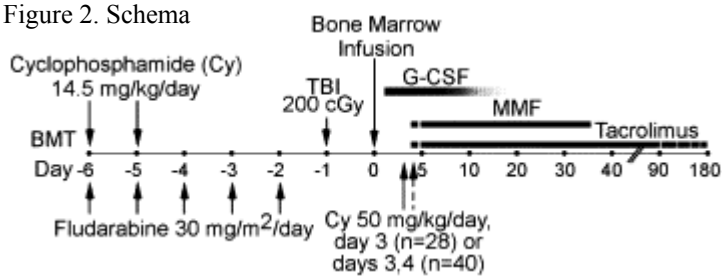
Initial results of haploHSCT with post-transplant cyclophosphamide at JHU

In light of these observations, a non-myeloablative conditioning regimen was developed at Johns Hopkins University (JHU) for transplantation of T cell replete, unmanipulated marrow from haploidentical first-degree relatives [36]. The main goal of this study was to titrate the dose of post-transplantation Cy (PT/Cy) given in conjunction with pre-transplantation fludarabine and total body irradiation (TBI). All patients received mycophenolate mofetil and tacrolimus, beginning on day 4 or 5 and terminating on days 35 and 50-180, respectively, to reduce the incidence and severity of GVHD (see Figure 2). At the same time, a trial was being conducted in Seattle utilizing Cy 50mg/kg IV on day 3 only and MMF three times a day. All study subjects had poor risk hematologic malignancies. A total of 68 patients were consecutively enrolled on these two protocols. The median times to neutrophil and platelet recovery for all patients were 15

and 24 days, respectively. Graft failure occurred in a total of 15/84 patients (18%): 6 of 19 (32%) in the Hopkins group that received only one dose of PT/Cy, 3 of 26 (12%) in the Seattle group that received one dose of PT/Cy, and 6 of 39 (15%) in the Hopkins group that received 2 doses of PT/Cy, indicating that 2 doses of post-transplant cyclophosphamide was more efficacious at preventing engraftment failure. All but two of the patients with graft failure experienced recovery of autologous hematopoiesis, with median times to neutrophil and platelet engraftment of 15 days (range 11-48 days) and 24 days (range 15-395 days), respectively. Graft rejection occurred in 9 of 66 evaluable patients (13%). All but 1 patient with graft failure experienced recovery of autologous hematopoiesis with median times to neutrophil and platelet engraftment of 15 days (range: 11-42 days) and 28 days (range: 0-395 days), respectively.

For the entire population of patients transplanted in these two studies, the probabilities of grades II-IV and III-IV aGVHD by day 200 were 34% and 6%, respectively. There was no statistically significant difference in the probability of aGVHD between patients who received 1 versus 2 doses of post transplantation Cy. However, the incidence of extensive cGVHD at 1 year in the group of patients who received 2 doses of post transplantation Cy (5%) was suggestively lower than the incidence of extensive cGVHD in the group of patients who received 1 dose of post transplantation Cy (25%; hazard ratio [HR] 0.21; 95% confidence interval [CI] 0.04-1.01; P = .05). The probabilities of NRM at 100 days and at 1 year after transplantation were 4% and 15%, respectively, and the probabilities of relapse at 1 and 2 years after transplantation were 51% and 58%, respectively. At a median follow-up among survivors of 745 days (range: 112-1483 days), the actuarial OS at 1 and at 2 years was 46% and 36%, respectively. The actuarial EFS at 1 and at 2 years was 34% and 26%, respectively. OS and EFS were not statistically significantly different between groups (data not shown).

Figure 2. Schema



Furthermore, CMV reactivation was observed in 17 of 45 (38%) high-risk patients with a median time to reactivation of 34 days. Proved or probable invasive mold infections post transplant, all caused by *Aspergillus* sp, were observed in 5 of 68 (7%) patients. Two patients died from *Aspergillus* infection: 1 while persistently neutropenic following graft failure, and 1 with fungal sinusitis. There was no CMV-associated mortality[36].

Results of haploHSCT with post-transplant cyclophosphamide in the BMT-CTN

Based on the promising results using post-transplant Cy in the two pilot studies, two Phase 2 trials (Protocols 0603, 0604) were conducted by Blood and Marrow Transplant Clinical Trials Network for adults with leukemia or lymphoma and no suitable related donor were conducted and published recently[37]. Either double umbilical cord blood (dUCB) or haploidentical bone marrow was utilized as stem cell source with RIC using cyclophosphamide, fludarabine, and 200 cGy of total body irradiation.

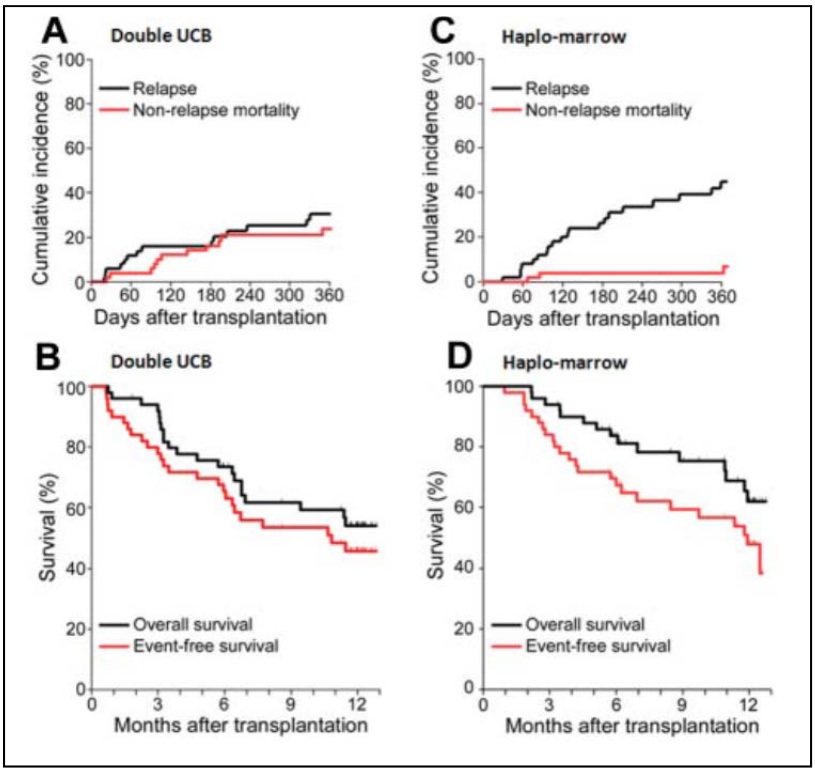


Figure 3. Results of BMT-CTN dUCBT and haploBMT trials

The 1-year probabilities of overall and progression-free survival were 54% and 46%, respectively, after dUCB transplantation (n = 50) and 62% and 48%, respectively, after haploBMT (n = 50). The cumulative incidence of neutrophil recovery was 94% after dUCB and 96% after haploBMT. The 100-day cumulative incidence of grade II-IV acute GVHD was 40% after dUCB and 32% after haploBMT. The 1-year cumulative incidences of nonrelapse mortality and relapse after dUCB transplantation were 24% and 31%, respectively, with corresponding results of 7% and 45%, respectively, after haploBMT (Figure 3). In summary trend towards reduced NRM for haploBMT was balanced by increased relapse as compared to dUCB to yield almost equivalent 1 yr overall and progression free survival (Figure 3).

Post-transplant Cy in matched HSCT with full-intensity conditioning

Relapse was the major source of treatment failure for patients in each of the haploHSCT studies described above. Potential explanations are that the transplantation conditioning intensity was not sufficient to achieve adequate tumor cytoreduction or to augment a graft-versus-host reaction through epithelial tissue damage.

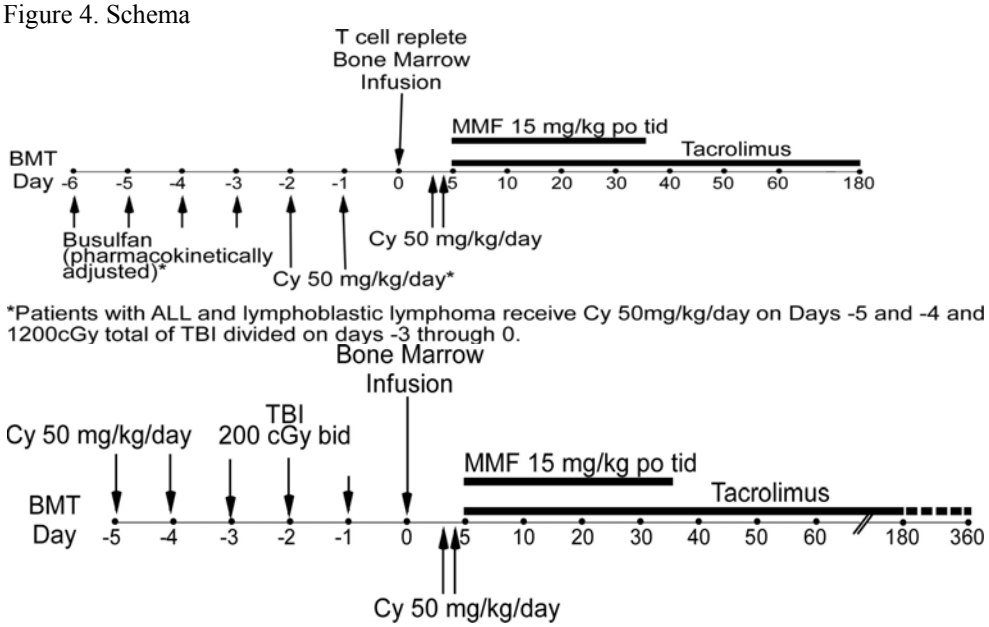
Post-transplant Cy in combination with Bu Cy myeloablative conditioning was used for HLA-matched HSCT at JHU in study J0373, which enrolled 117 patients (median age 50, range 21-66) with advanced hematologic malignancies[38]. These patients received HLA-matched related (n=78) or unrelated (n=39) bone marrow transplants after conditioning with busulfan on days -7 to -3 and Cy (50 mg/kg/day) on days -2 and -1, +3, and +4 and no additional GVHD prophylaxis. The incidences of acute grades II through IV and grades III through IV GVHD for all patients were 43% and 10%, respectively. The nonrelapse mortality at day 100 and 2 years after transplantation were 9% and 17%, respectively. The actuarial overall survival and event-free survivals at 2 years after transplantation were 55% and 39%, respectively, for all patients and 63% and 54%, respectively, for patients who underwent transplantation while in remission. With a median follow-up of 26.3 months among surviving patients, the cumulative incidence of chronic GVHD is 10%. Seven pediatric patients with high risk hematologic malignancies were treated in the same way and all patients engrafted, none of the patients had aGVHD or cGVHD

and there were no TRMs. Two out of the eight patients relapsed. One patient with early ALL relapse who was transplanted without evidence of disease but with aplasia after reinduction chemotherapy relapsed early post transplant and one patient with AML relapsed two years post BMT. These results suggest that high-dose post transplantation cyclophosphamide is an effective single-agent prophylaxis of acute and chronic GVHD after BuCy conditioning and HLA-matched BMT[39].

Myeloablative conditioning with post-transplant Cy for haploHSCT

The combination of myeloablative conditioning with a haploidentical graft source and post transplant Cy was studied at JHU. This trial combined the Bu/Cy myeloablative preparative regimen utilized in the matched setting (J0373) with the post transplantation immunosuppression utilized in the nonmyeloablative setting (J9966 and J0457: tid MMF and FK506), along with post-

Figure 4. Schema



transplantation Cy on days +3 and +4 utilized in both trials in an effort to achieve the same low toxicity profile while augmenting disease response and decreasing relapse rates[39]. All patients with high risk hematologic malignancies received a busulfan and cyclophosphamide preparative regimen except those with ALL or lymphoblastic lymphoma who received a TBI based preparative regimen (Figure 4). The primary objective was to establish feasibility (day 60 engraftment). Ninety-six patients were evaluated on this protocol. Median age of the patients is 42y (1y-65y). Median total nucleated cell (TNC)/kg cells infused $4.8x10^8$. Disease breakdown included 42 patients with AML, 21 patients with ALL, 2 patients with bilineage leukemia, 12 patients with MDS, 4 patients with CML, 11 patients with lymphoma and 4 patients with other diagnosis. Donor engraftment at Day 60 was observed in 91% of patients (80/88 evaluable patients). The median time to neutrophil recovery is 24 days and platelet recovery is 29 days. The cumulative incidence of TRM at 100 days was 6%, and at 1 year 11%. The cumulative incidence of acute GVHD grades 2-4 was 17% at 100 days and severe acute GVHD grades 3-4 was 7% at 100 days, and chronic GVHD (cGVHD) at one year was 15%, with 5% moderate-severe cGVHD. The one-year cumulative incidence of relapse in this group of patients with high-risk malignancy is 36%. Overall survival at 1 year is 72% and EFS is 56%. CMV

reactivation was seen in 22% at risk, and hemorrhagic cystitis was seen in 24% of patients (all BK+ and 70% grades 1-2).

