BLOOD AND MARROW TRANSPLANT CLINICAL TRIALS NETWORK

A Randomized, Multicenter, Phase III Trial of Tacrolimus/Methotrexate versus Post-Transplant Cyclophosphamide/Tacrolimus/Mycophenolate Mofetil in Non-Myeloablative/Reduced Intensity Conditioning Allogeneic Peripheral Blood Stem Cell Transplantation

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Companion Study: Microbiome and Immune Reconstitution in Cellular Therapies and Hematopoietic Stem Cell Transplantation (Mi-Immune)

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BLOOD AND MARROW
TRANSPLANT
CLINICAL TRIALS NETWORK

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PROTOCOL SYNOPSIS

A Randomized, Multicenter Phase III Trial of Tacrolimus/Methotrexate versus Post-Transplant Cyclophosphamide/Tacrolimus/Mycophenolate Mofetil in Non-Myeloablative / Reduced Intensity Conditioning Allogeneic Peripheral Blood Stem Cell Transplantation

Co-Principal Investigators: Javier Bolaños-Meade, MD and Shernan Holtan, MD

Study Design: The study is designed as a randomized, phase III, multicenter trial comparing two acute graft-versus-host disease (aGVHD) prophylaxis regimens: tacrolimus / methotrexate (Tac/MTX) versus post-transplant cyclophosphamide / tacrolimus / mycophenolate mofetil (PTCy/Tac/MMF) in the setting of reduced intensity conditioning (RIC) allogeneic peripheral blood stem cell (PBSC) transplantation.

Primary Objective: The primary objective of the trial is to compare 1 year GVHD-free, relapse-free survival (GRFS) between the two GVHD prophylaxis regimens. An event for this time—to-event outcome is defined as grade III-IV aGVHD, chronic GVHD requiring systemic immune suppression, disease relapse or progression, or death by any cause.

Secondary Objective: Secondary objectives are to describe for each treatment arm rates of grade II-IV and III-IV aGVHD, rates of Minnesota high risk aGVHD, chronic GVHD, immunosuppression-free survival at 1 year, hematologic recovery (neutrophil and platelet), donor cell engraftment, disease relapse or progression, transplant-related mortality, rates of grade 3+ toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, incidence of grade 2-3 infections, immune reconstitution, and overall survival.

Eligibility Criteria:

Eligible patients are at least 18.0 years of age undergoing allogeneic PBSC transplant for the treatment of acute leukemia and chronic myelogenous leukemia with no circulating blasts and less than 5% blasts in the bone marrow; or myelodysplasia/chronic myelomonocytic leukemia with no circulating blasts and less than 10% blasts in the bone marrow; chronic lymphocytic leukemia/small lymphocytic lymphoma, follicular lymphoma, Hodgkin lymphoma, diffuse large B cell lymphoma, mantle cell lymphoma, peripheral T-cell lymphoma,

angioimmunoblastic T-cell lymphoma and anaplastic large cell lymphoma sensitive to chemotherapy who are eligible for an allogeneic hematopoietic cell transplantation (HCT). Patients are eligible only if receiving a RIC regimen.

Patients must have a related or unrelated PBSC donor. Sibling donor must be a 6/6 match for HLA-A and HLA-B at intermediate or higher resolution, and DRB1 at high resolution using DNA-based typing; must be willing to donate peripheral blood stem cells; and meet institutional criteria for donation. Unrelated donor must be a 7/8 or 8/8 match at HLA-A, -B, -C, and -DRB1 at high resolution using DNA-based typing; must be willing to donate PBSCs; and be medically eligible to donate stem cells according to NMDP criteria.

Treatment Description: Patients will be randomized to receive one of 2 specified GVHD prophylaxis regimens: Tac/MTX or PTCy/Tac/MMF. MTX will be dosed at 15 mg/m^2 Day +1, and 10 mg/m^2 Days +3, +6, and +11. PTCy will be dosed at 50 mg/kg on Days +3 and +4, followed by Tac/MMF. MMF will be dosed at 15 mg/kg every 8 hours from Day +5 to Day +35.

Accrual Objective: The clinical trial will enroll 428 patients, or 214 per arm.

Accrual Period: The estimated accrual period is 36 months.

Study Duration: Patients will be followed for 1 year post-PBSC transplant.

Interim Analysis: The study will include one interim analysis for efficacy, for the primary endpoint at the time when the required total number of events is reached. Z test statistic for comparing the two treatment arms will be compared to the critical values and results will be reviewed by the NHLBI-appointed Data and Safety Monitoring Board (DSMB). If the test statistic is outside the continuation range, the DSMB will be consulted on the discontinuation of the trial.

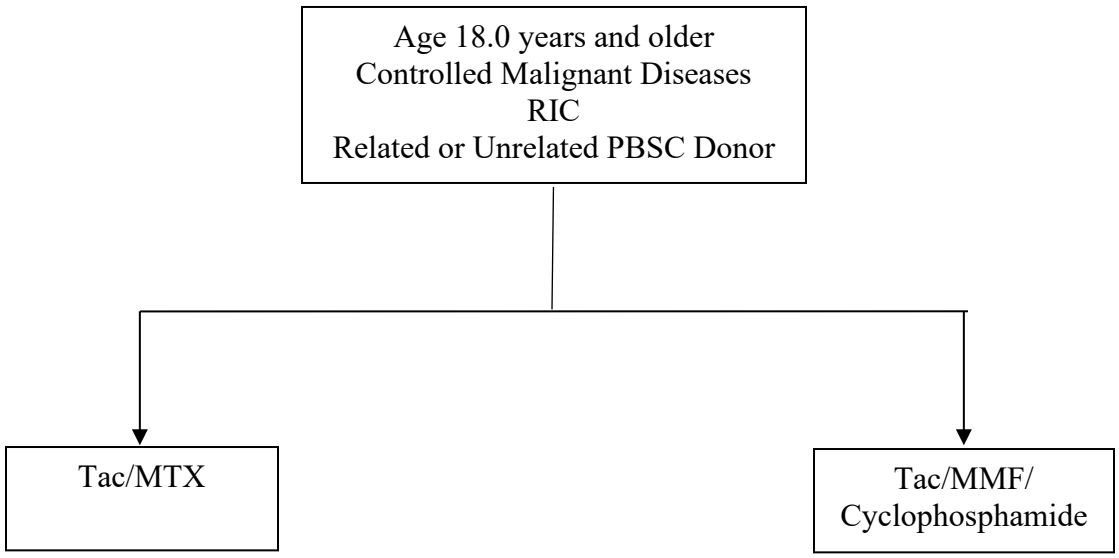
Stopping Guidelines: Monitoring of the key safety endpoint of death will be conducted. The rate of overall mortality will be monitored up to Day 100 post-randomization separately in each of the 2 treatment arms. Each month, the null hypothesis that the Day 100 mortality rate

	is less than or equal to 15% is tested using a truncated Sequential Probability Ratio Test (SPRT) for censored exponential data.
Correlative Studies:	Completed through the Mi-Immune study (Appendix J), with additional blood samples processed and stored for future research.
Companion Study:	**Microbiome and Immune Reconstitution in Cellular Therapies and Hematopoietic Stem Cell Transplantation (Mi-Immune)**
Co-Principal Investigator:	Leslie S. Kean MD, PhD, Miguel-Angel Perales, MD, and Ami Bhatt MD, PhD
Primary Objective:	The goal of this protocol is to test the primary hypothesis that the engraftment stool microbiome diversity predicts one-year non-relapse mortality in patients undergoing reduced intensity allogeneic HCT. Our study will be powered to test this hypothesis. Patients will be primarily recruited through co-enrollment on BMT CTN 1703.
Secondary Objectives:	To establish a cohort of biologic samples and a linked clinical and molecular dataset from patients and donors from BMT CTN 1703. The product of this study will be a shared biospecimen and data resource for conducting future allogeneic HCT mechanistic studies. To study the diversity of the immune repertoire post-transplant and correlate with clinical outcomes, as well as study the impact on immune recovery of the method of GVHD prophylaxis and other patient and transplant factors. To study the diversity and composition of the gut microbiome post-transplant and correlate with clinical outcomes, as well as study the impact on the microbiome of the method of GVHD prophylaxis and other patient and transplant factors.
Eligibility Criteria:	Eligible patients are at least 18 years of age and enrolled on the BMT CTN 1703 PROGRESS III study. Eligible donors are at least 18 years of age.
Treatment Description:	Conditioning regimens, GVHD prophylaxis, and other supportive care will be those described in BMT CTN 1703.
Accrual Objective:	A minimum of 300 patients will be enrolled (in collaboration with protocol BMT CTN 1703).

Accrual Period: The estimated accrual period is 36 months.

Study Duration: Patients will be followed clinically for 12 months post-HCT; long-term follow-up data will be collected through usual procedures of the Center for International Blood and Marrow Transplant Research (CIBMTR). A 24-month biological sample will be collected as well.

Outline of Treatment Plan



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CHAPTER 1

1 BACKGROUND AND RATIONALE

1.1 Introduction

Acute Graft-versus-Host-Disease (GVHD) is an important cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT). Clinically significant grade II-IV acute GVHD occurs in 34-40% of patients undergoing HLA-matched related donor HCT, 47-52% of HLA-matched unrelated donor HCT, and is further increased in those lacking HLA-matched donors.[1,2,3,4]

Acute GVHD is mediated by donor-derived T cells that are reactive against recipient antigens expressed in the context of the major histocompatibility complex (MHC or HLA). These antigens, termed minor histocompatibility antigens (mHA), are small peptides with immunogenic single nucleotide polymorphisms (SNPs) capable of eliciting potent T cell immune responses upon presentation by antigen presenting cells (APCs). A number of these mHAs have been identified.[5] In fact, mismatches of known mHA among HLA identical donor-recipient pairs have been associated with the development of acute GVHD after stem cell transplantation.[6]

Approximately 40% of patients with acute GVHD will have durable responses to corticosteroid therapy.[7] The prognosis of the 60% of patients without long-lasting response is poor.[8] A strategy that minimizes the incidence of acute GVHD, without other adverse effects, would be an effective approach to improve survival after allogeneic transplantation.

Acute GVHD incidence can be decreased with various pharmacologic agents. Early transplants were done using post-transplant methotrexate to prevent GVHD; in the 1980s cyclosporine was shown to be superior to methotrexate and in 1986 the combined use of cyclosporine and methotrexate was shown to be superior to single agent prophylaxis.[9] More recently, other calcineurin-inhibitors, such as tacrolimus have been developed as acute GVHD prophylactic agents due to favorable toxicity profiles in comparison with cyclosporine.[10,11]

Phase III trials comparing tacrolimus/methotrexate (Tac/MTX) versus cyclosporine/methotrexate for related and unrelated donors have been performed. In the unrelated donor setting, the incidence of grade II-IV acute GVHD was 56% among the 46 patients randomized to tacrolimus arm versus 74% among the 63 patients randomized to cyclosporine arm.[12] The combination of tacrolimus/methotrexate remains the standard for acute GVHD prophylaxis, despite its limited efficacy. Hence for many years, a calcineurin inhibitor-based doublet has been considered the standard of care for acute GVHD prophylaxis.

In order to test novel regimens incorporating agents promising in the phase II setting, BMT CTN 1203 was completed. This phase II multicenter trial randomly assigned patients (pts) 1:1:1 to Tac/MTX /maraviroc 300mg PO BID Day -3 to +30; Tac/MTX/bortezomib 1.3mg/m2 on Days +1, +4, and +7; or post-transplant cyclophosphamide (PTCy) 50mg/kg/day on Days +3 and +4 plus Tac/MMF(mycophenolate mofetil), each compared with a nonrandomized prospective

contemporaneous Tac/MTX treated control cohort. Pts aged 18-75 with hematologic malignancies eligible for RIC alloHCT were accrued. All pts received peripheral blood stem cell (PBSC) grafts from 6/6 HLA matched related or 7-8/8 HLA matched unrelated donors. Controls had the same eligibility, but enrollment was done at centers not participating in the trial.

In BMT CTN 1203, the regimen of PTCy/Tac/MMF was the most promising compared to controls (detailed in later paragraphs) and thus has been selected for a randomized phase III study in comparison with Tac/MTX in BMT CTN 1703.

1.2 Cyclophosphamide, Tacrolimus, and Mycophenolate Mofetil (PTCy/Tac/MMF)

1.2.1 Rationale of Post-transplant Cyclophosphamide

High dose cyclophosphamide is a potent immunosuppressor that has been successfully used to prevent GVHD in unrelated, HLA-matched sibling and haploidentical bone marrow/PBSC transplants in single center as well as in multicenter studies.[13,14,15,16,17,50] Cyclophosphamide administered early post HCT preferentially kills allo-reactive T cells while sparing resting, non allo-reactive T cells leading to suppression of GVHD as well as graft rejection.[18] The paragraphs that follow highlight the growing experience with PTCy as GVHD prophylaxis.

1.2.2 Post-transplant Cyclophosphamide in Alternative Donor Transplantation

Based on promising pre-clinical results at Johns Hopkins, a Phase I/II clinical trial of haploidentical BMT to treat high-risk hematologic malignancies was initiated in 1999. Following a non-myeloablative regimen of fludarabine, cyclophosphamide, and low-dose total body irradiation (TBI), GVHD prophylaxis consisted of cyclophosphamide (Cy) given on Days +3 and +4 post-transplant, tacrolimus, and mycophenolate mofetil (MMF).[15] Primary graft failure occurred in 13% of patients, and was fatal due to infection in one patient in whom autologous hematopoiesis failed to occur. In general, complete T-cell engraftment was observed by Day +28 or the grafts were rejected. Cumulative incidences of grades II-IV and grades III-IV acute GVHD by Day 200 were 34% and 6%, respectively. There was lower incidence of extensive chronic GVHD among recipients of two versus one dose of post-transplantation Cy (5% versus 25%; p=.05). There was no difference in the incidence of severe acute GVHD with one or two doses of post-transplant Cy. The cumulative incidences of non-relapse mortality and relapse at 1 year were 15% and 51%, respectively. Actuarial overall and event-free survivals (EFS) at two years after transplantation were 36% and 26%, respectively. Patients with lymphoid malignancies appeared to have an improved EFS compared to those with myeloid malignancies (p=.02).

The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) sponsored a multicenter Phase II trial of haploidentical BMT (BMT CTN 0603) for high-risk hematologic malignancies modeled after the Hopkins approach. This was published along with a similar study using cord grafts without post-transplant Cy (BMT CTN 0604).[13] The 1-year probabilities of overall and progression-free survival were 54% and 46% after cord transplantation and 62% and 48% after haploidentical bone marrow transplantation. The Day +56 cumulative incidence of neutrophil recovery was 94% after dUCB and 96% after haploidentical marrow. The 100-day cumulative

incidence of grade II-IV acute GVHD was 40% with cord blood and 32% with haploidentical bone marrow. The 1-year cumulative incidences of non-relapse mortality and relapse after cord transplantation were 24% and 31%, respectively; corresponding rates after haploidentical bone marrow transplantation were 7% and 45%.

1.2.3 Post-transplant Cyclophosphamide as GVHD Prophylaxis after PBSC transplantation

Post-transplant Cy as GVHD prophylaxis was developed initially for haploidentical BM transplantation after nonmyeloablative conditioning but, recently, several studies have extended the approach to PBSC transplantation. To date, haploidentical nonablative transplantation with post-transplant Cy has used bone marrow as the graft source. Use of PBSC instead of marrow may allow wider applicability of this approach but there is concern about higher risks of acute and chronic GVHD due to the 5-10-fold higher number of T-cells in the allograft. Groups in Houston and Seattle/London reported small studies in which PBSC were substituted for BM with post-transplant Cy in the haploidentical donor setting.[16,19] In both studies, the incidences of severe acute GVHD, chronic GVHD and non-relapse mortality at 1 year with PBSC were comparable to the rates seen with BM. A recent large series compared clinical outcomes in 481 patients receiving haploidentical bone marrow (BM) grafts versus 190 patients receiving haploidentical PBSC grafts. There were no significant differences in OS or NRM, but leukemia patients receiving BM had a lower risk of acute and chronic GVHD (HR = 0.45, $p<0.001$ and HR = 0.35, $p<0.001$ respectively), and patients with leukemia receiving BM had a higher risk of relapse (HR 1.73, $p=0.002$).[20]

PTCy has been extended beyond haploidentical donor grafts and into matched related/unrelated PBSC transplantation in recent reports. The Seattle group published experience with PTCy/cyclosporine/MMF as GVHD prophylaxis in the myeloablative transplantation setting, with previously tested regimen that showed excellent rates of engraftment (>97%), low relapse at 2 years (17%), low 2-year non-relapse mortality (14%), no grade III-IV acute GVHD, and low rates of chronic GVHD (16%).[21] Moiseev et al described similar outcomes in a prospective study, with <10% grade III-IV acute GVHD and non-relapse mortality <20%.[50] In BMT CTN 1203, a reduced intensity setting, PTCy/Tac/MMF showed the most favorable results compared to controls (details in 1.3). Thus, experience and interest in extending PTCy in to the matched PBSC transplantation setting continues to grow.

1.3 Benchmark Analysis and Composite Endpoint

In order to better evaluate the efficacy of novel approaches for GVHD prophylaxis, a benchmark analysis was performed using data from the Center for International Blood and Marrow Transplant Research (CIBMTR) for patients who received a RIC HCT.