In a recent report from Italy, 50 patients received unmanipulated haploHSCT following myeloablative conditioning with busulfan, fludarabine, and thiotepa followed by post transplant Cy, CSA, and MMF[40]. This was a high-risk population of patients with lymphoid and myeloid malignancies, 54% of whom were not in remission at the time of transplant and 20% of whom had received a prior alloHSCT. Engraftment was 90%; grade 2-4 acute GvHD 12%, and moderate chronic GvHD 10%. The 2-year DFS was 68% for those transplanted in CR and 37% for those not in remission at transplant, with overall TRM of 18% and a relapse rate of 26%. Bashey et al from Atlanta treated 20 patients who did not have suitable matched related or unrelated donors with a myeloablative preparative regimen consisting of fludarabine, busulfan, pretransplant cyclophosphamide (14.5mg/kg) and post transplant cyclophosphamide (50mg/kg/dx2) with haploidentical unmanipulated peripheral blood stem cells for adults with hematological malignancies. Donor engraftment occurred in all 20 patients with full donor T cell and myeloid chimerism by day 130. The cumulative incidence of grades II-IV and III-IV acute graft-versus-host disease (aGVHD) was 30% and 10%, respectively. The cumulative incidence of chronic GVHD (cGVHD) was 35%. Nonrelapse mortality (NRM) at 100 days and 1 year was 10% for all patients and 0% for standard-risk patients. With a median follow-up of 20 months, the estimated 1-year overall survival (OS) and disease-free survival (DFS) was 69% and 50%, respectively, for all patients, and 88% and 67% for standard-risk patients[41].

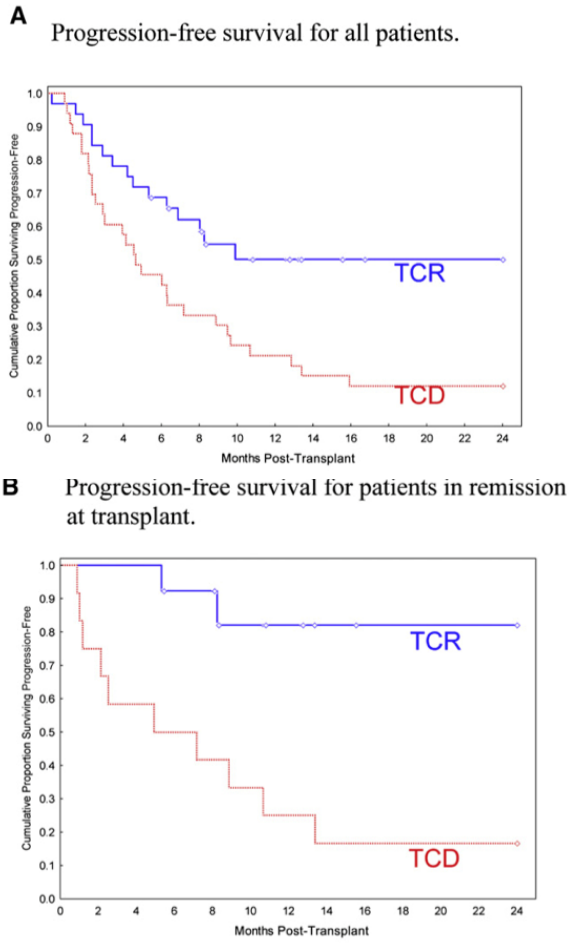


Figure 5. Comparison of progression-free survival in haploSCT patients receiving FMT condition and either T-cell replete (TCR) or T-cell depleted (TCD grafts. A) PFS for all patients. B) PFS for patients in remission at the time of transplant.

MDACC recently published their results of a myeloablative haploBMT with fludarabine, melphalan, and thiotepa (FMT), in order to compare the outcomes with concurrent TCD aploHSCT that received the same FMT conditioning regimen[42]. They analyzed 65 consecutive adult patients with hematologic malignancies who received T-cell replete (N = 32) or TCD (N = 33) haploHSCT. The TCR group received post-transplantation treatment with Cy, tacrolimus, and MMF. Patients with TCD received antithymocyte globulin followed by infusion of $CD34^{+}$selected cells with no post-transplantation immunosuppression. The majority of patients in each group had active disease at the time of transplantation. Engraftment was achieved in

94% of TCD versus 81% of TCR (NS). The TCR transplants were superior, however, in NRM at 1 year (16% versus 42%, P = .02), overall survival (64% versus 30%, P = 0.02) and progression-free survival (50% versus 21%, P = 0.02) whether or not the patients were in remission at the time of transplant (**Figure 5**). The TCR cohort had a non-significant trend toward reduced grade II-IV aGVHD (20% versus 11%, P = 0.20), and a significant decrease in cGVHD (7% versus 18%, P = 0.03). Improved reconstitution of T cell subsets, NK cells, and a lower rate of infection were observed in the TCR post-transplant Cy group.

Experience in pediatric patients receiving haploHSCT with post-transplant Cy

Twenty-nine pediatric patients ≤24 years of age have been treated at JHU using a myeloablative haploHSCT with the post-transplant Cy platform depicted in Figure 4. Median age for this group of patients was 14y (range 1y-24y). Median time to neutrophil recovery was 24 days and platelet recovery was 29 days. Donor engraftment at Day 60 was 96%. The three year overall survival is 75%. Acute GvHD grades 2-4 occurred in 16%, and aGvHD grades 3-4 in 7%. The cumulative incidence of chronic GvHD is 25%, 12% moderate-severe. The cumulative incidence of TRM is 11% and relapse at 3 years 25%[43].

Stem cell source- bone marrow vs. mobilized peripheral blood

Some studies have demonstrated that peripheral blood grafts have a higher sustained engraftment rate than marrow grafts in the matched unrelated setting after nonmyeloablative conditioning. (85% vs. 56%, p=.007)[44]. In one trial, a multivariate statistical analysis identified significantly increased risks of graft rejection for patients who received marrow instead of peripheral blood (PB) grafts (p=.003) and those without preceding chemotherapy (p=.003). However, the same study also showed the probability of severe GVHD grades III-IV was higher among PB recipients (11% vs. 0%, p=.05). Additionally, Anasetti et al conducted a phase III multicenter trial randomizing patients to receive PBSC versus bone marrow from unrelated donors that enrolled 551 patients. There was no significant survival difference between PBSC and BMT from unrelated donors. There was a slightly higher rate of graft failure with bone marrow (9% vs. 3%, p=0.002) but a higher rate of cGVHD with PBSC versus BM (53% vs. 41%, p=0.01)[45]. Given our successful engraftment rates with BM on the nonmyeloablative and myeloablative haploidentical HSCT protocols and the myeloablative matched related or unrelated protocols as well as the increased risk for severe GVHD using haploidentical donors, this trial will be restricted to BM as the stem cell source.

Use of a Cy/TBI prep in ALL patients

Preparative regimens utilizing busulfan and Cy versus Cy and TBI have been compared in children receiving bone marrow transplants for hematologic malignancies. It has been shown that for pediatric patients (age < 20 years) with acute lymphoblastic leukemia, a TBI based prep regimen resulted in superior overall survival (55% v 40%, p=0.003) and three-year leukemia free survival (50% v 35%, p=0.005). In addition, treatment related mortality was higher in the Bu/Cy group (RR, 1.68, p=0.012), as were death and treatment failure (p=0.017 for death and p=0.006 for relapse)[46]. Thus, patients with acute lymphocytic leukemia will receive a Cy/TBI based preparative regimen on this trial.

Shortened Duration Tacrolimus

Kasamon et al presented the JHH data using shortened duration tacrolimus at the American Society of Hematology 2016 annual meeting. In this trial, tacrolimus was discontinued at Day 60 or Day 90 after nonmyeloblative haploBMT instead of the historical Day +180. Of the 47 pts in the Day 90 cohort (median follow-up 44 months), 23 (49%) stopped tacro early as planned. Safety stopping criteria were not met. Of these 23 patients, 16 (70%) had no safety events before Day 180, 5 (22%) developed grade 2 acute GVHD (1 complicated by severe chronic GVHD) and 2 (9%) developed grade 3-4 acute GVHD. Of the 55 patients in the Day 60 cohort (median follow-up 14 months), 38 (69%) stopped tacro early as planned, and safety stopping criteria were likewise not met. Of these 38 patients, 25 (66%) had no safety events before Day 180, 1 developed graft failure, 9 (24%) developed grade 2 acute GVHD and 3 (8%) developed grade 3-4 acute GVHD. In both cohorts, the D 180 cumulative incidence (CuI) of grade 2-4 acute GVHD was < 40% and was < 10% for grade 3-4 acute GVHD and NRM. The 1-year CuI of any chronic GVHD was 11% for the Day 90 arm and 13% for the Day 60 arm (12% historically). Risks of acute GVHD, chronic GVHD, graft failure and NRM appear similar to historical outcomes with tacro until Day 180. These data show that many pts (60% in this trial) can discontinue tacro without taper well before Day 180. The favorable toxicity profile of the PTCy platform, coupled with the feasibility and safety of early tacro cessation, provides an ideal setting to incorporate posttransplantation approaches for relapse reduction (Kasamon, ASH abstracts, Blood 2016 128:831). JHH has since adopted Day +90 as its timepoint to stop and/or wean tacro (over 4 weeks) after myeloablative and/or nonmyeloablative haploBMT.

Second Transplants for Relapsed Disease

Relapse of the primary malignancy after hematopoietic stem cell transplantation is a common cause of transplant failure. In the pediatric population one in three children will likely relapse following HSCT. In a retrospective study of 77 consecutive patients aged 2 to 51 years who relapsed after allogeneic HSCT, pediatric patients fared much better than the adults[47]. Disease Free Survival (DFS) in the 19 pediatric patients in this study was 44%. In a subsequent dedicated pediatric study of 25 patients, the 10 year survival was 48%[48]. Second allogeneic HSCT has been considered as a potential treatment option for patients who relapse following a first transplant. A recent retrospective multi-center pediatric study including 532 patients[49] evaluated the outcome of the 160 patients (30%) who relapsed. The treatment options after relapse included: (a) Palliative therapy with non-curative intent (n = 43); (b) Salvage chemotherapy without a second HSCT (n = 55), and (c) Salvage chemotherapy followed by second HSCT (n = 62). The one-year DFS of the patients who underwent a second transplant was 50%, and this dropped to 35% at year two. In comparison, those who did not undergo second transplant had a 9% and 2% one and two year DFS, respectively (P=<0.0001). After the second transplant, 43 (69%) of the patients died; 24% from non-relapse causes and the remaining from relapse of primary disease. Patients with NRM died at a median of 2 months. A multivariate analysis of this cohort showed that the outcome of the second transplant improved in proportion to the length of time to relapse following the first transplant. Furthermore, a time to relapse of more than twelve months from the first transplant, and receiving a second transplant were the only significant factors influencing overall outcome. In a second retrospective analysis at a single institution, 40 of 93 (43%) patients experienced relapse after a first alloHCT. Eleven patients underwent a second alloHCT. The three-year overall survival probability in patients who underwent a second transplant was 27% (95% CI 6.5-54%) versus 5.4% (95% CI 0-20%,) for those who did not receive a second transplant. Eight (72%) died from post-HCT complications

including infection, multisystem organ failure, and sinusoidal obstructive syndrome. (N. Shah; personal communication, manuscript submitted)

In this protocol, we plan to include patients who have relapsed after a 1st HSCT as separate cohort, based on the experience with second transplant in the pediatric setting and the low rates of TRM and infectious complications thus far in the myeloablative haploidentical setting. Indeed, two pediatric second transplant recipients on the JHH protocol are long-term survivors. Outcome in second transplant recipients has historically been considerably worse because of both TRM and relapse compared with patients who undergo their first HSCT. Thus, this small cohort of patients would be considered separately from patients receiving a first transplant, and providing descriptive outcome results with appropriate stopping rules for excessive TRM as described in the statistical section.

Summary

Multiple institutions have demonstrated the safety and feasibility of haploidentical HSCT after myeloablative conditioning with post-transplant Cy, and JHH has preliminary data demonstrating promising results in children. We propose a multi-institutional phase II study in children with high-risk leukemias in 1st CR, acute leukemias in 2nd CR, MDS, and JMML. The myeloablative conditioning regimen prescribed will be TBI-based for lymphoid leukemias and busulfan-based for myeloid leukemias, or for lymphoid leukemias in which a TBI-based regimen was used for the first transplant. Our goal is to establish an easily exportable, inexpensive platform for haplotransplantation that has a safety profile equivalent to matched related and unrelated BMTs. The primary objective will be to estimate the incidence of 6-month non-relapse mortality, hypothesizing that NRM is < 18%. This is slightly higher than the 15% Johns Hopkins has seen in the combined adult and pediatric data on the myeloablative haploidentical BMT protocol with PT/Cy, and within the range of published TRM data for myeloablative haploBMT within PT/Cy as mentioned above (10-18%).

3.0 SELECTION OF PATIENTS AND DONORS

3.1 Recipient eligibility

3.1.1 Patient age 0.5-25years

3.1.2 Lack of a suitable HLA-matched related donor

3.1.3 **Patients must have a first-degree related donor or half-sibling who is at minimum HLA haploidentical to be enrolled.** The donor and recipient must be identical at at least one allele of each of the following genetic loci: HLA-A, HLA-B, HLA-Cw, HLA-DRB1, and HLA-DQB1. A minimum match of 5/10 is therefore required, and will be considered sufficient evidence that the donor and recipient share one HLA haplotype.