The CIBMTR maintains an outcomes registry that prospectively collects data from all centers performing allogeneic HCTs and almost all centers performing autologous HCTs in the United States and about 100 non-US centers. Centers must report all consecutive patients and provide longitudinal follow-up on those patients according to set timelines that include a pre-transplant report, a 100-day report, a 6-month report and an annual report through 6 years post-transplant followed by a biannual report in perpetuity. Data are reported on two tracks: a "Transplant Essential Data" track and a "Comprehensive Report Form" track. Centers provide a pretransplant

Transplant Essential Data form for all patients. Data from this form are used to select patients for the Comprehensive Report Form track using a weighted random selection that over selects patients with rare diseases or procedures or for the purposes of specific studies. For example, most patients on BMT CTN trials are selected for the Comprehensive Report Form track so that data collected by the CIBMTR can supplement clinical trial data collected through AdvantageEDC or Advantage eClinical and can allow for long-term follow-up of trial patients for specific late effects of treatment. Longitudinal data are collected for patients on both the Transplant Essential Data and Comprehensive Report Form Track; the data differ in quantity and granularity. Data quality is ensured by computerized error checks and on-site audits.

The objective of the benchmark analysis was to select promising approaches to be further studied and to explore novel endpoints that could not only assess GVHD, but also the complex relationships between relapse and GVHD as well as prolonged use of immune suppression. The control population selected from the CIBMTR database was comprised of patients who received HCT in a US center from 2006 to 2009 and who received tacrolimus and methotrexate as their sole GVHD prophylaxis. Data from single institution studies of the two agents to be tested in this protocol were also studied. Populations differed according to disease, donor, conditioning intensity, disease risk and patient age. Each institutional cohort was compared with the CIBMTR controls, adjusting for differences in baseline populations using multivariate regression techniques. Table 1.3A summarizes the results of both univariate and multivariate analyses for the 2 arms proposed in BMT CTN 1703.

Table 1.3A: Univariate and Multivariate Results from the Benchmark Analysis

Outcome		**Tac+MTX Control** (95%CI)	**PTCy** (95%CI)
Grade III-IV Acute GVHD	HR	1.00	0.90 (0.58-1.4)
	Incidence at 6 mo (95%CI)	25% (23-26%)	23% (15-33%)
CGVHD	HR	1.00	0.24 (0.14-0.41)
	Incidence at 12 mo (95%CI)	45% (43-46%)	13% (7-20%)
Overall Survival	HR	1.00	1.07 (0.82-1.4)
	Probability at 12 mo (95%CI)	60% (58-61%)	57% (47-66%)
Disease free survival	HR	1.00	1.21 (0.94-1.56)
	Probability at 12 mo (95%CI)	52% (51-53%)	46% (37-55%)

HR=Hazard Ratio, CI=Confidence Interval. The event for overall survival was death and the event for disease free survival was death or relapse. A hazard ratio (HR) greater than 1 implies that a specific group has more events at any time compared to the Tac+MTX Control reference group (indicated by a HR of 1.00).

We remain interested in evaluating a composite endpoint that would better reflect reductions in either or both acute and chronic GVHD as well as the sometimes opposite effects of reducing GVHD on transplant-related mortality and relapse. In BMT CTN 1203, PTCy/Tac/MMF was the only study arm that showed an improved GVHD- Free-Relapse-Free Survival (GRFS) relative to controls. The study design provided an 81-87% power to identify a superior treatment if 1-year GRFS was 15% better than control. One-sided testing was used. 92 pts were assigned to PTCy/Tac/MMF; 89 to Tac/MTX/bortezomib; 92 to MTX/Tac/maraviroc; and 224 controls received Tac/MTX. For the primary 1-year GRFS endpoint, only the MMF/Tac/PTCy arm was superior to control (p 0.04, Table 1.3B). The cumulative incidence of grade II-IV aGvHD through Day 180 was not different for each arm vs. control; however, for grade III-IV aGvHD and GVHD-free survival, PTCy/Tac/MMF was superior to control (p 0.006, p 0.01 respectively). While there was no difference in overall cGvHD incidence, for cGVHD requiring SIS, the PTCy/Tac/MMF arm was superior to control (p 0.04). Treatment-related mortality at 1 year was 11% for PTCy/Tac/MMF vs 16% in controls and 17% in the other 2 arms, but was not statistically different. There were no differences in engraftment, relapse/progression, and overall survival.

Table 1.3B: Multivariate Outcomes

s		Controls	Bortezomib	Maraviroc	PTCy
GRFS	HR (90% CI)	1	0.82 (0.62-1.08)	1.02 (0.79-1.32)	0.72 (0.54-0.94)
	P		0.2407	0.8689	0.0467
	Adjusted 1 yr KM estimates	0.32 (0.26-0.39)	0.42 (0.33-0.54)	0.32 (0.23-0.43)	0.46 (0.37-0.57)
aGvHD II-IV	HR (90% CI)	1	0.80 (0.53-1.20)	0.98 (0.68-1.43)	0.83 (0.56-1.22)
	P		0.3694	0.9578	0.4334
	CuI at 180 days (90% CI)	31 (26-36)%	26 (19-34)%	32 (24-40)%	27 (20-35)%
aGvHD III-IV	HR (90% CI)	1	0.51 (0.25-1.04)	0.58 (0.29-1.15)	0.13 (0.03-0.44)
	P		0.1204	0.1945	0.0066
	CuI at 180 days (90% CI)	13 (9-16)%	8 (4-13)%	9 (4-14)%	2 (0-5)%
cGvHD	HR (90% CI)	1	1.07 (0.76-1.51)	1.23 (0.90-1.70)	0.74 (0.51-1.08)
	P		0.7352	0.2717	0.2029
	CuI at 1 yr (90% CI)	38 (32-43)%	39 (30-47)%	44 (35-53)	37 (20-36)%
cGvHD requiring immunosuppression	HR (90% CI)	1	0.70 (0.45-1.07)	0.85 (0.57-1.26)	0.57 (0.36-0.89)
	P		0.1749	0.5063	0.0413
	CuI at 1 yr (90% CI)	32 (27-37)%	22 (16-30)%	27 (20-35)%	19 (12-26)%
Relapse/progression	HR (90% CI)	1	0.94 (0.60-1.46)	1.209 (0.80-1.81)	1.24 (0.84-1.83)
	P		0.8188	0.4415	0.3458
	CuI at 1 yr (90% CI)	25 (20-30)%	22 (15-30)%	28 (20-36)%	27 (20-35)%
Treatment related mortality	HR (90% CI)	1	1.06 (0.63-1.78)	0.97 (0.57-1.65)	0.62 (0.34-1.11)
	P		0.8465	0.9327	0.1799
	CuI at 1 yr (90% CI)	16 (12-21)%	17 (11-24)%	17 (11-24)%	11 (6-17)%
Disease-free survival	HR (90% CI)	1	0.97 (0.70-1.35)	1.14 (0.83-1.57)	0.93 (0.68-1.29)
	P		0.9086	0.4685	0.7449
	1 yr KM estimates	59 (53-64)%	60 (52-69)%	56 (47-64)%	62 (53-70)%
GvHD free survival	HR (90% CI)	1	0.83 (0.62-1.11)	0.98 (0.75-1.28)	0.63 (0.46-0.85)

	P		0.3078	0.9130	0.0121
	Adjusted 1 yr estimates (90% CI)	0.39 (0.33-0.46)	0.46 (0.37-0.58)	0.40 (0.31-0.51)	0.56 (0.47-0.67)

1.4 Studies of the Microbiome and Immune Reconstitution in Transplant Patients

1.4.1 Microbiome Studies

The microbiome, consisting of a varied community of microbes (bacteria, viruses, fungi, microeukaryotes, and sometimes multicellular parasites), exists in niches across the human body. The skin, lung, nares, vagina, and gastrointestinal tract are among the most heavily colonized, with the largest number of microorganisms inhabiting the colonic lumen. Intestinal microbiota interact with and regulate host immunity.[26] While the majority of the over trillion organisms that live within a healthy human colon are nonpathogenic members of the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria, alterations in the balance of these microorganisms have been associated with adverse outcomes ranging from GVHD and infection to relapse post-HCT.[27] This clinical association between intestinal microorganisms and HCT outcomes has been investigated for decades – and has informed the still controversial practices of infection prophylaxis, gut decontamination, the "neutropenic diet," and isolation of patients in laminar air flow rooms. Single-institution studies have demonstrated that low microbial diversity in the stool after allogeneic HCT is associated with poor survival .[28,29] Additionally, specific alterations have been associated with increased risk of acute GVHD, infectious outcomes and most recently, relapse.[30-32] While these findings are compelling, most studies have been carried out in single institutions and thus the generalizability of these proposed microbial biomarkers is unclear. This is particularly important as there is known geographic variation of the intestinal microbiome and practice variability in antibiotic use for prophylaxis and treatment from institution to institution, in part due to different antibiograms.
Growing understanding of the positive roles of the microbiome in regulating immune maturation in developing humans, maintenance of host barriers against pathogens, and nutrition suggest that the health of the microbiome must be carefully considered in allogeneic HCT patients.