3.1.4 An unrelated donor search is not required for a patient to be eligible for this protocol, or a donor search and donor mobilization may be abandoned if the clinical situation dictates an urgent transplant. Clinical urgency is defined as high likelihood that greater 6-8 weeks will be required to proceed to transplant or a low-likelihood of finding a matched, unrelated donor.

3.1.5 Patients must have at least one of the following high-risk conditions listed below (criteria are consistent with existing criteria within COG protocols):

a) Acute lymphocytic leukemia (ALL) in CR1* as defined by at least one of the following:
i. hypodiploidy
ii. induction failure
viii. MRD after consolidation

b) Acute myeloid leukemia (AML) in CR1* with high risk features defined as:
i. High allelic ratio FLT3/ITD+
ii. Monosomy 7
iii. Del (5q)
iv. Standard risk cytogenetics with positive minimal residual disease at the end of Induction I chemotherapy (for patients being treated on or according to COG AAML1031 who have had MRD studies sent to Seattle or performed at their local institution where the flow assay is sensitive enough to detect $\geq$ 0.1% blasts)

c) Acute Leukemias in 2nd or subsequent CR (CR$\geq$2)

d) Mixed phenotype/Undifferentiated Leukemias in 1st or subsequent CR*

e) Secondary or therapy related leukemias in CR $\geq$ 1

f) NK cell leukemia or NK cell lymphoblastic leukemia/lymphoma CR $\geq$ 1

g) Myelodysplastic syndrome (MDS)
h) JMML
i) Prior transplant eligible if $\leq$18yo, $\geq$1 year has elapsed since BMT, and patient is off immunosuppression for $\geq$ 3 months with no GVHD. Patients who have had a prior chemotherapy based preparative regimen are allowed to receive a TBI based prep, regardless of their disease.

j) No known active CNS involvement or extramedullary involvement by malignancy. Such disease treated into remission is permitted.

* Remission is defined as morphology with < 5% blasts with no morphological characteristics of acute leukemia (e.g., Auer Rods) in a bone marrow with > 20% cellularity.

3.2 Criteria for recipient ineligibility

3.2.1 Patients will not be excluded on the basis of sex, racial or ethnic background.

3.2.2 Poor cardiac function: left ventricular ejection fraction <50% as determined by MUGA or ECHO or a shortening fraction below 27%.

3.2.3 Poor pulmonary function:

a) For patients receiving a TBI based preparative regimen: FEV_1, FVC, and DLCO (corrected for Hgb) $\leq$ 60% by pulmonary function tests (PFTs).

b) For patients receiving a non-TBI based preparative regimen: FEV_1, FVC, and DLCO $\leq$50% predicted (corrected for hemoglobin) for patients who have not received thoracic or mantle irradiation.

c) For patients who have received thoracic or mantle irradiation, FEV1 and FVC <70% predicted or DLCO $\leq$ 50 of predicted. For children unable to perform PFTs because of developmental stage pulse oximetry $\leq$ 92% on RA,: no evidence of dyspnea at rest, no exercise intolerance.

d) For children who are unable to cooperate for PFTs, required criteria are: no evidence of dyspnea at rest, no exercise intolerance, and not requiring supplemental oxygen therapy.

3.2.4 Poor liver function defined as bilirubin $\geq$2 mg/dl (not due to hemolysis, Gilbert's or primary malignancy) or ALT or AST $\geq$ 3 x laboratory upper normal limits.

3.2.5 Poor renal function: Creatinine clearance (calculated creatinine clearance is permitted) < 60 mL/min based on Traditional Cockcroft-Gault formula:

(140 - age (yrs) x Body Weight (kg) (Smaller of Actual Weight and IBW)) /
72 x Serum creatinine (mg/dl)

- Multiply by 0.85 if female
- Intended for ages >18,y serum creatinine 0.6-7 mg/dl

For patients <18 years: CrCl will be estimated by the Schwartz formula. A measured CrCl or a GFR may be substituted to determine the subject's CrCl.

Schwartz equation: CrCl (ml/min/1.73m2)=[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

3.2.6 HIV-positive

3.2.7 Positive leukocytotoxic crossmatch. Specifically, complement dependent cytotoxicity and flow cytometric crossmatch assays must be negative, and the mean (or median) fluorescence intensity (MFI) of any anti-donor HLA antibody by solid phase immunoassay should be <2000. If a screening assay against pooled HLA antigens is used, positive results must be followed with specificity testing using a single antigen assay. The MFI must be <2000 unless the laboratory has validated higher threshold values for reactivity for HLA antigens, such as HLA-C, DQ, and DP, that may be enhanced in concentration on the single antigen assays. Consult with PI for the clinical

significance of any anti-donor antibody. If centers are unable to perform this type of testing, please contact the PI to make arrangements testing.

3.2.8 Women of childbearing potential who currently are pregnant (B-HCG^{+}) or who are not practicing adequate contraception or who are breastfeeding

3.2.9 Uncontrolled viral, bacterial, or fungal infections (currently taking medication and with progression of clinical symptoms or findings). Patients with symptoms consistent with RSV, influenza A, B, or parainfluenza at the time of enrollment will be assayed for the above viruses and if positive are not eligible for the trial until they are no longer symptomatic (patients may have continued assay positivity for a period of time post resolution of symptoms secondary to the nature of the assay.

3.3 Criteria for donor eligibility

3.3.1 Age $\geq$0.5 years

3.3.2 Donors must meet the selection criteria as defined by the Foundation for the Accreditation of Hematopoietic Cell Therapy (FACT).

3.3.3 The following criteria, in order of importance, should also be used for donor selection:

a) Medically and psychologically fit and willing to donate

b) The patient must lack antibodies against donor HLA molecules potentially clinically significant (see section 6.1.3j)
c) ABO compatibility (in order of priority)

i. Compatible or minor ABO incompatibility
ii. Major ABO incompatibility

d) CMV status

i. For a CMV seronegative recipient, use a CMV seronegative donor
ii. For a CMV seropositive recipient, use a CMV seropositive donor

3.3.4 If there is more than one donor option based on the above criteria, additional suggested criteria to consider (in no order of priority as none of these characteristics have been shown to make a difference in the setting of haploBMT with PT/Cy) include:

a) Younger adults age $\geq$18 years and non-obese donors should be preferred.

b) If all else is equal, male donors may be preferred over nulliparous female donors who may be preferred over multiparous female donors.

c) If all other criteria equal and if the patient and family express a strong preference for a particular donor, that donor should be selected.

3.3.5 Please contact Dr. Heather Symons at the Coordinating Center
Institution with any donor selection questions. Dr. Symons can help with donor selection upon request.

4.0 Treatment Plan

4.1 Indwelling central venous catheter

Placement of a double lumen central venous catheter will be required for administration of IV medications and transfusion of blood products, as per standard BMT requirements. This catheter may be removed and replaced as clinically indicated. However, the graft **MUST** be infused through a central line.

4.2 Pre-treatment Evaluation

All patients will require documentation of a detailed history and physical examination and standard BMT evaluation of cardiac, pulmonary, liver and renal function. All patients with leukemia will undergo a bone marrow aspirate and biopsy for morphological, cytogenetic and flow cytometric evaluation.

4.3 Preparative regimen administration:

4.3.1 Appropriate seizure prophylaxis (Keppra preferred) for patients above 10 years of age who are receiving busulfan will be administered. If institutional practice is to provide seizure prophylaxis for patients <10years of age, that is not a protocol violation.
4.3.2 IV Busulfan will be administered at a starting dosage of:
≤12 kg: 1.1mg/kg/dose Q 6 hours IV (each dose over 2 hours) for 4 days (16 doses)
>12 kg: 0.8mg/kg/dose Q 6 hours IV (each dose over 2 hours) for 4 days (16 doses)

OR
≤12 kg: 1.1mg/kg/dose Q 6 hours IV (each dose over 2 hours) for 4 days (16 doses)
< 6 years of age and >/= 12 kg: 32mg/m2/dose Q 6 hours IV (each dose over 2 hours) for 4 days (16 doses)
6 years of age and older: 0.8mg/kg/dose Q 6 hours IV (each dose over 2 hours) for 4 days (16 doses)

It is recommended that the dose be based on the lesser of ideal body weight and actual body weight, however, dosing per institutional standards is allowed.

For q 6 hour dosing, IV Busulfan is diluted in 5% Dextrose or NS for IV infusion over 2 hours. For accurate pharmacokinetics, it is recommended that the IV tubing be primed with drug, and connected as close as possible to the patient's central venous catheter. At the conclusion of the 2 hour infusion, it is recommended that the tubing must be disconnected so that no additional drug is given. With IV administration, blood samples will be drawn according to institutional protocol. The first dose should be administered in the evening. Busulfan kinetics should be drawn with the first dose. Samples will be sent to a reference laboratory to quantify each

individual patient's busulfan concentrations and subsequently undergo pharmacokinetic modeling to determine the individual's AUC and clearance. Target AUC is 800-1400, target Css is 600-900.

IV Busulfan may be given once daily as a 3 hour infusion as per institutional standards as long as pharmacokinetics are drawn around a test dose or the first dose. For 24 hr dosing, target AUC is 3600-6000 uMol*min per day based on the dose being infused over 3 hours.

If dose adjustments are made, repeat kinetics should be performed if at all possible to demonstrate the target AUC or Css was obtained.

4.3.3 Cyclophosphamide will be given at a dose of 50 mg/kg/day IV over 1-2 hours x 2 days on day -2 and day -1. Dosing of cyclophosphamide is based on the LESSER of ideal body weight and actual body weight (ABW). Hyperhydration and maintenance of significant urine output after administration is required. Recommendations include starting intravenous fluids at least 2 hours prior to cyclophosphamide and continuing for at least 8 hr post-cyclophosphamide. Fluids may be tailored to the individual patient and this will not result in any protocol violations. Recommended hydration: Hydration with ½ NS at 2x maintenance IV will be started 2 hours prior to cyclophosphamide and continued for 8 hours post-cyclophosphamide.

Ideal Body Weight (IBW) Children (1-18 years)

IBW (kg) =((height in cm)2 x 1.65) / 1000 Children (5 feet and taller)
IBW (male) = 39 + (2.27 x height in inches >60)
IBW (female) = 42.2 + (2.27 x height in inches >60) Adults (18 years and older)
IBW (male) = 50 + (2.3 x height in inches >60)
IBW (female) = 45.5 + (2.3 x height in inches >60)

Mesna will be given to prevent hemorrhagic cystitis at ≥80% of cyclophosphamide dosing per institutional standards.
Recommended but not mandatory MESNA administration:
Mesna 10 mg/kg lesser of IBW or ABW/dose IV mixed with cyclophosphamide over 1 hour, followed immediately by Mesna 40mg/kg lesser of IBW or ABW IV continuous infusion between hours 1-13 once daily.

It is recommended that patients achieve and maintain a urine output over ≥ 3ml/kg/hr before administering cyclophosphamide and throughout administration. Urinalysis will be performed to detect evidence of hemorrhagic cystitis, a known complication of high-dose Cy therapy.

4.3.4 TBI will be given to those patients with acute lymphocytic leukemia, or for patients who have had a prior non-TBI-based BMT. Individual institutions can also choose to give a TBI based regimen for patients with mixed phenotype leukemia. All patients will receive 1200 cGy of total body irradiation (either 200 cGy bid x 3 days beginning on Day -3 or 150 cGy bid x 4 days beginning on Day -4.). If TBI is over 4 days, then cyclophosphamide will be given on Days -6 and -5). Patients will be simulated for radiation prior to the start of their transplant preparative

regimen and the final TBI dose rate, field arrangements, treatment distance, field size, and beam energy will follow the recommendations of radiation oncology in adherence to institutional guidelines. TBI will be given at low-dose rate.
Important considerations for TBI:
- Effort should be made to avoid interruptions in TBI administration
- The inter-fraction interval shall be no less than 5 hours between treatments (start to start)
- A mid-plane dose rate of between 6 and 15cGy per minute is required

Restricting the lung dose to $\leq$ 800 cGy is required on this protocol. This requirement applies to both AP/PA and lateral treatments. To limit overall total lung dose to $\leq$ 800 cGy, partial transmission lung blocks can be used. For AP/PA treatments the lung blocks may be placed in both AP and PA fields or alternatively in just the AP or PA field alone. Partial transmission lung blocks can be used to limit the overall total lung dose.

Institutions are allowed to use their preferred method of beam attenuation to achieve the dose reduction to lungs.

Any patient who is felt to warrant additional cranio or craniospinal irradiation based on CNS disease risk or testicular radiation will be addressed on a case by case basis by the oncology and radiation oncology teams and should be discussed with the PI. TBI may be given before Cy (TBI Days -5 through -3 or -6 through -3 and Cy Days -2 and -1 for scheduling issues. TBI may also be given once a day in the pm on Day -3 (200 cgY), twice a day on Days -2 and -1 (200 cGy twice a day) and once a day on Day 0 in the am (200cGy) for scheduling issues.