While a substantial investment was made by the NIH Common Fund in characterizing the "normal human microbiome" in the Human Microbiome Project (HMP) phase I and diseased human microbiome in the HMP phase II, no broad scale microbiome characterization effort has yet been made in the setting of cancer. Indeed, iHMP (or HMP2) prioritized multi-omic characterization of longitudinal samples from diseased individuals from three categories: pre-term birth, type 2 diabetes mellitus, and inflammatory bowel disease. As recently outlined in the NIH Emerging Themes workshop on microbiome research (NIH, Bethesda, MD; August 15 – 18, 2018), the field has recognized the strong importance of longitudinal sampling, high quality metadata collection, and simultaneous microbial and immunologic characterization.

Novel methods being pioneered and perfected in microbiome research are likely to facilitate translational breakthroughs – these methods allow (1) detailed taxonomic classification of microorganisms at the strain level, (2) metabolic characterization of the small molecules and proteins that a microbial community makes, (3) measurement of microbial genomic evolution in clinical time courses, and (4) culturing of previously fastidious organisms from the microbiome

for *in vitro* investigation and cultivation as potential therapeutic/adjuvant live bacterial clinical interventions. A well-curated, longitudinal biospecimen collection will facilitate validation of existing microbial biomarkers of disease and discovery of novel microbial biomarkers of disease that are generalizable. Particularly compelling is the fact that both preclinical and clinical studies support the role of the microbiome as a potentially modifiable biomarker of disease.[33] In fact, the microbiome has already been causally associated with opportunistic infections such as *C. difficile* colitis, in which fecal microbiota transplantation is highly safe and effective.[34] Furthermore, inflammatory diseases, such as IBD, are clearly modulated by the microbiome.[35]

Given recent reports suggesting that alterations in the microbiome can impact the efficacy of immunologic therapies, there is a clear imperative that the strong and growing link between the microbiome and cancer outcomes be investigated.[36,37] As the BMT CTN carries out a multitude of interventional clinical trials and has a strong track record for longitudinal sampling, high quality metadata collection, and immunologic characterization of patients post-HCT, the BMT CTN is ideally suited to lead the first cancer-associated effort in this space.

1.4.2 Protective Immunity

The reconstitution and regain of function of a donor-derived immune system is of utmost importance for the recovery and long-term survival of patients after allogeneic HCT. Dysfunctional immune reconstitution after transplant is associated with several transplant-related complications and adverse transplant outcome. However, although the phenomenology of the many defects in protective immunity (both against infectious pathogens and against leukemia relapse) is well-documented, the causative molecular mechanisms remain unknown.[38] To address these questions, several groups, including investigators at FHCRC, MSKCC, and Stanford, have begun to perform detailed assessments of immunologic reconstitution after allogeneic HCT including the application of new T Cell Receptor (TCR) and B Cell Receptor (BCR) deep-sequencing technologies[39-46] These technologies allow the investigation of the breadth and depth of post-transplant immune reconstitution at a level of molecular detail not previously possible and hold the promise of deepening our understanding of the impact of infectious pathogens on global immune health and immune reconstitution. The widespread application of these technologies, and their intersection with detailed assessment of immune phenotype and function can provide novel insights about the state of immune health in transplant patients, and holds the promise of identifying patients in need of novel interventions to improve their post-transplant immune reconstitution.

Accumulating evidence in recent years have indicated that the host microbiome has a tremendous influence on the generation and shaping of immune cell repertoire.[47] Additionally, the microbiota affects the immune response against cancer. For example, there are studies demonstrating the association between intestinal microbiota and relapse after HCT[32], or studies demonstrating that immune responses against cancer triggered by immune checkpoint inhibition are associated with the abundance of distinct members of intestinal microbiota.[48,49]

1.4.3 Microbiome and Immune Reconstitution in Cellular Therapies and Hematopoietic Stem Cell Transplantation (Mi-Immune)

Mi-Immune represents a companion study, where additional key precision medicine and systems biology questions will be explored. The goal of this companion study is several-fold: First, it is to test the primary hypothesis that the engraftment stool microbiome diversity (determined by 16s rRNA sequencing analysis of the sample closest after, but within 14 days of neutrophil engraftment) predicts 1-year non-relapse mortality in patients undergoing reduced-intensity allogeneic HCT. Second, it is to perform additional analyses on patient samples to answer key questions concerning: a) the impact of the stool microbiome on transplant outcome as well as the impact of transplant on the microbiome and the associated downstream impact on patient health; and b) reconstitution of the T cell repertoire after transplant. Third, it is to establish a cohort of biologic samples collected prospectively from patients treated on BMT CTN 1703 that will be a shared biospecimen resource for conducting future allogeneic HCT correlative studies. The resulting dataset will become an additional resource generated by the trial and will be made available to the biomedical community. Mi-Immune is designed to link molecular data and biospecimens with high quality clinical phenotype and outcomes data to identify risk factors for development and severity of key complications after allogeneic HCT.

1.5 Rationale for a Randomized Trial

This multicenter Phase III clinical trial will evaluate 2 acute GVHD prophylaxis approaches for their efficacies in improving the proportion of patients who do not develop severe acute GVHD, chronic GVHD that requires systemic therapy, disease progression, or relapse by one year post-transplant. Selection of the most promising therapy will be made based on the magnitude of difference in the primary endpoint.

CHAPTER 2

2 STUDY DESIGN

2.1 Study Overview

The BMT CTN 1703 study is a Phase III randomized, open label, multicenter trial comparing PTCy/Tac/MMF versus Tac/MTX for GVHD prophylaxis in patients with controlled malignant diseases receiving an allogeneic PBSC transplant after a RIC regimen. The primary endpoint is GRFS at 1 year.

The study will collect stool, urine, and blood biospecimens, as well as detailed clinical data on infections and antibiotic exposures in an attempt to understand the microbial and immune recovery predictors of HCT outcomes. Mi-Immune component of the study will form the correlative science arm of the BMT CTN 1703 study (PROGRESS III), which will compare two acute GVHD prophylaxis regimens: Tac/MTX versus PTCy/Tac/MMF in the setting of RIC allogeneic PBSC transplantation.

2.2 Hypothesis and Specific Objectives

2.2.1 Primary Hypothesis

BMT CTN 1703: At 1 year, PTCy/Tac/MMF GRFS will be 15% or greater compared to Tac/MTX GRFS.

Mi-Immune study: The engraftment stool microbiome diversity (determined within 14 days after neutrophil engraftment) predicts 1-year non-relapse mortality.

2.2.2 Study Objectives

BMT CTN 1703: The primary objective of the randomized trial is to compare 1-year GRFS after HCT between PTCy/Tac/MMF versus Tac/MTX. An event for this time to event outcome is defined as grade III-IV acute GVHD, chronic GVHD requiring systemic immune suppression, disease relapse or progression, or death by any cause. Secondary objectives are grade II-IV and III-IV acute GVHD per the NIH Consensus Conference Criteria on Acute GVHD Grading by Day +180, rates of Minnesota standard and high risk acute GVHD by Day +180, rates of NIH mild, moderate, and severe chronic GVHD (defined by the NIH Consensus Conference Criteria) at 1 year, systemic immunosuppression-free survival at 1 year, hematologic recovery including neutrophil engraftment (first day of ANC greater than or equal to 500 for 3 consecutive days), platelet engraftment (first day of sustained platelet greater than or equal to 20,000, or greater than or equal to 50,000 with no platelet transfusion in preceding 7 days), lymphocyte recovery (first day of sustained absolute lymphocyte count greater than or equal to 1000), proportion of patients with full (at least 95% or more) or mixed (5.0-94.9%) total donor chimerism or graft rejection (less than 5% total donor chimerism) at Day +100, disease relapse or progression at 1 year, non-relapse mortality at 1 year, incidence of biopsy-confirmed PTLD at 1 year, incidence of infections at 1 year, overall survival at 1 year, and patient-reported outcomes (PRO) at baseline, Day 100, Day 180, 1 year.

Mi-Immune study: The goal of this protocol is to test the primary hypothesis that the engraftment stool microbiome diversity (determined by 16s rRNA sequencing analysis of the sample closest after, but within 14 days of neutrophil engraftment) predicts 1-year non-relapse mortality in patients undergoing reduced-intensity allogeneic HCT. Mi-Immune secondary and exploratory objectives are addressed in Chapter 2 of Appendix J.

2.3 Patient Eligibility

2.3.1 Inclusion Criteria

1. Age 18.0 years or older at the time of enrollment on Segment A
2. Patients with acute leukemia or chronic myelogenous leukemia with no circulating blasts and with less than 5% blasts in the bone marrow
3. Patients with myelodysplasia/chronic myelomonocytic leukemia with no circulating blasts and with less than 10% blasts in the bone marrow (higher blast percentage allowed in MDS due to lack of differences in outcomes with <5% vs. 5-10% blasts in this disease)
4. Patients with relapsed chronic lymphocytic leukemia/small lymphocytic lymphoma with chemosensitive disease at time of transplantation
5. Patients with lymphoma [follicular lymphoma, Hodgkin lymphoma, diffuse large B cell lymphoma, mantle cell lymphoma, peripheral T-cell lymphoma, angioimmunoblastic T-cell lymphoma and anaplastic large cell lymphoma] with chemosensitive disease at the time of transplantation
6. Planned reduced intensity conditioning regimen (see eligible regimens in Table 2.4a)
7. Patients must have a related or unrelated peripheral blood stem cell donor as follows:
    a. Sibling donor must be a 6/6 match for HLA-A and -B at intermediate (or higher) resolution, and -DRB1 at high resolution using DNA-based typing and must be willing to donate peripheral blood stem cells and meet institutional criteria for donation.
    b. Unrelated donor must be a 7/8 or 8/8 match at HLA-A, -B, -C and –DRB1 at high resolution using DNA-based typing. Unrelated donor must be willing to donate peripheral blood stem cells and meet NMDP criteria for donation.
8. Cardiac function: Left ventricular ejection fraction at least 45%
9. Estimated creatinine clearance acceptable per institutional guidelines
10. Pulmonary function: DLCO corrected for hemoglobin at least 40% and FEV1 predicted at least 50%
11. Liver function acceptable per institutional guidelines
12. Karnofsky Performance Score at least 60%
13. Female patients (unless postmenopausal for at least 1 year before the screening visit, or surgically sterilized), agree to practice two (2) effective methods of contraception at the same time, or agree to completely abstain from heterosexual intercourse, from the time of signing the informed consent through 12 months post-transplant.

14. Male patients (even if surgically sterilized), of partners of women of childbearing potential must agree to one of the following: practice effective barrier contraception, or abstain from heterosexual intercourse from the time of signing the informed consent through 12 months post-transplant.
15. **Plans for the use of post-transplant maintenance therapy must be disclosed upon enrollment and must be used irrespective of the outcome of the randomization. Please note that THIS DOES NOT INCLUDE INVESTIGATIONAL AGENTS** and maintenance therapy with investigational treatment requires approval by the study chairs.
16. Voluntary written consent obtained prior to the performance of any study-related procedure that is not a part of standard medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care

2.3.2 Exclusion Criteria

1. Prior allogeneic transplant
2. Active CNS involvement by malignant cells
3. Patients with secondary acute myeloid leukemia arising from myeloproliferative disease, including CMML
4. Patients with uncontrolled bacterial, viral or fungal infections (currently taking medication and with progression or no clinical improvement) at time of enrollment.
5. Presence of clinically significant fluid collection (ascites, pleural or pericardial effusion) that interferes with methotrexate clearance or makes methotrexate use contraindicated
6. Patients seropositive for human immunodeficiency virus (HIV) with detectable viral load. HIV+ patients with an undetectable viral load on antiviral therapy are eligible.
7. Myocardial infarction within 6 months prior to enrollment or New York Heart Association (NYHA) Class III or IV heart failure, uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia.
8. Female patients who are pregnant (as per institutional practice) or lactating
9. Patients with a serious medical or psychiatric illness likely to interfere with participation in this clinical study
10. Patients with prior malignancies except resected non-melanoma skin cancer or treated cervical carcinoma in situ. Cancer treated with curative intent ≥ 5 years previously will be allowed. Cancer treated with curative intent < 5 years previously must be reviewed and approved by the Protocol Officer or Chairs.
11. Planned use of ATG or alemtuzumab in conditioning regimen

2.4 Treatment Plans

It is recommended that adjusted ideal body weight be used when calculating conditioning regimen chemotherapy doses.

Ideal Body Weight (IBW) Formulas:

Males IBW = 50 kg + 2.3 kg/inch over 5 feet

Females IBW = 45.5 kg + 2.3 kg/inch over 5 feet

Adjusted Ideal Body Weight Formula:

AIBW = IBW + [(0.25) x (ABW* - IBW)]

2.4.1 Conditioning Regimens

Eligible patients will receive a reduced intensity or nonmyeloablative conditioning regimen according to Table 2.4A. Other regimens deemed to be RIC by the transplant center and not included in Table 2.4A should be submitted to the Protocol Coordinator for consideration by the Protocol Chairs and/or Officer.

*ABW: Actual Body Weight

Table 2.4A: Conditioning Regimens[1]

Reduced Intensity Conditioning	Nonmyeloablative Conditioning
Fludarabine/Busulfan (Flu/Bu) • Fludarabine (120-180 mg/m^2) • Busulfan (less than or equal to 8 mg/kg PO or 6.4 mg/kg IV)	**Fludarabine/Cyclophosphamide (Flu/Cy)** • Fludarabine (90-120 mg/m^2) • Cyclophosphamide (120 mg/kg or 2250 mg/m^2)
Fludarabine/Melphalan (Flu/Mel) • Fludarabine (120-180 mg/m^2) • Melphalan (less than or equal to 150 mg/m^2)	**Fludarabine /Total Body Irradiation (Flu/TBI)** • Fludarabine (90 mg/m^2) • TBI (200 cGy)
	Fludarabine/ Cyclophosphamide/TBI (Flu/Cy/TBI) • Fludarabine (150 mg/m^2) • TBI (200 cGy) • Cyclophosphamide (29-50 mg/kg)

[1]Addition of antithymocyte globulin or alemtuzumab is not allowed.

Fludarabine and busulfan (Flu/Bu)

The recommended Flu/Bu regimen is the following:

- Days -6 to -2: Flu (30 mg/m^2/day, total dose of 150 mg/m^2)

  Busulfan Options

  Busulfan without PK - Days -5 to -4: Busulfan (4 mg/kg/day PO or 3.2 mg/kg/day IV or 130 mg/m^2/day IV; total dose of 8 mg/kg PO or 6.4 mg/kg IV or 260 mg/m^2 IV respectively)

  Busulfan with PK – target doses to area under the curve of 4000µMol/min (course AUC 16000µMol/min) or less is allowed.

  The sequence of fludarabine and busulfan administration may follow institutional practice.

Fludarabine and melphalan (Flu/Mel)

The recommended Flu/Mel regimen is the following:

- Days -5 to -2: Flu (30 mg/m^2/day, total dose of 120 mg/m^2)

- Day -1: Mel (140 mg/m^2)

The sequence of fludarabine and melphalan administration will be done according to institutional standards as long as the prescribed doses are the same as the recommended regimen above. Dividing the dose of melphalan into two days is allowed.

Fludarabine and cyclophosphamide (Flu/Cy)

The recommended Flu/Cy regimen is the following:

- Days -5 to -3: Flu (30 mg/m^2/day, total dose of 90 mg/m^2)
- Days -4 to -2: Cyclophosphamide (750 mg/m^2/day, total dose of 2250 mg/m^2)

  Alternatively:

- Days -3 to -2: Cyclophosphamide (60 mg/kg/day, total dose of 120 mg/kg)

The sequence of fludarabine and cyclophosphamide administration will be done according to institutional standards as long as the prescribed doses are the same as the recommended regimen above. Hydration and Mesna may be administered per institutional guidelines. Addition of rituximab conditioning in patients with lymphoproliferative disease is allowed. Dose and intervals of rituximab conditioning can be determined according to institutional guidelines. However, rituximab use after stem cell infusion is not permitted.

Fludarabine and total body irradiation (Flu/TBI)

The recommended Flu/TBI regimen is the following:

- Days -5 to -3: Flu (30 mg/m^2/day, total dose of 90 mg/m^2)
- Day -1 or 0: TBI 200 cGY (pre-stem cell infusion)

Fludarabine, total body irradiation and cyclophosphamide (Flu/Cy/TBI)

The recommend Flu/Cy/TBI regimen is the following:

- Days -6 to -5: Cy (14.5 mg/kg/day, total dose of 29 mg/kg, **or** single dose 50 mg/kg on day -6 [Minnesota regimen])
- Days -6 to -2: Flu (30 mg/m^2/day, total dose of 150 mg/m^2)
- Day -1: TBI 200 cGY

Hydration and Mesna may be administered per institutional standards. Fludarabine can be dose adjusted per institutional standards.

2.4.2 Hematopoietic Stem Cell Transplantation (HCT)

Mobilized PBSC is the only allowed graft source for patients enrolled in this clinical trial.

Donors will undergo G-CSF and/or plerixafor mobilization according to local institutional and donor center practices. PBSC will be collected by apheresis according to local institutional guidelines. Plasma and red cell depletion are allowed for volume reduction or ABO incompatibility but any other form of graft manipulation (including ex-vivo T cell depletion) is **not** permitted.

The target stem cell dose is a minimum of 2 x 10^6/kg and a maximum 10 x 10^6/kg (actual body weight) $CD34^+$ cells. The maximum CD34+ cell dose is 10 x 10^6/kg.

Up to two leukapheresis procedures may be performed to obtain the minimum $CD34^+$ cell target. If, after two leukapheresis procedures, fewer than 2 x 10^6/kg $CD34^+$ cells have been collected, transplant centers will have the discretion to continue PBSC cell harvesting or to proceed to bone marrow harvesting to obtain sufficient cells. A third leukapheresis procedure is discouraged. If bone marrow harvesting is needed in order to meet the desired cell dose, the transplant center needs to notify the Protocol Coordinator, in addition to the Protocol Chairs and/or Officer.