4.3.5 Unmanipulated bone marrow on Day 0 must be infused at least 24 hours after the last dose of Cy. Graft may be infused on the same day as TBI administration as long as there is 4-6 hours between administration of TBI and infusion of bone marrow.

One day of rest may be added between Days -1 and Day 0 prior to bone marrow infusion, depending on donor availability, operating room schedules, and as clinically indicated.

4.4 Marrow processing and infusion

On Day 0, patients will receive unprocessed marrow unless there is a major ABO incompatibility, in which case red blood cells will be depleted from the donor marrow using institutional practices. Minor ABO incompatible grafts will have plasma removed. Institutional practices will determine if there will be processing for minor ABO incompatibilities. Donor bone marrow will be harvested with a target yield of 4×10^8 nucleated cells/kg recipient IBW, and a recommended minimum yield of 2.5×10^8 nucleated cells/kg of recipient IBW. We recommend taking no more than 10 mL per aspirate. In addition to calculating the total nucleated cell dose /kg, a sample of the product to be infused will be sent for flow cytometry to determine the content of $CD34^+$cells. The use of cryopreserved marrow is not permitted.

4.5 Post-transplantation cyclophosphamide

Cyclophosphamide will be given at a dose of 50 mg/kg/day IV over 1 hour x 2 days on day +3 and day +4 post-transplant. Begin Day +3 cyclophosphamide 60-72 hours after the start of bone marrow infusion. Dosing of cyclophosphamide is based on the LESSER of ideal body weight and actual body weight (ABW). Hyperhydration and maintenance of significant urine output after administration is required. Recommendations include starting intravenous fluids at least 2 hours prior to cyclophosphamide and continuing for at least 8 hr post-cyclophosphamide. Fluids may be tailored to the individual patient and this will not result in any protocol violations. Recommended hydration: Hydration with ½ NS at 2x maintenance IV will be started 2 hours prior to cyclophosphamide and continued for 8 hours post-cyclophosphamide.

Mesna will be given to prevent hemorrhagic cystitis at ≥80% of cyclophosphamide dosing per institutional standards.
Recommended but not mandatory MESNA administration:
Mesna 10 mg/kg lesser of IBW or ABW/dose IV mixed with cyclophosphamide over 1 hour, followed immediately by Mesna 40mg/kg lesser of IBW or ABW IV continuous infusion between hours 1-13 once daily.

It is recommended that patients achieve and maintain a urine output over ≥ 3ml/kg/hr before administering cyclophosphamide and throughout administration. Urinalysis will be performed to detect evidence of hemorrhagic cystitis, a known complication of high-dose Cy therapy.

It is crucial that no immunosuppressive agents are given until 24 hours after the completion of the post-transplant Cy. This includes corticosteroids as antiemetics. Corticosteroids used for adrenal support or during a medical emergency (e.g. treatment of anaphylaxis) will not be a violation of the protocol.

4.6 GVHD prophylaxis

On day +5, patients will begin prophylaxis with Tacrolimus and Mycophenolate Mofetil (MMF). Begin these at least 24 hours after the last PT/Cy infusion.

The recommended starting dose of tacrolimus is 0.015mg/kg IBW/dose IV over 4 hours every 12 hours. Serum trough levels of tacrolimus should be measured around D+7 and the dose should be adjusted based on this level to maintain a level of 5-15 ng/ml (or institutional equivalent). Institutional standards (i.e. continuous infusion, etc.) of administering tacrolimus is allowed as long as tacrolimus is started on Day +5 and trough levels are maintained between 5-15 ng/ml (or institutional equivalent). Tacrolimus should be converted to oral dosing when patient has a stable, therapeutic level and is able to tolerate food or other oral medications. For pediatric patients, the oral dosing is approximately two to four times the IV dosing. It is recommended that serum trough levels should be checked at steady state after any dose modification and when switching from IV to oral to ensure therapeutic trough concentrations. Serum trough concentrations should be checked at a minimum weekly thereafter and the dose adjusted accordingly to maintain a level of 5-15 ng/ml. Tacrolimus can be discontinued or weaned as early as Day +90 if the patient has full engraftment and no evidence of GVHD. If weaning, please

wean by approximately 25% per week such that tacro is discontinued after 4 weeks. At the latest, tacrolimus will be discontinued after the last dose around Day 180, or may be continued if active GVHD is present. This should be discussed with the PI. Tacrolimus may also be discontinued early if patients relapse. This should also be discussed with the PI.

Mycophenolic acid mofetil (MMF) will be given at a dose of 15mg/kg/dose po or IV TID (based upon actual body weight) with the maximum total daily dose not to exceed 3 g/day (1 g po/IV TID). MMF prophylaxis will be discontinued after the last dose on D35 unless there is concern about engraftment and/or GVHD. Concerns should be discussed directly with the PI.

4.7 Supportive care

Patients will receive transfusions, nutritional support, infection prophylaxis and treatment, and other supportive care according to standard of care and institutional guidelines. Infection prophylaxis should include, but is not limited to, agents or strategies (e.g. PCR screening and preemptive therapy) to reduce the risk of bacterial, herpes simplex, CMV, Pneumocystis jiroveci, and fungal infections. GCSF is not routinely given prior to engraftment on this protocol. Reasons to give GCSF prior to engraftment can include circumstances such as neutropenia with severe infection. The PI should be notified. If GSCF will be given, the dose should be 5 mcg/kg daily. After engraftment, filgrastim may be given for severe neutropenia (ANC < 500/μL), without discussion with the PI. Other reasons for neutropenia should be considered such as bactrim or other myelosuppressive drugs. Other growth factors (GM-CSF, erythropoietin) should be given only if clinically indicated.

4.8 Anti-ovulatory treatment

Menstruating females must begin an anti-ovulatory agent per institutional guidelines before starting the preparative regimen.

4.10 Post-BMT intrathecal chemotherapy

Post-BMT intrathecal chemotherapy may be given or not given as per institutional practice. Intrathecal chemotherapy (IT) may not be started before Day +30 and, ideally, should begin when platelets are $\geq$ 50,000 without supportive transfusions. IT chemo may be started with platelet transfusion support if platelets have not recovered by Day +60.

4.11 Correlative Laboratory Studies (Recommended, Not Required; consent required)

a. <u>NK cell reconstitution studies:</u>

10cc peripheral blood will be collected in a sodium heparin tube and kept at room temperature at the following time points: Day 30+/-3, 60+/-7, 100+/-15, 180+/-30, and 365+/-60 after transplantation.

Specimens should be sent via Fed Ex overnight Mon-Thurs to:
Lee Lab
Attn: Robin Nakkula

Research Institute of Nationwide Children's Hospital
700 Children's Drive, WA4112
Columbus, OH 43205
Email: Robin.Nakkula@nationwidechildrens.org
Phone: 614-355-1538

Please email both Robin.Nakkula@nationwidechildrens.org and Dean.Lee@nationwidechildrens.org in advance to let them know when samples will be arriving.
FedExAcct #2169-7339-9. Include in the note section "NCH Fund No 18951016"

b. Immune reconstitution of T cells, B cells.
When possible, 1 cc/kg of peripheral blood to max of 50mls EACH will be collected from the recipient in sodium heparin tube and kept at room temperature. Because of this, if samples for Johns Hopkins need to be drawn on a different day than the sample for Nationwide (10cc), that is ok. Time points: Prior to preparative regimen, days 30+/-3, 60+/-7, 180+/-30, and 365+/-60 after transplantation. When possible, 1cc/kg of peripheral blood to max of 50mls from the donor should also be collected prior to BMT.
1. Flow cytometry for B, T cells
2. Complete blood count with differential
3. Additional relevant immunologic/biologic testing

Specimens should be sent via Fedex overnight, Mon-Thurs (please also avoid holiday arrival) to:
Leo Luznik, MD
Cancer Research Bldg., (CRB-I) Rm. 290
1650 Orleans St.
Baltimore, MD. 21287

Phone: (410) 502-7732
Lab: (410) 955-8567
E-mail: luznile@jhmi.edu

Please email luznile@jhmi.edu at least several days prior to shipping so they know to expect the sample.

FedExAcct #5403-2938-9

4.12 Relapse prevention therapies post-BMT: Relapse prevention therapies, i.e TKIs for Ph+ leukemias, sorafenib for FLT-3 –ITD leukemias, etc are allowed on this trial.

5.0 DATA MONITORING AND MANAGEMENT

This study will be conducted in compliance with the JHM-IRB approved SKCCC Coordinating Center Operations Manual. The protocol chair, Dr. Heather Symons, will serve as liaison and will coordinate protocol development, submission, approval, amendments, results reporting and publications.

5.1 General Guidelines

Eligible patients will be registered on study centrally at the Sidney Kimmel Comprehensive Cancer Center.

Patients should be registered by two weeks before the start of prep regimen. Following registration, patients should begin protocol treatment within two weeks. Issues that would cause treatment delays should be discussed with the Principal Investigator. The Study Coordinator should be notified of cancellations as soon as possible.

5.2 Registration Process

A centralized, 3-part registration procedure will be used. After eligibility screening, patients who are selected to participate will be registered with the Coordinating Center (SKCCC) and with their study site/institution. A record of patients who fail to meet entry criteria (i.e., screen failures) will be maintained by each individual site. Patient registration must be complete before beginning any treatment or study activities.

5.2.1 Coordinating Center (SKCCC) registration

Prior to protocol enrollment and initiation of treatment, subjects must sign and date an IRB-approved consent form. To initiate registration, study sites/institutions should forward copies of the signed informed consent form, the research authorization/HIPAA form, the institutional registration form, plus any required laboratory tests to the coordinating center or sponsor by fax or email. Upon receipt of these forms, research personnel at the coordinating center will confirm patient eligibility with study personnel, assign a unique patient study identification number, and complete patient registration. Treatment must not commence until the site has received their patient's identification number. All patients must be registered centrally at SKCCC.

To register a patient, participating sites must send the documents by email (crocc@jhmi.edu) and CC the Lead Study Coordinator to the Coordinating Center. The Coordinating Center fax (410-502-9933) may be used if email is not available.

- Signed patient consent form
- Registration Form
- Eligibility Checklist
- Copies of all source documentation of all clinical studies confirming eligibility and HLA typing results

The Coordinating Center will review the documents to confirm eligibility. To complete the registration process the Coordinating Center will:

- Assign a patient study number
- Register the patient on the study with SKCCC's CRO Coordinating Center
- Fax or e-mail the patient study number to the participating site

The Johns Hopkins Pediatric Oncology Research Team will not register their patients through the SKCCC's CRO Coordinating Center. Eligibility for Hopkins subjects will be reviewed and confirmed internally by the Pediatric Oncology Research Team and verified by the Study PI. A subject number, however, *must be requested upon consent and prior to enrollment* from the

SKCCC's CRO Coordinating Center via email or telephone. The SKCCC CRO Coordinating Center Lead Study Coordinator will provide this number to the study team.

5.2.2 Institutional registration

Patient registration at each study site/institution will be conducted according to the institution's established policies. Before registration, patients will be asked to sign and date an Institutional Review Board (IRB)-approved consent form and a research authorization/HIPAA form. Patients must be registered with their local site/institution and also with the sponsor before beginning any treatment or study activities.

5.3 Data Reporting and Regulatory Requirements

5.3.1 Multicenter Guidelines

Principal Investigator/Protocol Chair is responsible for performing the following tasks:

- Coordinating development of the protocol as well as its subsequent amendments.
- Taking responsibility for the overall conduct of the study at all participating institutions and for monitoring the progress of the study.
- Reviewing and ensuring reporting of Serious Adverse Events (SAE)
- Reviewing data from all sites.

Coordinating Center

The Coordinating Center (SKCCC) is responsible for performing the following tasks:

- Ensuring that IRB approval has been obtained at each participating site prior to the first patient registration at that site, and maintaining copies of IRB approvals from each site.
- Managing central patient registration.
- Collecting and compiling data from each site
- Establishing procedures for documentation, reporting, and submitting of AE's and SAE's to the Protocol Chair, and all applicable parties.
- Facilitating audits by securing selected source documents and research records from participating sites, or by auditing at participating sites.
- Ensuring that all participating institutions are using the correct version of the protocol.
- Ensuring that each participating institution has a FWA number
- Ensuring that participating sites are accruing a representative sample consistent with the estimated population of the site
- Preparing all submitted data for review by the protocol chair

Participating Sites

- Participating sites are responsible for performing the following tasks:
- Securing IRB approval of the protocol and all subsequent amendments.
- Implementing and adhering to the guidelines of Good Clinical Practice (GCP). ·
- Submitting data to the Coordinating Center.
- Registering all patients with the Coordinating Center by promptly submitting

the patient registration form, eligibility checklist, and signed informed consent.

- Providing sufficient experienced clinical and administrative staff and adequate facilities and equipment to conduct a collaborative trial according to the protocol.
- Maintaining regulatory binders on site and providing copies of all required documents to the Coordinating Center.
- Collecting and submitting data, including the reporting of all adverse events and serious adverse events, in accordance with the schedule specified in the protocol.
- Following the protocol as written
- Verifying the current active version of the protocol with the Coordinating Center.