If more than 10 x 10^6/kg $CD34^+$ stem cells are collected, the excess will either be discarded or cryopreserved for future use, but will not be administered to the patient. PBSC will be administered on Day 0 to all patients according to individual institutional guidelines after appropriate processing and quantification has been performed by the local laboratory. If two leukapheresis procedures are performed to obtain the minimum $CD34^+$ cell dose target, the two products must be combined and infused together on one day. Stem cells are administered through an indwelling central venous catheter.

2.4.3 Tacrolimus/Methotrexate

Tacrolimus will be given per institutional practices, orally at a dose of 0.05-0.06 mg/kg/day or intravenously at a dose of 0.02-0.03 mg/kg/day starting on Day –3. The dose of tacrolimus may be rounded to the nearest 0.5 mg for oral formulations. Subsequent dosing will be based on blood levels per institutional guidelines with a suggested range of 5-15. If patients are on medications which alter the metabolism of tacrolimus (e.g. concurrent CYP3A4 inhibitors), the initial starting dose and subsequent doses should be altered as per institutional practices. Tacrolimus taper can be initiated at a minimum of 90 days post HCT if there is no evidence of active GVHD. The rate of tapering will be done according institutional practices and patients should be off tacrolimus by Day 180 post HCT if there is no evidence of active GVHD.

Dose reductions should be made if toxicity is present or whole blood levels are above the recommended range, in the absence of toxicity. Patients with severe intolerance of tacrolimus may be placed on cyclosporine (trough level of 200-400 ng/mL) or sirolimus (trough level of 5-10 ng/mL).

Methotrexate will be administered at the doses of 15 mg/m^2 IV bolus on Day +1 and 10 mg/m^2 IV bolus on Days +3, +6 and +11 after hematopoietic stem cell infusion. Methotrexate should be dose reduced, given with leucovorin rescue, or held for complications such as severe mucositis per institutional guidelines.

2.4.4 Tacrolimus/Mycophenolate Mofetil/Cyclophosphamide

Tacrolimus

Tacrolimus will be given per institutional practices, orally at a dose of 0.05-0.06 mg/kg/day or intravenously at a dose of 0.02-0.03 mg/kg/day starting Day +5. Starting tacrolimus dose can be modified to account for possible drug interactions (e.g. concurrent CYP3A4 inhibitor use) according to institutional guides. Serum levels of tacrolimus will be measured at Day +7 and then should be checked weekly thereafter, and the dose adjusted accordingly to maintain a suggested level of 5-15 ng/mL. Tacrolimus taper can be initiated at a minimum of 90 days post HCT if there is no evidence of active GVHD. The rate of tapering will be done according to institutional practices but patients should be off tacrolimus by Day 180 post HCT if there is no evidence of active GVHD.

Dose reductions should be made if toxicity is present or whole blood levels are above the recommended range, in the absence of toxicity. Patients with severe intolerance of tacrolimus may be placed on cyclosporine (trough level of 200-400 ng/mL) or sirolimus (trough level of 5-10 ng/mL).

Mycophenolate mofetil (MMF)

MMF will be given at a dose of 15 mg/kg/dose TID (based upon actual body weight) with the maximum total daily dose not to exceed 3 grams (1g TID, IV or PO). MMF prophylaxis will start Day +5 and discontinue after the last dose on Day +35 or may be continued if active GVHD is present.

Cyclophosphamide

Hydration prior to cyclophosphamide may be given according to institutional standards. A recommended approach is as follows: Patients are instructed to increase fluids overnight before cyclophosphamide administration. Hydration with normal saline at 3 ml/kg/hr IV will be started 2 hours prior to cyclophosphamide, then the rate will be reduced to 2 ml/kg/hr for 1 hour pre-cyclophosphamide and continued at 2 ml/kg/hr for 8 hours post-cyclophosphamide.

Mesna will be given in divided doses IV 30 min pre- and at 3, 6, and 8 hours post-cyclophosphamide or administered per institutional standards. Mesna dose will be based on the cyclophosphamide dose being given. The total daily dose of Mesna is equal to 80%-100% of the total daily dose of cyclophosphamide.

Cyclophosphamide [50 mg/kg IBW; if ABW < IBW, use ABW] will be given on Day +3 (between 60 and 72 hours after the start of the PBSC infusion) and on Day +4 post-transplant (approximately 24 hours after Day +3 cyclophosphamide). Cyclophosphamide will be given as an IV infusion over 1-2 hours (depending on volume).

It is crucial that no systemic immunosuppressive agents, such as corticosteroids, are given from **day 0 until 24 hours** after the completion of the post-transplant cyclophosphamide (Day +5). This

rule applies only to the post-transplant cyclophosphamide arm of the study**This includes corticosteroids as anti-emetics, however, replacement doses of chronic glucocorticoids for adrenal insufficiency are allowed.**

2.5 Supportive Care

All supportive care will be given in keeping with local institutional practice. Supportive care will be administered in a similar fashion to patients randomized to both arms of the study.

2.5.1 Growth Factors

G-CSF may be given per institutional guidelines.

2.5.2 Blood Products

Transfusion thresholds for blood product support will be consistent with standard institutional guidelines. All blood products will be irradiated.

2.5.3 Prophylaxis Against Infections

Patients will receive infection prophylaxis according to institutional guidelines. Infection prophylaxis will 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, HHV-6, EBV, Pneumocystis jirovecii, and fungal infections:

- Antifungal therapy: Prophylaxis with fluconazole or other antifungal agents can be given as per local institutional guidelines.
    - **Fluconazole, voriconazole and other azoles** (CYP3A4 inhibitors) are expected to increase serum tacrolimus levels, therefore, dosages of tacrolimus should be adjusted accordingly.
- **CMV:** CMV monitoring will be done according to institutional guidelines. It is recommended that weekly assessment for CMV be done through Day 60 post-transplant. Use of letermovir is allowed. Any reactivation and/or CMV disease will be captured in this study. An Infection form must be submitted in Advantage eClinical.

2.5.4 Intravenous Immune Globulin (IVIG)

IVIG administration will be according to local institutional standard practice.

2.6 Participant Risks

2.6.1 Therapy Toxicities

All toxicities will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.

2.6.2 Tacrolimus

Tacrolimus side effects include:

- Cardiovascular: pericardial effusion, hypertension (which may cause arrhythmia, angina, myocardial infarction)
- Cutaneous: itching, rash
- Endocrine and metabolic: hyperglycemia, hypomagnesemia, hypokalemia, hyperkalemia, hypophosphatemia, hyperlipidemia
- Gastrointestinal: constipation, diarrhea, nausea, vomiting, anorexia, bowel perforation, dyspepsia
- General: fatigue
- Hematologic: anemia, thrombocytopenia, leukopenia, thrombotic microangiopathy
- Hepatic: liver dysfunction
- Neurologic: paresthesia, headache, tremor, encephalopathy/posterior reversible encephalopathy syndrome (PRES), dizziness, insomnia, confusion, altered mental status, seizure, blindness
- Pulmonary: pleural effusion, dyspnea
- Renal: renal impairment which may require dialysis, peripheral edema
- Miscellaneous: infection, post-transplant lymphoproliferative disorders, allergic reaction, secondary malignancy

2.6.3 Methotrexate

Methotrexate side effects include:

- Cardiac and vascular: thrombosis
- Cutaneous: photosensitivity, rash, alopecia, erythema multiforme
- Gastroinstestinal: nausea, vomiting, diarrhea, pain, anorexia, ulcers, gastrointestinal bleeding, mucositis
- General: fatigue
- Hematologic: anemia, thrombocytopenia, leukopenia
- Pulmonary: pulmonary fibrosis
- Renal: nephrotoxicity
- Miscellaneous: secondary malignancy, infection

2.6.4 Mycophenolate mofetil (MMF)

MMF side effects include:

- Cardiac and vascular: hypertension, hypotension, tachycardia, edema
- Cutaneous: rash

- Endocrine and metabolic: hypocalcemia, hypokalemia, hyperuricemia, hyperkalemia, hypomagnesemia
- Gastrointestinal: nausea, vomiting, dyspepsia, abdominal pain, diarrhea
- Hematologic: leukopenia, thrombocytopenia, anemia
- Neurologic: headache, tremors, insomnia, dizziness, progressive multifocal leukoencephalopathy (PML)
- Pulmonary: dyspnea, cough, interstitial lung disease
- Miscellaneous: change in vision, infection, secondary malignancy, arthralgia, myalgia

2.6.5 Cyclophosphamide

Cyclophosphamide side effects include:

- Cardiac and vascular: heart failure (which can result in edema, effusion, dyspnea)
- Cutaneous: alopecia, rash, hyperpigmentation of skin and nails
- Gastrointestinal: nausea, vomiting, anorexia, mucositis, stomatitis, abdominal pain, diarrhea
- General: lethargy
- Hematologic: leukopenia, thrombocytopenia, anemia
- Pulmonary: pulmonary fibrosis
- Endocrine: amenorrhea, gonadal function impairment, sterility, syndrome of inappropriate antidiuretic hormone secretion (SIADH) – with associated cerebral edema
- Genitourinary: hemorrhagic cystitis
- Miscellaneous: infection, allergic reaction including anaphylaxis, secondary malignancy

2.6.6 Busulfan

Busulfan side effects include:

- Cardiac: arrhythmia, edema, hypertension, tachycardia, pericardial effusion, heart failure, hypotension
- Cutaneous: rash, hyperpigmentation, pruritis
- Gastrointestinal: constipation, diarrhea, dyspepsia, nausea, vomiting, anorexia, mucositis, dysphagia
- General: chills, fever, pain, fatigue
- Genitourinary: gonadal function impairment, menopause
- Endocrine: hypomagnesemia, hyperglycemia, hypokalemia, hypocalcemia
- Hematologic: anemia, thrombocytopenia, leukopenia
- Hepatic: hepatic impairment, hepatic sinusoidal obstruction syndrome

- Neurologic: dizziness, headache, insomnia, seizure, anxiety, depression
- Pulmonary: cough, nasal congestion, hemoptysis, interstitial pulmonary fibrosis
- Renal: hematuria, renal impairment
- Miscellaneous: infection, allergic reaction including anaphylaxis, visual impairment

2.6.7 Fludarabine

Fludarabine side effects include:

- Gastrointestinal: vomiting, anorexia, nausea, mucositis
- General: fatigue, fever, chills
- Hematologic: anemia, thrombocytopenia, neutropenia
- Hepatic: increased liver function tests
- Neurologic: paresthesia, confusion, seizure, agitation, visual disturbances, pain
- Pulmonary: cough, shortness of breath
- Renal: renal impairment, hematuria
- Miscellaneous: infection, general organ damage

2.6.8 Melphalan

Melphalan side effects include:

- Cardiac and vascular: edema, heart failure, vasculitis
- Gastrointestinal: mucositis, nausea, vomiting, diarrhea
- General: fatigue
- Hematologic: anemia, thrombocytopenia, neutropenia
- Hepatic: abnormal liver function tests, hepatitis
- Pulmonary: shortness of breath, pulmonary fibrosis
- Renal: renal impairment
- Miscellaneous: allergic reaction including anaphylaxis, secondary malignancy

2.6.9 Total Body Irradiation (TBI)

Low Dose TBI side effects include:

- Cutaneous: erythema, hyperpigmentation, alopecia
- Gastrointestinal: nausea, vomiting, diarrhea, parotitis, mucositis, abdominal cramping
- General: fever, fatigue
- Genitourinary: gonadal impairment
- Hepatic: hepatic sinusoidal obstruction syndrome
- Hematologic: myelosuppression, anemia, thrombocytopenia
- Pulmonary: interstitial pneumonitis
- Renal: nephropathy
- Miscellaneous: infection, short stature, vertebral deformities, cataracts, secondary malignancy, hormonal impairment

2.7 Study Drug Supply

Tacrolimus, methotrexate, cyclophosphamide and mycophenolate mofetil are commercially available agents and will not be provided by the study. Please administer as described in Section 2.4.

CHAPTER 3

3 STUDY ENDPOINTS

3.1 Primary Endpoint

BMT CTN 1703: The primary endpoint is 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 immune suppression, disease relapse or progression, or death by any cause.

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 chronic GVHD severe enough to warrant any additional systemic treatment(s). Also, continuation of study-mandated GVHD prophylaxis beyond Day 180 in the presence of chronic GVHD will also be considered an event with time to event determined as date of chronic GVHD onset.

Mi-Immune: The primary endpoint is non-relapse mortality within one year. This endpoint is defined according to the BMT CTN 1703 protocol. This primary endpoint will be associated with the engraftment stool microbiome diversity (grouped by tertiles) for the primary analysis of this study. Mi-Immune secondary endpoints are addressed in Chapter 3 of Appendix J.

3.2 Secondary Endpoints

3.2.1 Acute GVHD

Cumulative incidences of grade II-IV and III-IV acute GVHD will be determined. Acute GVHD will be graded according to the BMT CTN Technical MOP (see Appendix G). The time of onset of acute grades II-IV and III-IV acute GVHD will be recorded, as well as the maximum grade achieved. Within the acute GVHD endpoint, the proportion of patients with visceral involvement (liver or gut) will be described.

Cumulative incidences of Minnesota standard and high risk acute GVHD will also be determined.

3.2.2 Chronic GVHD

The cumulative incidence of chronic GVHD will be determined. Data will be collected directly from providers and chart review as defined by the NIH Consensus Conference Criteria. Eight organs will be scored on a 0-3 scale to reflect degree of chronic GVHD involvement. Liver and pulmonary function test results, and use of systemic therapy for treatment of chronic GVHD will also be recorded. These data will allow calculation of the NIH global severity scores of mild, moderate and severe chronic GVHD, which has been associated with transplant related mortality and overall survival. Assessment of chronic GVHD will occur up to 1 year post-transplant.

3.2.3 Systemic Immunosuppression-free Survival

Patients who are alive, relapse-free, and do not need ongoing immune suppression to control GVHD at one year post-transplant are considered successes for this endpoint. Immune suppression is defined as any systemic agents used to control or suppress GVHD. Corticosteroid doses greater than 10 mg will be considered active systemic immune suppression treatment. Patients who

discontinued immune suppression within 15 days or less prior to the 1-year time point will be considered to be on immune suppression for this endpoint.

3.2.4 Hematologic Recovery

Hematologic recovery will be assessed according to neutrophil and platelet counts recovery after transplant. Neutrophil recovery is defined as achieving an absolute neutrophil count (ANC) greater than or equal to 500/mm^3 for three consecutive measurements on three different days. The first of the three days will be designated the day of neutrophil recovery. The competing event is death without neutrophil recovery. For patients who never drop ANC below 500/mm^3, the date of neutrophil recovery will be Day +1 post-transplant.

Platelet recovery is defined by two different metrics: the first day of a sustained platelet count greater than or equal to 20,000/mm^3 or greater than or equal to 50,000/mm^3 with no platelet transfusions in the preceding seven days. The first day of sustained platelet count above these thresholds will be designated the day of platelet engraftment. For patients who never drop their platelet count below 20,000/mm^3 or 50,000/mm^3, the date of platelet recovery will be Day +1 post HCT.

Lymphocyte recovery will be defined as the first day of sustained absolute lymphocyte count greater than or equal to 1000/mm^3.

3.2.5 Donor Cell Engraftment

Donor cell engraftment will be assessed with donor/recipient chimerism studies. Chimerism may be evaluated in bone marrow, whole unfractionated blood or blood cell fractions, including CD3 and CD33 or CD15 fraction. For the purpose of this protocol, mixed chimerism is defined as the presence of donor cells, as a proportion of total cells to be less than 95% but at least 5% in the bone marrow or peripheral blood. Full donor chimerism is defined as greater than or equal to 95% of donor cells. Mixed and full chimerism will be evidence of donor cell engraftment. Donor cells of less than 5% will be considered as graft rejection. The proportion of patients with each level of chimerism described above will be described as part of this outcome. For sorted blood cell fractions, CD3+ donor cell chimerism will be used to define the donor/recipient chimerism status.

3.2.6 Disease Relapse or Progression

Relapse is defined by either morphological or cytogenetic evidence of acute leukemia or MDS consistent with pre-transplant features, or radiologic evidence of lymphoma, documented or not by biopsy. Progression of disease applies to patients with lymphoproliferative diseases (lymphoma or chronic lymphocytic leukemia) not in remission prior to transplantation. The event is defined as increase in size of prior sites of disease or evidence of new sites of disease, documented or not by biopsy.

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

- Reappearance of leukemia blast cells in the peripheral blood; or,
- Greater than 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

Lymphoproliferative Diseases – Relapse or progression will be diagnosed when there is:

- Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased FDG uptake in a previously unaffected site will only be considered relapsed or progressive disease after confirmation with other modalities. In patients with no prior history of pulmonary lymphoma, new lung nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the PET without histologic confirmation.
- At least a 50% increase from nadir in the sum of the product diameters of any previously involved nodes, or in a single involved node, or the size of other lesions (e.g., splenic or hepatic nodules). To be considered progressive disease, a lymph node with a diameter of the short axis of less than 1.0 cm must increase by at least 50% or more and to a size of 1.5 x 1.5 cm or more than 1.5 cm in the long axis.

  Lesions should be PET positive if observed in a typical FDG-avid lymphoma or the lesion was PET positive before therapy unless the lesion is too small to be detected with current PET systems (less than 1.5 cm in its long axis by CT).
- In addition to the criteria above, patients with CLL who present in complete remission prior to transplantation may fulfill the relapse definition if there is reappearance of circulating malignant cells that are phenotypically characteristic of CLL.

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.