5.4 Data Entry

Data collected during this study will be entered into a secure database.

5.4.1 Case Report Forms

Case report forms will be generated by the Coordinating Center for the collection of all study data. Investigators will be responsible for ensuring that the CRFs are kept up-to-date.

5.4.2 Source Documents

Study personnel will record clinical data in each patient's source documents (i.e., the patient's medical record). Source documentation will be made available to support the patient research record. Study monitors will review entries on the CRFs at regular intervals, comparing the content with source documents.

5.4.3 Data Submission

All data will be collected on case report forms. Case report forms will be provided to participating sites by the Coordinating Center. A primary research data file (research chart) will be maintained at each site, and must include completed case report forms and copies of required source documentation. Copies of the completed case report forms and source documents should be submitted to the Coordinating Center according to the following recommended schedule via email (crocc@jhmi.edu).

Baseline/ On Study Forms	Submit within one week of screening
Treatment Forms	Submit within 4 weeks post BMT
Follow-up Forms	Submit within 4 weeks of contact/visit date
Off Study Form	Submit within 4 weeks of discontinuing study

5.4.4 Record Retention

The investigator will maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. After study closure, the investigator will maintain all source documents, study-related documents, and the CRFs. Because the length of time required for retaining records depends upon a number of regulatory and legal factors, documents should be stored until the investigator is notified that the documents may be destroyed. In this study, records are to be retained and securely stored for a minimum of 7 years after the completion of all study activities.

5.5 Data Management

5.5.1 Research Program Coordinators

A Lead Research Program Coordinator at the Coordinating Center will be assigned to the study and will manage the study activities at each of the participating sites. The responsibilities of the Research Program Coordinator include project compliance, data collection, data entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol team.

5.6 Study Monitoring and Quality Assurance

Regularly scheduled registration reports will be generated to monitor patient accruals and the completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and the extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period, and potential problems will be brought to the attention of the Principal Investigator for discussion and action.

Random-sample data quality monitoring will be conducted by the Coordinating Center at least every 6 months; and protocol compliance audits will be conducted by the Coordinating Center at least once a year and more frequently if indicated. Audits by the Coordinating Center may entail (1) faxing source documents and research records for selected patients from participating sites to the coordinating center for audit, or (2) on-site auditing of selected patient records at participating sites.

All clinical work conducted under this protocol is subject to Good Clinical Practice (GCP) guidelines. This includes inspection of study-related records by the Coordinating Center, sponsor, its designee, or health authority representatives at any time.

5.7 Data Safety Monitoring Board (DSMB)

5.7.1 Role of the DSMB

This study will be monitored by a DSMB. The primary role of the DSMB will be to monitor the conduct of the study as it pertains to TRM based on the protocol statistical guidelines and stopping rules (Section 13.0). A secondary role will be to monitor protocol specific adverse events and all SAEs.

5.7.2 DSMB Membership
The PBMTC will be providing DSMB oversight.

5.7.3 Operations and responsibilities of the DSMB
The DSMB will meet after the first 5 patients have reached day 100 after HCT. Future meetings will be held at 6-month intervals. At each meeting the DSMB will review the data related to patient safety. The safety report to the DSMB will include a summary of all protocol-specific adverse events as defined in Section 5.9.1, all SAEs and all unanticipated problems.

Based on the information available at each of these meetings, the DSMB is charged with deciding whether a) no action is required, b) the protocol should be modified, c) further enrollment should be suspended pending additional review, or d) the protocol should be closed. The DSMB will keep minutes of its meetings and provide its findings in a letter to the Coordinating Center within 10 working days. An exception to this time requirement will occur if the DSMB recommends study termination, suspension of enrollment or a protocol modification that significantly affects patient safety. In these cases, the DSMB will be expected to provide their recommendations to the Protocol Chair/Principal Investigator within 24 hours. Copies of the letter will be also distributed to the local PI at the participating institutions. An ad hoc meeting or discussion with the DSMB will be arranged as deemed necessary by the protocol PIs. Each institution will forward the minutes to their IRB per local institutional practice.

5.7.4 Reporting data to the DSMB

The Coordinating Center will be responsible for providing the DSMB with a summary of acute GVHD and safety data sufficient to meet their responsibilities. The participating centers are required to provide the Coordinating Center with completed case report forms and SAE reports in a timely manner to meet their obligations to the DSMB.

This is a DSMP Level I study under the SKCCC Data Safety Monitoring Plan (12/6/2012). The Clinical Research Office will perform an audit after the first subject has been treated and then periodically depending on the rate of accrual and prior audit results. All trial monitoring and reporting will be reviewed annually by the SKCCC Safety Monitoring Committee. The PI is responsible for internally monitoring the study. Data must be reviewed to assure the validity of data, as well as, the safety of the subjects. The PI will also monitor the progress of the trial, review safety reports, and clinical trial efficacy endpoints and to confirm that the safety outcomes favor continuation of the study.

5.8 Adverse Event Reporting

Allogeneic hematopoietic cell transplantation (HCT) is an intrinsically complex procedure associated with a variety of previously well-described adverse events, most of which are non-serious without long-term sequelae. **Therefore, for this study, adverse events (AEs) requiring reporting will be limited to post transplant Cy-related events, Serious AEs and Unanticipated Problems as defined in the sections below. Only Adverse Events in any way realted to Cy-infusion and not related to the BMT will be captured on the Adverse Event Log. Additionally, only clinically significant laboratory results considered in any way related to the Cy-infusion, and not at all related to the BMT are also to be captured on the Adverse Events Log. Serious Adverse Events (SAEs) must be individually assessed by the PI on the Adverse Event Log.**

5.8.1 Definition and Documentation of Adverse Event (AE)

An adverse event is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during treatment, whether or not considered causally related to the treatment. There are many expected toxicities of allogeneic BMT. The following are examples of toxicities that are serious but not unexpected: Grade 4 cytopenias;

neutropenic fever and sepsis; bacterial, fungal, or viral (including CMV, BK virus) infection; severe mucositis; pulmonary toxicities; bleeding without hemodynamic compromise.

Toxicities should be described according to the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE), version 4.0, which can be accessed and downloaded via the website:
http://ctep.cancer.gov/reporting/ctc.html.

As the primary research intervention in this study is the administration of cyclophosphamide after transplantation, adverse event reporting will focus on Cy related events. Patients will be assessed for the following events:

- Failure to engraft neutrophils (>500/mcl) by day 60 after transplantation
- Other unexpected grade 3-5 events (per CTCAE, v. 4.0 possibly, probably, and definitely related to the cyclophosphamide
- Grades 3-4 aGVHD
- Failure to detect donor chimerism >5%, as defined by the BMT-CTN and as per section 8.1.4 in whole blood at Day 60
- Grade 3-5 infections (per CTCAE, v4.0)
- Cardiac: (per CTCAE, v. 4.0)
  Clinical Heart Failure or Cardiomyopathy:
  Left Ventricular dysfunction: Grade 3- 5
- Gastrointestinal: (per CTCAE, v. 4.0)
  Mucositis/stomatitis (clinical exam): Grade 3- 5
- Hemorrhage/Bleeding; (per CTCAE, v. 4.0)
  Hemorrhage, GU, - Bladder, Grade 2 – 5 (and not related to infection)

Attribution of the event to the investigational product may be characterized as follows:

- definitely related, clearly associated with study drug/treatment
- probably related, likely associated with study drug/treatment
- possibly related, may be associated with study drug or other treatment
- unlikely to be related, or
- definitely not related to the study drug/treatment

For reporting purposes, an AE should be regarded as possibly, probably, or definitely related to the regimen if the investigator believes that at least one of following criteria are met:
a) There is a clinically plausible time sequence between onset of the AE and the administration of the study drug or treatment;
b) There is a biologically plausible mechanism for the study drug or treatment causing or contributing to the AE;
c) The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures

In those cases where the NCI criteria do not apply, intensity will be defined as:
· Mild: awareness of symptom or sign, but easily tolerated
· Moderate: discomfort is enough to cause interference with normal activities

· Severe: inability to perform normal daily activities
· Life threatening: immediate risk of death from the reaction as it occurred

Adverse events will be documented on AE logs and submitted to the Coordinating Center on a monthly basis. Adverse events will be collected through the time of discharge from the transplant center, but no longer than 100 days after transplantation.

5.8.2 Definition and reporting of Serious Adverse Event (SAE)
A serious adverse event is an AE that fulfills one or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires or prolongs a hospital stay**
  Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Represents a significant medical condition which, without urgent medical intervention, would lead to one of the above outcomes.

Life-threatening means that the AE represented an immediate threat of death without medical intervention.

All events that meet the definition of an SAE in section 5.8.2, regardless of whether they are related or unrelated to the protocol intervention will be reported to the Coordinating Center. SAEs will be reported to the Coordinating Center through the time of discharge from the transplant center, but no longer than 100 days after transplantation, with the exception of any deaths, which will be reported throughout the duration of the study. SAE reports also must be submitted to the local IRB per the institutional requirements.

**The following hospitalizations are NOT considered SAEs:

- Admissions as per protocol for a planned medical/surgical procedure
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission for the purpose other than remedying ill heath state and was planned prior to entry into the study [Documentation is required in these cases].
- Admissions encountered from another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g. lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative)
- Admission directly related to the BMT but not to the PTCy

5.8.3. Role of Protocol Chair/Principal Investigator in adverse event reporting
The Protocol Chair/Principal Investigator is ultimately responsible for the required reporting of all adverse events.

5.8.4 Role of Coordinating Center in adverse event reporting

The Coordinating Center is the central location for the collection and maintenance of documentation of adverse events and is responsible for submitting adverse event reports to the Protocol Chair promptly. The Coordinating Center will maintain documentation of all adverse events for each participating site. Adverse event reports submitted to the Coordinating Center must be signed and dated by the participating site's Principal Investigator. The Coordinating Center will provide appropriate forms to be used by all participating sites for reporting adverse events.

Documentation of all SAEs must include:

- Subject ID number, and initials
- Date of the event
- Description of the event
- Description of site's response to the event
- Assessment of the subject's condition
- Subject's status on the study (on study, in follow-up, off study)
- Attribution of event to study drug

5.8.5. Role of Participating Sites in adverse event reporting

Participating sites are responsible for reporting adverse events to the local IRB, per local institutional requirements, and to the Coordinating Center as follows:

Fatal Events whether anticipated or unanticipated, and whether or not related to the study must be reported to the Coordinating Center within **24 hours** of the participating site Principal Investigator's learning of the event.

Other Serious Adverse Events must be reported to the Coordinating Center within 72 business hours of the participating site Principal Investigator's learning of the event.

Serious Adverse Event reports are to be emailed (crocc@jhmi.edu) or faxed (410-502-9933) to the Coordinating Center. Follow-up reports are faxed, mailed, or sent electronically to the Coordinating Center as necessary.

The investigator must also report follow-up information about SAEs within the same time frames. Investigators must follow patients with SAEs until the event has resolved or the condition has stabilized. If the patient is lost to follow-up with an ongoing SAE, this should be captured accordingly on a follow-up SAE report.

If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided within the same time frames described above.

All SAEs must be collected whether or not they are considered causally related to the investigational product. Investigators and other site personnel also are responsible for reporting all casually related SAEs to their IRB and the Protocol Chair. Site PIs and other investigators must provide expedited reports of all SAEs to the protocol chair. It is the responsibility of the protocol chair to determine whether the SAE is related to the study and whether it is unexpected

by virtue of greater severity or higher frequency when evaluated in the context of prior expectations and experience in the study to date.

5.9 GVHD

A major toxicity of allogeneic BMT from an unrelated or mismatched donor is GVHD. Acute graft-versus-host disease (GVHD) shall be graded clinically according to the criteria developed by the consensus conference on acute GVHD5 (Appendix 3). *All suspected cases of acute GVHD should be confirmed histologically by biopsy of an affected organ (skin, liver, or gastrointestinal tract).* Diarrhea and/or hyperbilirubinemia in a patient with histologically documented skin GVHD may be assumed to be a manifestation of visceral GVHD and will be graded as such. All patients with histologically documented, clinical grade >2 acute GVHD should receive initial treatment according to institutional preference. If skin GVHD resolves with treatment but suspected visceral GVHD does not, biopsy of the affected organ (liver or gastrointestinal tract) should be obtained to rule out other causes of hyperbilirubinemia and/or diarrhea. Steroid refractory acute GVHD will be treated according to institutional preferences. In patients who develop GVHD, the GVHD assessment questionnaire must be completed at the time of onset, weekly until GVHD resolves, and at Day 60+/-7 days, regardless of whether or not the patient has GVHD. The following information shall be collected on all patients with acute GVHD:

- Date of onset (defined as the date of first biopsy confirming GVHD)
- Initial overall clinical grade
- Maximum overall clinical grade
- Date of onset of grade III-IV acute GVHD, if any

Chronic graft-versus-host disease (cGVHD) shall be graded clinically according to the criteria developed by the NIH consensus conference on chronic GVHD[48] (Appendix 4). *All suspected cases of chronic GVHD should be confirmed histologically by biopsy of an affected organ whenever possible.* Mild chronic GVHD involves only 1 or 2 organs or sites (except the lung), with no clinically significant functional impairment (maximum of score 1 in all affected organs or sites). Moderate chronic GVHD involves (1) at least 1 organ or site with clinically significant but no major disability (maximum score of 2 in any affected organ or site) or (2) 3 or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). A lung score of 1 will also be considered moderate chronic GVHD. Severe chronic GVHD indicates major disability caused by chronic GVHD (score of 3 in any organ or site). A lung score of 2 or greater will also be considered severe chronic GVHD.

The following information shall be collected on all patients with chronic GVHD:

- Date of onset (defined as the date of first biopsy confirming GVHD, if possible or the first day of onset of clinical symptoms if no biopsy is performed)
- Initial overall clinical score
- Maximum overall clinical score

5.10 Non-relapse mortality (NRM)

Causes of NRM, i.e., death in the absence of relapse or disease progression, will be documented as important indicators of procedure-associated toxicity, particularly as these causes relate directly or indirectly to GVHD. Analysis will stratify mortality with respect to the peri-transplant period (<100 d post-BMT) or later times post-BMT.

5.11 Protocol Deviations

When an emergency occurs that requires a deviation from the protocol for patient safety, a decision will be made as soon as possible to determine whether or not the subject (for whom the deviation from protocol was effected) is to continue in the study. The subject's medical records will completely describe the deviation from the protocol and state the reasons for such deviation. In addition, the Investigator will notify their local IRB as required in writing of the deviation from protocol. The Transplant Center Local Principal Investigator is responsible for notifying the Protocol Coordinator as soon as possible of the deviation. Major deviations in this protocol consist of modifications of the preparative regimen or a last minute change of stem cell source or significant delay in stem cell source infusion.
Non-emergency minor deviations from the protocol will be permitted with approval of the Protocol Coordinator. Modifications of GVHD prophylaxis/immune suppression necessary for good patient care are not considered protocol deviations.

6.0 PATIENT MONITORING

The following parameters will be obtained according to this schedule: (for details of these evaluations, see text sections 6.1-6.2, or additional sections when indicated).

	Initial	Allowable time frame from date of consent[1]	Day 30 +/- 3	Day 60 +/-7	Day 100 +/- 15	Day 180 +/- 30	Day 365 +/- 60
History and Physical	X	Within 30 days					
Performance status	X	Within 30 days		X	X	X	X
Disease Staging[2]	X	Within 30 days		X[8]	X		
RFLP or FISH studies[3]	X	Within 30 days					
CBC & Diff	X	Within 7 days	X	X	X	X	X
Reticulocyte count	X	Within 7 days					
Comprehensive Metabolic Panel	X	Within 7 days	X	X	X	X	X
Chimerism analysis[4]			X	X	X	X	X
MRD testing[5]	X	Within 30 days		X			
EKG	X	Within 30 days					
ECHO	X	Within 30 days					
HepB Ag, HBC Ab, HCV Ab, HSV IgG, CMV IgG, RPR, HIV, VZV IgG (if possible)	X	Within 30 days					
Anti-Donor HLA antibody/ lymphocyto-toxic screen	X	Within 30 days					
PFTs (Spirometry	X	Within 30 days					

and DLCO)							
GVHD assessment[7]				X			
Immune Reconstitution Studies (with consent)[6]	X	Within 30 days for patient and donor	X	X		X	X
NK Reconstitution (with consent)[6]			X	X	X	X	X

[1]Baseline laboratory tests and radiology studies time frame will follow BMT standards.

[2] See section 6.1.3 h

[3] RFLP or FISH studies will be drawn as a baseline for subsequent engraftment studies. May also be done via microsatellite technique or str analysis.

[4] Donor chimerism as per institutional standards on bone marrow when disease restaging is being done and on peripheral blood if bone marrow not being done. If bone marrow chimerism is performed, peripheral blood chimerism does not need to be performed. We recommend T cell engraftment (whole blood and CD3+) if possible, but this is not required.

5Recommended, but not required. See section 6.1.3 h

[6] Please see section 4.10 for details

[7] GVHD assessment should be completed at time of onset and weekly until GVHD resolves. Assessment should also be completed at Day 60 regardless of whether the patient has GVHD or not.

[8] Day 60 disease staging may be done as early as Day 30 +/-3 days

6.1 Pre-transplant Evaluation

Potential subjects must meet all of the eligibility criteria. These represent the basic baseline studies required on all patients prior to starting their preparative regimen. Additional investigations may be clinically indicated in certain individuals. Other baseline studies may be required for the purposes of non-preparative regimen protocols on which the patient is enrolled. In this case, such requirements will be stipulated in the pertinent protocols.

6.1.1. Thorough general medical evaluation which should include:

a) Physical examination

6.1.2. Baseline investigations including:

a) Hematologic
   i. CBC with platelets, differential, reticulocyte count
   ii. ABO and Rh typing

b) Chemistries
   i. Comprehensive chemistry panel including electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, calcium

c) Cardiac
   i. EKG
   ii. Echocardiogram or MUGA scan with Left Ventricular Ejection Fraction (LVEF)

d) Pulmonary

i. Pulmonary function tests including at least FEV1, FVC, and DLCO (pediatric patients under the age of 7-8 are excluded from this test)

e) Immunologic / Infections
i. HBsAg, anti-HBC, anti-HCV
ii. RPR
iii. HIV antibody
iv. Serology for CMV, HSV, and VZV
v. HLA typing/lymphocytotoxic antibody screen

f) Donor chimerism studies will be drawn as a baseline for subsequent engraftment studies when the donor and patient are the same gender.

h) Disease specific staging studies:
i. Acute myeloid leukemia
- Bone marrow aspirate
- Flow cytometry
- Comprehensive cytogenetics
- FISH of a preexisting known chromosomal abnormality
- CSF cytology, if previously involved
- MRD testing*

ii. Acute lymphocytic leukemia
- Bone marrow aspirate
- CSF cytology
- Flow cytometry
- Comprehensive cytogenetics
- FISH of a preexisting known chromosomal abnormality
- MRD testing*

iii. Myelodysplasia
- Bone marrow aspirate and biopsy
- Cytogenetics
- Flow cytometry
- FISH of a preexisting known chromosomal abnormality

*MRD is strongly recommended but not required. Testing should be done at Institutions capable of detecting MRD at a level of 0.01% for ALL and 0.1% for AML. If this is not possible at the home Institution, 2cc BM can be sent for MRD testing to an Institution with this capability.

Preferably (but not mandatory), ALL specimens can be sent to:

Michael Borowitz, MD, PhD

Johns Hopkins Medical Institution
Flow Cytometry Lab
Weinberg Building - Room 2300
401 N Broadway
Baltimore, MD 21231-2410
Phone: (410) 614-2968
Fax: (410) 502-1493
Email: mborowit@jhmi.edu

and AML specimens can be sent to:

Michael Lokin, PhD
Hematologics, Inc.
3161 Elliott Avenue, Suite 200
Seattle, WA 98121

i.) Immune Reconstitution Study blood (with consent)
-50ml of blood in a preservative free heparinized sterile syringe from adult recipients and 1cc/kg to a maximum of 50mls of blood from pediatric recipients and donors.

j) Anti-donor HLA Antibody Testing: Positive anti-donor antibody is defined as a positive crossmatch test of any titer (by complement dependent cytotoxicity or flow cytometric testing) or the mean fluorescence intensity (MFI) of any anti-donor HLA antibody by solid phase immunoassay >3000. Consult with Dr. Heather Symons at the Coordinating Center for the clinical significance of any anti-donor antibody.

6.2 Post-transplant Evaluation

6.2.1. Day 0 through Day 60 evaluation. These represent the minimum required. More frequent determinations and additional investigations may be indicated by the clinical condition of the patient.

1. CBC daily with a WBC differential once the total WBC is greater than 100 until ANC > 500 for three days or two consecutive measurements over a three day period. Then, CBC weekly with differential.
2. Comprehensive metabolic panel (electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, calcium) once a week.
3. Patients will have evaluations for infectious complications as clinically indicated. Surveillance cultures according to institutional protocol.
4. Evaluations by history and physical examination for GVHD will be performed as per BMT unit standards.

6.2.2 Evaluations on day 30 (+/-3 days)

1. CBC and differential and comprehensive panel (electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, calcium).

2. GVHD assessment/ History and Physical for GVHD
3. Immune Reconstitution and NK Studies: see section 4.10
4. Donor chimerism evaluation on bone marrow and peripheral blood [T cell engraftment (whole blood and CD3+) if possible].

6.2.3 Evaluations on day 60 (+/-7 days)

1. Donor chimerism evaluation on bone marrow and peripheral blood [T cell engraftment (whole blood and CD3+) if possible].
2. Disease staging (may be done as early as Day 30 +/- 3 days). (see section 6.1.3 h). MRD testing recommended but not required.
3. CBC and white blood cell differential, comprehensive panel (electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, calcium)
4. GVHD assessment/ History and Physical for GVHD
5. Performance status
6. Immune Reconstitution and NK Studies: see section 4.10

6.2.4 Evaluations on day 100 (+/- 15 days)

1. Donor chimerism evaluation on bone marrow and peripheral blood [T cell engraftment (whole blood and CD3+) if possible]
2. Disease staging. (see section 6.1.3 h). MRD testing recommended but not required.
3. CBC and white blood cell differential, comprehensive panel (electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, calcium)
4. GVHD assessment/ History and Physical for GVHD
5. Performance status
6. NK Studies: see section 4.10

6.2.5 Evaluations on day 180 (+/-30 days)

1. History and physical examination.
2. Donor chimerism on bone marrow (if performed) and T cell engraftment f/u on peripheral blood [T cell engraftment (whole blood and CD3+) if possible]. Only peripheral blood is required at this time point.
3. CBC and white blood cell differential, comprehensive panel (electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, calcium)
4. GVHD assessment/ History and Physical for GVHD
5. Performance status
6. Immune Reconstitution and NK Studies: see section 4.10

6.2.6 Evaluations on Day 365 (+/- 60 days)

1. RFLP or FISH for donor chimerism on bone marrow (if performed) and T cell engraftment f/u on peripheral blood [T cell engraftment (whole blood and CD3+) if possible]. Only peripheral blood is required at this time point.

2. CBC and white blood cell differential, comprehensive panel (electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, total protein, albumin, calcium)
3. GVHD assessment/ History and Physical for GVHD
4. Performance status
5. Immune Reconstitution and NK Studies: see section 4.10

7.0 RISKS AND TOXICITIES

7.1 Busulfan (Bu)

Busulfan Toxicities:

a) Nausea and vomiting - this occurs in some patients. It is usually mild but antiemetics may be required.
b) Hematologic - the doses of busulfan are expected to produce marrow ablation from which the patient would not be expected to recover without marrow rescue.
c) Pulmonary complications - diffuse interstitial pneumonitis with fibrosis has been reported as a complication of busulfan therapy when the drug is given in small doses over long periods of time. Changes in pulmonary function tests may occur following bone marrow transplantation. To date, no specific pulmonary complications have been attributed to busulfan as used in the transplant setting at this institution.
d) Seizures - preliminary studies suggest that significant levels of Busulfan are attained in the cerebrospinal fluid. In the past about 10% of our patients have experienced grand mal seizures during Busulfan administration. This toxicity is avoided by the use of keppra as outlined in the treatment plan.
e) Other toxic effects which may be produced by Busulfan include erythematous skin rash, hyperpigmentation, hepatic dysfunction, amenorrhea, testicular atrophy, gynecomastia, myasthenia symptoms, cataract and atrophic bronchitis associated with cytologic dysplasia.

7.2 Cyclophosphamide (Cytoxan)

Cyclophosphamide is an alkylating agent whose metabolites form cross-links with DNA resulting in cell cycle-nonspecific inhibition of DNA synthesis and function. Commercial supply of cyclophosphamide will be used.

Cyclophosphamide Toxicities:

a) Hematologic: Leukopenia, anemia
b) Dermatologic: Alopecia
c) Gastrointestinal: Nausea, vomiting, increased AST, ALT, mucositis, diarrhea
d) Neurologic: Headache, dizziness
e) Cardiovascular: Cardiac necrosis rarely with high dose cyclophosphamide
f) Renal: Hemorrhagic cystitis, SIADH
g) Other: teratogenic, may cause secondary neoplasms, anaphylaxis (rare)
h) Fluid retention. Cy has anti-diuretic effect usually counteracted by furosemide administration. Careful physical examination should be made and accurate weights should be determined to detect fluid overload early.

i) Cardiomyopathy. At doses greater than 200mg/kg, Cy can cause fatal myocardial necrosis with clinical heart failure. Non-specific ST changes on EKG are not unusual but a decrease in voltage is significant.
j) Hemorrhagic cystitis. Hematuria is not uncommon at this dose level, but is usually not symptomatic or severe unless there is inadequate diuresis. An occasional patient will get severe cystitis despite prophylactic measures.

7.3 TBI

Early side effects (< 1 month): Most patients experience some degree of nausea, vomiting, and diarrhea either during or immediately after treatment. Fever that develops soon after TBI is not uncommon. Other side effects include skin erythema, parotid gland swelling, diminished salivary gland function, stomatitis, mouth ulcers, sore throat, generalized weakness and fatigue and alopecia. Myelosuppression occurs promptly following TBI and doses of 1000cGy or more are assumed to cause permanent bone marrow aplasia and would be lethal without BMT.

Intermediate side effects (1-4 months): Interstitial pneumonia can be seen with a fatal incidence of up to 5-10%. Other side effects may include graft versus host disease and infection from prolonged immunoincompetence.

Late effects (>4months): An increased risk of sterility and cataracts is known; the risk of developing a second malignancy may be increased. Additional complications for long term survivors may include cardiac, pulmonary, liver, and kidney damage as well as hearing loss. Changes in hormone levels may also occur.

7.4 Mesna (sodium-2-mercapto ethane sulphonate)

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

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

7.5 Tacrolimus (FK-506, Prograf®)

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

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

Drug interactions: Tacrolimus is well absorbed orally. Tacrolimus is extensively metabolized by the cytochrome P-450 (CYP3A4) system and metabolized products are excreted in the urine.

Drugs that may increase tacrolimus levels include tri-azole drugs (especially voriconazole and posaconazole), nephrotoxic drugs, calcium channel blockers, cimetidine and omeprazole, metoclopramide, macrolide antibiotics, quinupristin/dalfopristin, danazol, ethinyl estradiol, methylprednisolone, and HIV protease inhibitors. Drugs that may decrease tacrolimus levels include some anticonvulsants (phenobarbital, phenytoin, carbamezepine), caspofungin, rifamycins, and St. John's Wort.

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

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

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

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

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

7.6 Mycophenolate Mofetil (MMF, Cellcept®)

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

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

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

8.0 STUDY PARAMETERS

8.1 Hematologic parameters

8.11 Neutrophil recovery: The cumulative incidence of neutrophil engraftment from the time of transplant will be estimated using the cumulative incidence function with death and relapse prior to engraftment as the competing risk. The definition of neutrophil engraftment is a post-nadir ANC > 500/mm3 for three consecutive measurements on different days. The first of the three days will be designated as the day of neutrophil recovery.

8.12 Platelet recovery: The cumulative incidence of platelet engraftment from the time of transplant will be estimated using the cumulative incidence function with death and relapse prior to engraftment as the competing risk. The definition of platelet engraftment is sustained platelet count > 20,000/mm^3 and > 50,000/mm^3 with no platelet transfusions in the preceding seven days. The first of three consecutive measurements on different days will be designated as the day of initial platelet recovery.

8.13 Donor chimerism: Mixed donor chimerism is defined as > 5%, but < 95%, donor. Full donor chimerism is defined as > 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.

It is recommended (not required) that chimerism determinations from T cells (CD3+ sorted) and whole blood (total nucleated cells) will be made from peripheral blood per Section 6.2, 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, (v) other institutional standards. Chimerism should also be determined from the bone marrow at required timepoints.

8.14 Graft failure: < 5% donor chimerism in blood and/or bone marrow by ~Day 60 and on all subsequent measurements. This time point was chosen based on the median and range of donor engraftment seen on the Hopkins and other single institutional published studies of myeloablative haploBMT with PT/Cy.

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.

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

Patients who have relapsed or died prior to day 60 will not be evaluable for full donor chimerism, as these are competing risk factors.

8.2 Graft-versus-host Disease

8.21 Acute GVHD: The cumulative incidence of acute GVHD Grades 2-4 and 3-4 will be assessed according to the BMT-CTN Manual of Procedures (MOP): https://web.emmes.com/study/bmt2/public/MOP/BMTCTNTechnicalMOPv3.pdf. Standard assessment criteria is also listed in Appendix 4.(Appendix 4). It is strongly recommended, when possible (not required), that biopsies be taken for histological confirmation of GVHD of an affected organ (e.g., skin, liver, or gastrointestinal tract). Date of symptom onset, date of biopsy confirmation of GVHD, maximum clinical grade, sites affected, and dates and types of treatment will be recorded. Dates of symptom onset of grade II or higher GVHD and grade III-IV GVHD will be recorded.

The cumulative incidences of acute grade II-IV and grade III-IV GVHD will be determined through competing risk analysis. Relapse/progression, graft failure, or death without aGVHD are considered competing risks for aGVHD. . In addition, aGVHD will be reported with only graft failure and death regarded as competing risks.

Recommended primary systemic treatment for acute GVHD will be corticosteroids. However, initiation of calcineurin inhibitors or other systemic immunosuppressants will be per discretion of the treating physician. All efforts should be made to record the systemic immunosuppressants used, timing of their administration and duration of treatment administered beyond the originally planned prophylaxis regimen We will characterize the duration, number, and type of steroid and non-steroid immunosuppressants used to treat aGVHD.

8.22 Chronic GVHD: The cumulative incidence and severity of chronic GVHD will be assessed according to the BMT-CTN Manual of Procedures (MOP): https://web.emmes.com/study/bmt2/public/MOP/BMTCTNTechnicalMOPv3.pdf using the NIH consensus criteria.[51] Date of onset, date of biopsy confirmation (if any), dates and types of treatment, and extent will be recorded. The cumulative incidence of chronic GVHD (overall and according to extent) will be determined through competing risk analysis. Relapse/progression, graft failure, or death without cGVHD are considered competing risks for cGVHD. The cumulative incidence of cGVHD will be described at 6 months and 1 year post transplant.

Use of systemic immunosuppressive therapy for treatment of chronic GVHD is at the discretion of the treating physicians. The event of interest is the development of any chronic GVHD severe enough to warrant systemic therapy, which includes corticosteroids (prednisone dose ≥0.5mg/kg/day or equivalent), any systemic immunosuppressive agent or extracorporeal photopheresis. Use of topical immunosuppressive agents is not necessary for triggering this endpoint. Patients who continue on immunosuppressive therapy beyond day 180 due to manifestation of chronic GVHD will also be considered an event for the primary endpoint.

We will characterize the duration, number, and type of steroid and non-steroid immunosuppressants used to treat cGVHD.

8.3 Disease and survival endpoints

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

8.3.2 Event-free survival: Interval from Day 0 to date of first objective disease progression or relapse, an unplanned therapeutic maneuver for disease persistence, death from any cause, or last patient evaluation. Patients without events will be censored at the last date they were assessed and deemed event-free. EFS will be estimated using the Kaplan-Meier method and EFS at one and two years after transplantation will be estimated along with 90% confidence intervals.

8.3.3 Disease-Free Survival (DFS): Disease free survival is the time from date of transplant to death or relapse/progression, whichever comes first. The event for this endpoint is relapse/progression or death. Patients alive and disease free will be censored at last follow-up

8.3.4 Overall survival: Interval from Day 0 to date of death from any cause or last patient contact. Overall Survival (OS) will be estimated using the Kaplan-Meier method. OS at one and two years after transplantation will be estimated along with 90% confidence intervals.

8.3.5 GVHD/relapse or progression-free survival (GRFS): An event for this time to event outcome is defined as grade III-IV acute GVHD, chronic GVHD requiring systemic immunosuppressive treatment, disease relapse or progression, or death by any cause. Patients will be followed up for at least one year for this endpoint.

8.3.6 Nonrelapse mortality: The cumulative incidence of death without evidence of disease progression or relapse will be characterized at days 100, 180, and 1 year post-transplant. An event for this endpoint is death without evidence of disease progression or recurrence Relapse/progression is a competing risk for NRM.

8.3.7 Relapse or progression: Relapse is defined by either morphological or cytogenetic evidence of acute leukemia or MDS consistent with pretransplant features. High clinical suspicion of relapse will most likely lead to a disease-specific evaluation, for example a bone marrow aspirate for patients with leukemia or myelodysplasia. Cytogenetic studies or decreasing donor chimerism also increase the suspicion of relapse as well and can sometimes detect asymptomatic relapse in routine protocol-related marrow samples. If there is evidence of early relapse and no evidence of GVHD following BMT, immunosuppressive therapy may be discontinued earlier than indicated in Section 4.6, after discussion with PI. If there is evidence of donor chimerism and no evidence of GVHD, patients may be eligible for subsequent donor lymphocyte infusions.

Acute leukemia and MDS – Relapse will be diagnosed when there is:

- Reappearance of leukemia blast cells in the peripheral blood; or,
- >5% blasts in the bone marrow, not attributable to another cause (e.g. bone marrow regeneration)
- The appearance of previous or new dysplastic changes (MDS specific) within the bone marrow with or without falling donor chimerism; or
- The development of extramedullary leukemia or leukemic cells in the cerebral spinal fluid or
- The reappearance of cytogenetic abnormalities present prior to transplantation

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

Institution of any therapy to treat persistent, progressive or relapsed disease, including the withdrawal of immunosuppressive therapy or donor lymphocyte infusion, will be considered evidence of relapse/progression regardless of whether the criteria described above were met.

8.3.8 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.

8.3.9 Primary cause of death. Primary cause of death will be classified as follows:

- **Relapse/Primary disease:** If the patient relapsed/progressed after day 0 prior to death, the primary cause of death is relapse/progression, even if they subsequently developed GVHD, organ toxicities or infections that may have contributed to subsequent death.
- **GVHD:** Death from acute or chronic GVHD, in the absence of relapse or AML/MDS disease progression.
- **Infection:** Death from documented viral, bacterial or fungal infections in the absence of GVHD or relapse/disease progression.
- **Organ toxicity:** Death from major organ toxicities not attributable to AML/MDS, infection or GVHD.
- **Other:** Any other causes of death than those listed above.

9.0 STATISTICAL CONSIDERATIONS

Overall Study Design

This study is a single-arm Phase II trial with the primary objective being to assess nonrelapse mortality at 180 days (NRM_{180}) of a myeloablative preparative regimen and post transplantation cyclophosphamide with a partially HLA-mismatched donor in patients with hematologic

malignancies who have not had a bone marrow transplant before (cohort 1). NRM_{180} is defined from transplant date to date of death without evidence of disease progression or relapse at 180 days. Patients who relapse or progress will be counted as competing risks events. Patients with acute lymphoblastic leukemia will receive a Cy/TBI preparative regimen. All other patients will receive a busulfan/cytoxan preparative regimen. In the Johns Hopkins Hospital Phase 2 single institutional study (n=85), there was no difference in transplant toxicities including engraftment, NRM, or GVHD in patients treated with Cy/TBI versus Bu/Cy therefore all patients will be analyzed together.

We will enroll a second cohort of patients who have relapsed after bone marrow transplant and will be receiving this regimen as their second or greater transplant. Given these patients are expected to have a significantly higher NRM based on reports in the literature, their NRM will be descriptive only.

9.1 Accrual

We plan to accrue 31 patients over approximately one-two years in cohort 1. Cohort 2 (descriptive only) expects to accrue 5-7 patients. Subjects will be followed for the primary endpoint of NRM_{180}, which will be described at 180 days.

9.2 Sample Size Considerations

Cohort 1 will accrue all eligible patients who are receiving their first bone marrow transplant. Previous literature using haploidentical bone marrow transplant without post-transplant Cy suggests that NRM_{180} is 35% or higher in this patient population. Literature using post-transplant Cy in the myeloablative setting suggests a NRM of 6-20%. At our institution, NRM is around 13%. In this multicenter study, we are interested in showing that NRM_{180} will be no higher than 17%. NRM will be estimated through a cumulative incidence function, but for sample size considerations we performed a calculation using an exact binomial test to determine what difference we could detect with our projected sample size. A sample size of 28 patients who meet the inclusion criteria of cohort 1 and a one-sided type 1 error rate of 10%, would yield 80% power to test if NRM is significantly improved from 35% (historical rates of TRM in haploBMT without PT/Cy as in background section) to 17%, based on an exact binomial test. Because we expect approximately 10% of patients to experience a competing event (relapse), and these patients will not contribute in the risk set of NRM, thus we are inflating the sample size to 31.

Due to the small number of patients expected to enroll on this protocol who have already relapsed after their first BMT, we are not providing formal sample size estimates for cohort 2. We anticipate enrolling 5-7 patients who meet the inclusion criteria of cohort 2, and will examine their outcomes in an exploratory manner. As described in the background section, NRM for these patients has been 25-75%. We would like to see if this regimen seems feasible for those in need of a second BMT such that it would be explored further on a future clinical trial

9.3 Analysis of Primary Endpoint

Cumulative incidence of NRM will be estimated using Gray's method [50] with disease relapse or progression considered as competing events. The cumulative incidence of NRM for cohort 2

will be estimated using the same approach, though we expect the confidence intervals to be fairly wide. Cumulative incidence of NRM at 100 days, 180 days and one year will be calculated and reported with 90% confidence intervals for each cohort.

9.4 Analysis of Secondary Endpoints

Secondary endpoints such as donor engraftment, platelet and neutrophil engraftment, aGVHD, cGVHD, survival endpoints, and relapse will be analyzed, as per section 8.1.

9.4.1 Additional hematologic and non-hematologic toxicities: These will be recorded using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 at regular intervals as defined in the patient monitoring section (section 5.0). Toxicities will be tabulated by type and appropriate confidence intervals will be estimated.

9.4.2 Statistical Methodology: When appropriate, we will apply Gray's method[50] to examine differences in cumulative incidence functions with respect to the two cohorts of patients (BMT #1 vs. BMT $\geq$1) and also by regimen within cohort 1. The differences in overall survival and event-free survival will be explored as well using standard methods. Thus, we will report the cumulative incidence of NRM at 100 days, 180 days and one year with respect to patients based on received regimens. In addition, we will compare these groups with respect to overall survival and event free survival in an exploratory manner using standard methods.

9.5 Stopping Rules

Because patients with severe GVHD and engraftment failures who die will be captured in NRM, we will apply a stopping rule based on incidence of NRM. As outlined in Section 2.0 (Background), the overall NRM on the mini-haplo BMT BMT-CTN trial was 7%. Our institutional experience with myeloablative haploBMT has shown an overall NRM of 15% and a pediatric NRM of 6%. Other groups using PT/Cy after myeloablative haploBMT have shown NRM's in the range of 10-15%. Other haploidentical BMT trials without PT/Cy, both nonmyeloablative and ablative have shown NRM in the range of 30-40%, mostly from infectious causes. The working hypothesis of this trial is that the overall toxicity of fully ablative haploBMT in a national, multicenter trial is not significantly greater than the single institution experience with haploidentical BMT after myeloablative conditioning utilizing post-transplantation Cy, and less then what has been seen with other nonmyeloablative and ablative haploidentical BMT trials that do not utilize post-transplantation Cy. NRM will capture patients that have transplant related mortality including, but not limited to death from severe GVHD or engraftment failure.

Both cohorts will be monitored separately based on the observed NRM rate by day 100 (NRM_{100}). Within cohort 1, because we do not know how many patients will be accrued to each regimen at any one time and the NRM rate will be expected to be similar, we will separately monitor the NRM results of patients treated with Cy/TBI versus Bu/Cy but with the same stopping rule. We will monitor for NRM_{100} after every 2 patients enrolled at each treated regimen in cohort 1, and every patient in cohort 2. The stopping rule for NRM_{100} will hold enrollment if the posterior probability of NRM_{100} exceeding 25% within treated regimen in cohort 1 or 50% in cohort 2 is 0.75 or higher. The prior for this monitoring rule is a beta(1,3) for each regimen in cohort 1, and a beta(3,3) for cohort 2. This means that our prior guess at the proportion of NRM_{100} is 25% for each regimen in cohort 1 and 50% for cohort 2, and there is a

90% probability that these proportions are between 1.7-63.2% for each regimen in cohort 1 and 18.9-81.1% for cohort 2. In cohort 1, if both regimens trigger a review, then consideration would be given to stopping the trial; otherwise if only one regimen triggers a review, then accrual to this regimen will be temporarily halted and consideration will be given to trial modification. We expect approximately 1/3 of patients to be treated with Cy/TBI and the remaining 2/3 to be treated with Bu/Cy, but we have included stopping rules up to the full sample size for completeness in Table 1 for the stopping rules. Table 2 shows the operating characteristics based 5000 simulations. The probability of stopping in cohort 1 was estimated from the simulation results in two regimens, assuming the independent NRM_{100} event between two regimens, the same true toxicity rate in two regimens' groups, and under 10 and 21 patients in Cy/TBI amd Bu/Cy, respectively.

Table 1. Stopping rules for NRM_{100} for each cohort

Cohort 1			Cohort 2		
N patients who die due to transplant	Out of N patients	Probability of high NRM_{100}*	N patients who die due to transplant	Out of N patients	Probability of high NRM_{100}*
2	2	0.90	2	2	0.77
2	4	0.76	3	3	0.86
3	6	0.83	4	4	0.91
4	8	0.89	4	5	0.83
4	10	0.79	5	6	0.89
5	12	0.85	5	7	0.81
5	14	0.77			
6	16	0.83			
7	18	0.87			
7	20	0.80			
8	22	0.85			
8	24	0.79			
9	26	0.83			
9	28	0.77			
10	31	0.79			

* The probability of high NRM_{100} is the posterior probability of NRM_{100} being larger than 25% for Cohort 1 and 50% for Cohort 2 exceed 0.75

Table 2. The operating characteristics of the stopping rule are shown below and are based on 5000 simulations.

Cohort 1					**Cohort 2**			
Cy/TBI (assume n=10)			**Bu/Cy (assume n=21)**					
True NRM rate	**Probability of stopping**	**Average sample size**	**Probability of stopping**	**Average sample size**	**Probability of Stopping Cohort 1**	**True NRM rate**	**Probability of stopping**	**Average sample size**
10%	0.06	10	0.06	19	0.004	30%	0.11	7

15%	0.13	9	0.15	18	0.02	35%	0.15	6
20%	0.23	9	0.28	16	0.06	40%	0.22	6
25%	0.36	8	0.45	14	0.16	45%	0.29	6
30%	0.48	8	0.61	12	0.29	50%	0.37	6
35%	0.60	7	0.75	10	0.45	55%	0.45	5
40%	0.72	6	0.86	8	0.62	60%	0.56	5
						65%	0.65	5
						70%	0.74	4

10.0 INFORMED CONSENT/ASSENT

Patients eligible for marrow grafting are completely evaluated and presented at group conference. The group's recommendations are discussed with the patient. If the patient is approved for BMT, the marrow processing procedure itself, the risks of the preparative regimen, risks of BMT complications including infection and GVHD and alternate forms of therapy are presented as objectively as possible. For pediatric patients (<18 yr of age) assent is obtained from the patient and informed consent is obtained from all parents. Informed consent is obtained from the recipient using the forms approved by the JCCI IRB.

The requirement and process for assent will be at the discretion of the IRB at each site.

10.1 On-study date**:**
Date of consent signing.

10.2 Off-study date:
Upon completion of "Day 365" evaluations, patients have completed their treatment except for patient follow-up beyond day 365 which will consist of collecting information regarding ongoing engraftment, disease status, late effects of this protocol, acute and chronic graft vs.- host disease, immune reconstitution, additional therapies, and survival as per standard BMT long-term follow-up. Patients will go off study early in the event of:

1. Death
2. Disease progression and/or graft failure prior to day 100. In the event that a patient comes off study prior to day 60-100 due to disease progression, efforts will be made to obtain blood and bone marrow at the time of study discontinuation to assess chimerism, and to assess for the presence of GVHD. We will continue to update survival information on these patients.
3. Patient decision (or decision by a parent or guardian on behalf of a minor)
4. Unacceptable toxicity associated with protocol therapy, as determined by the treating physicians in consultation with the investigators.

APPENDIX 1

ECOG PERFORMANCE STATUS SCALE GRADE DESCRIPTION

0	Fully active, able to carry on all pre-disease activities without restriction.
1	Restricted in physically strenuous activities and able to carry out work of a light or sedentary nature, e.g. light housework, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

LANSKY PERFORMANCE STATUS SCALE

- 100 - fully active, normal
- 90 - minor restrictions in strenuous physical activity
- 80 - active, but tired more quickly
- 70 - greater restriction of play *and* less time spent in play activity
- 60 - up and around, but active play minimal; keeps busy by being involved in quieter activities
- 50 - lying around much of the day, but gets dressed; no active playing participates in all quiet play and activities
- 40 - mainly in bed; participates in quiet activities
- 30 - bedbound; needing assistance even for quiet play
- 20 - sleeping often; play entirely limited to very passive activities
- 10 - doesn't play; does not get out of bed
- 0 - unresponsive

KARNOFSKY PERFORMANCE STATUS SCALE

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

APPENDIX 2

NCI COMMON TOXICITY CRITERIA

The NCI common toxicity criteria can be accessed and downloaded via the website:
http://ctep.cancer.gov/reporting

APPENDIX 3

aGVHD STAGING

STAGE	Skin	GI	Liver
1	< 25% rash	Diarrhea 500-1000 mL/day or 280-555 mL/m^2 or persistent nausea	Bilirubin 2 - 3 mg/dl
2	25-50 %	Diarrhea 1000-1499 ml/day or 556-833 mL/m^2	Bilirubin 3 - 6 mg/dl
3	> 50 %	Diarrhea > 1500 ml/day or > 833 mL/m^2	Bilirubin 6 - 15 mg/dl
4	Generalized erythroderma with bullae	Large volume diarrhea and severe abdominal pain ± ileus	Bilirubin > 15 mg/dl

* For children with BSA < 1.5 m^2, diarrhea volume should be recorded using mL/m^2 scale.

For skin GVHD:
Use "Rule of Nines" or burn chart to determine extent of rash.

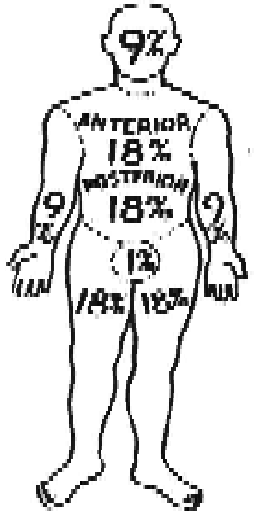
For liver GVHD:
Range of bilirubin given as total bilirubin. Downgrade one stage if an additional cause of hyperbilirubinemia is documented.

For gut GVHD:
Downgrade one stage if an additional cause of diarrhea is documented. Stage 1 is persistent nausea, vomiting and anorexia in the absence of other known cause unless histology is negative.

aGVHD GRADING

GRADE	Skin	Liver	Gut
0	None	None	None
I	Stage 1-2	None	None
II	Stage 3 and/or	Stage 1 and/or	Stage 1
III	None or Stage 3	Stage 2-3 or	Stage 2-4
IV	Stage 4 or	Stage 4	NA

Criteria for grading given as minimum degree of organ involvement required to confer that grade.



APPENDIX 4: NIH Consensus Scoring/Evaluation Forms for Chronic GVHD

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features ☐ Pruritus ☐ Hair involvement ☐ Nail involvement **% BSA involved** ☐	☐ No Symptoms	☐ <18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
EYES Mean tear test (mm): ☐ >10 ☐ 6-10 ☐ ≤5 ☐ Not done	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
GI TRACT	☐ No symptoms	☐ Symptoms such as dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%)	☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	☐ Bilirubin or enzymes > 5 x ULN

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
LUNGS†	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring 0_2)
FEV1 ☐				
DLCO ☐	☐ FEV1 > 80% **OR** LFS=2	☐ FEV1 60-79% **OR** LFS 3-5	☐ FEV1 40-59% **OR** LFS 6-9	☐ FEV1 ≤39% **OR** LFS 10-12
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
GENITAL TRACT	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

Other indicators, clinical manifestations or complications related to chronic GVHD (check all that apply and assign a score to its severity (0-3) based on its functional impact where applicable (none – 0,mild -1, moderate -2, severe – 3)

Esophageal stricture or web___	Pericardial Effusion___	Pleural Effusion(s)___
Ascites (serositis)___	Nephrotic syndrome___	Peripheral Neuropathy___
M yasthenia Gravis___	Cardiomyopathy___	Eosinophilia > 500µl___
Polymyositis___	Cardiac conduction defects___	Coronary artery involvement___
Platelets <100,000/µl ___	Progressive onset___	

OTHERS: Specify:_____

Organ scoring of chronic GVHD. *AP may be elevated in growing children, and not reflective of liver dysfunction. †Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptom or PFT scores the higher value should be used for final scoring. Scoring using the Lung Function Score (LFS) is preferred, but if DLCO is not available, grading using FEV1 should be used. The LFS is a global assessment of lung function after the diagnosis of bronchiolitis obliterans has already been established [29]. The percent predicted FEV1 and DLCO (adjusted for hematocrit but not alveolar volume) should be converted to a numeric score as follows: >80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; <40% = 6. The LFS = FEV1 score + DLCO score, with a possible range of 2-12.

GVHD indicates graft versus host disease; ECOG, Eastern Cooperative Oncology Group; KPS, Karnofsky Performance Status; LPS, Lansky Performance Status; BSA, body surface area; ADL, activities of daily living; LFTs, liver function tests; AP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal.

Mild chronic GVHD involves only 1 or 2 organs or sites (except the lung: see below), with no clinically significant functional impairment (maximum of score 1 in all affected organs or sites). Moderate chronic GVHD involves (1) at least 1 organ or site with clinically significant but no major disability (maximum score of 2 in any affected organ or site) or (2) 3 or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). A lung score of 1 will also be considered moderate chronic GVHD. Severe chronic GVHD indicates major disability caused by chronic GVHD (score of 3 in any organ or site). A lung score of 2 or greater will also be considered severe chronic GVHD.

Appendix 5:

Nomenclature for ABO Mismatching Observed and Theoretical Adverse Outcomes in Allogeneic BMT Reported in Previous Studies.

ABO Mismatch	Donor	Recipient	Know and Postulated Consequences
Minor	**O**	**A, B or AB**	**Recipient hemolysis**
	A, B	**AB**	**Reports of increased GVHD**
Major	**A, B or AB**	**O**	**Post transplantation pure red blood cell aplasia**
	AB	**A, B**	**Reports of impaired engraftment and increased GVHD**
Bidirectional	**A**	**B**	**Recipient hemolysis and red blood cell aplasia**
	B	**A**	**Reports of reduced overall survival**
			Reports of impaired engraftment and increased GVHD

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