3.2.7 Transplant-related Mortality

The cumulative incidence of TRM will be estimated at Days 100, 180 and 1 year after HCT. An event for this endpoint is death without evidence of disease progression or recurrence. Disease progression or recurrence will be considered competing events.

3.2.8 Toxicity

All grade 3-5 toxicities according to CTCAE, version 5.0 will be tabulated for each intervention arm. The proportion of patients developing at least a grade 3 or more AE across intervention arms will be compared.

3.2.9 Infections

The incidence of definite and probable viral, fungal and bacterial infections will be tabulated for each intervention arm. The cumulative incidence of treated CMV reactivation in the first 100 days

post-transplant will be described. All Grade 2 and 3 infections will be reported according to the BMT CTN Technical MOP up to 1 year posttransplant.

3.2.10 Disease-Free Survival

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.

3.2.11 Overall Survival

Overall survival is defined as the time interval between date of transplant and death from any cause. The event for this endpoint is death from any cause. Surviving patients will be censored at last follow-up.

3.2.12 PTLD

The incidence of post-transplant lymphoproliferative disease (PTLD) will be measured at one year post-transplant.

3.2.13 Patient-Reported Outcomes (PRO)

PRO will be measured at Baseline and then at Day 100, Day 180 and 1 year post-transplant using the Lee Chronic GVHD Symptom Scale, Hemorrhagic Cystitis symptom questions, and selected PROMIS subscales for gastrointestinal symptoms, physical function and satisfaction with participation in social roles. The instruments will be scored according to the recommendations of the developers. PRO data will be collected electronically, or on paper vial mail if requested by the patient. Whether collected electronically or on paper, PRO data will be entered in the CIBMTR's ePRO system. The PRO instruments will only be offered to English and Spanish speaking patients.

3.3 Endpoint Review Process

Upon completion of participant follow-up, an Endpoint Review Committee (ERC) will conduct an independent review of site-reported data on key study endpoints to determine the endpoint data to be presented in the primary manuscript and final analysis. This Committee will consist of members of the protocol team, including the Protocol Chairs, Protocol Officer, Operational Statistician, and Protocol Coordinator. Each participant's data will be reviewed by ERC clinicians. The adjudicated endpoint data for each participant will be determined by consensus of their reviewers.

The ERC will employ a risk-based strategy to the data review. First, a random sample of 20% of the total study population will be chosen. The key endpoints will be determined in two ways: using ERC adjudications and using the site-reported data. The concordance between the ERC adjudicated and site-reported endpoints will be computed. If the concordance is 90% or above, the ERC will end and site-reported endpoints will be used for all statistical analyses. If the concordance falls below 90%, a second random subset of 20% of study participants will be drawn from the unadjudicated participants. Then, the concordance between ERC adjudicated and site-reported endpoints in the combined first and second subsets will be computed. If the concordance is 90% or above, the ERC will end and site-reported data will be used for all statistical analyses. Otherwise, all remaining, unadjudicated participants will undergo review and the ERC adjudicated endpoints for the entire study population will be used in all statistical analyses.

Data will be obtained from the relevant case report forms and source documents and will be provided to reviewers in a blinded manner with respect to treatment assignment, treatment center, and participant identifier. These data will be kept confidential and will not be discussed outside the Committee or presented in a public forum.

CHAPTER 4

4 PATIENT ENROLLMENT AND EVALUATION

4.1 Approaching Patients, Eligibility Screening and Obtaining Consent

Patients will be approached for this study after the decision to proceed with transplantation is made and an HLA-matched PBSC donor is identified. Patients willing to participate in the trial will sign an NMDP IRB-approved consent form. Transplant physicians will evaluate the patient eligibility for randomization onto this study (see Section 2.2). Eligibility criteria will be verified, and ineligible patients will not be randomized, and no further follow-up will be obtained. Transplant center personnel will record the documentation of patient consent and register the patient in Segment 0 of the study in Advantage eClinical (Electronic Data Capture, an Internet-based data entry system). Patients also need to sign the CIBMTR "Protocol for a Research Database for Hematopoietic Cell Transplantation and Marrow Toxic Injuries" consent form because future laboratory correlative studies using any remaining stored research samples will require linking with clinical data collected CIBMTR. In cases where the HLA-matched donor is a related donor, the patient's donor who is 18 years of age or older should be approached to provide consent for the Mi-Immune study samples. Unrelated donors will be approached for consent for the Mi-Immune study by the NMDP.

4.2 Transplant Protocol Registration

Before randomization occurs, the transplant center must state through Advantage eClinical which conditioning regimen and which maintenance regimen (if any) will be used for the enrolled patient. Such a registration step will avoid potential biases that preferential use of a certain regimen on one treatment arm could confer to the study. At this stage, the transplant center will also verify that the patient is still a candidate for transplantation, and eligible for the trial. The transplant center will also record the donor's willingness to participate in the Mi-Immune study. At this point the patient is enrolled into Segment A of the study.

At the time of enrollment in Segment A, transplant center personnel will securely email or fax patient contact information to the CIBMTR Survey Research Group for purpose of contacting patients for PRO administration.

4.3 Randomization

Once the patient is deemed eligible and has given written informed consent, and the transplant center has confirmed patient eligibility and registered the patient's conditioning and maintenance regimen, randomization occurs. Patients should not be randomized more than 14 days prior to the planned initiation of conditioning. If initiation of conditioning has not started within 14 days of randomization, the Protocol Coordinator and Protocol Chairs and Officer must be notified. Refer to section 4.6.1 Patient Assessments-pre-transplant evaluations.

4.4 Treatment Scheduling

Treatment should be initiated as soon as possible after randomization. This will prevent patient attrition prior to transplant for reasons such as disease progression. Consequently, all treatments related to the transplant should be scheduled **prior** to randomization. This includes planning an admission date and ensuring that the PBSC donor can be mobilized and undergo apheresis in a coordinated fashion with the planned transplant.

4.5 Patient Evaluation

The patient pre-transplant evaluation must be completed within 60 days of randomization (exceptions noted in the Study Calendar). See section 4.6.1 Patient Assessments-pre-transplant evaluations. This step is necessary because patient organ function, infection status, and status of malignancy may vary over time. This evaluation will protect patients with a new contraindication to transplant from initiating transplant therapy at an unsafe time.

4.6 Study Monitoring

The follow-up schedule for scheduled study visits is outlined in Table 4.6A. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User's Guide.

TABLE 4.6A: FOLLOW-UP SCHEDULE

Study Visit	Target Day Post-Transplant
Day of Transplant	Day 0*
1 week	7 ± 3 days
2 weeks	14 ± 3 days
3 weeks	21 ± 3 days
4 weeks	28 ± 3 days
5 weeks	35 ± 3 days
6 weeks	42 ± 3 days
7 weeks	49 ± 3 days
8 weeks	56 ± 3 days
9 weeks	63 ± 3 days
10 weeks	70 ± 3 days
11 weeks	77 ± 3 days
12 weeks	84 ± 3 days
14 weeks	98 ± 7 days
6 months	180 ± 28 days
9 months	270 ± 28 days
12 months	365 ± 28 days
24 months	730 ± 60 days

*If infusion occurs over 2 days, the day of transplant should be recorded as the day the first infusion occurred.

4.6.1 Patient Assessments

Table 4.6B summarizes patient clinical assessments over the course of the study.

TABLE 4.6B: PATIENT AND DONOR CLINICAL ASSESSMENTS

	Pre-Conditioning	**Pre-infusion**	**0**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**98**	**180**	**270**	**365**	**730**
History, physical exam, weight and height[10]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Karnofsky performance status (see Appendix D)	X															X	X	X	X	
HCT-Specific Comorbidity Index score (see Appendix E)	X																			
Disease Risk Index (see Appendix F)	X																			
Donor and recipient HLA typing	X																			
CBC[1], differential, platelet count, and blood chemistries[2]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Infectious disease titers[3]	X																			
EKG and LVEF	X																			
DLCOcorr and FEV1predicted	X																			
Disease evaluation[4]	X															X			X	
Chest x-ray or chest CT	X																			
Pregnancy test[5]	X																			
GVHD assessments[6]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Toxicity assessments[7]							X				X					X	X	X	X	
Infection assessments	X		X		X		X		X		X		X		X	X	X	X	X	
Chimerism[8]	X						X									X				
Patient Mi-Immune research samples (see Appendix J)[9]	X[9]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Related donor CBC[11]		X																		
Related donor Mi-Immune research samples (see Appendix J)		X																		
Patient Reported Outcomes (see Appendix H)	X															X	X		X	

[1]CBC with differential performed three times weekly from Day 0 until ANC at least 500/mcLor greater for three days and platelet count at least 20,000/mcL or greater after nadir, while hospitalized. CBC then performed weekly through Day 84 post-transplant, then at Days 98, 180, 270, 365 and 730 post-transplant. A CBC needs to be collected at the same time as the scheduled Mi-Immune research samples and reported in Advantage eClinical.

[2]Blood chemistries include: serum creatinine, albumin, bilirubin, alkaline phosphatase, AST and ALT. Blood chemistries performed twice weekly until hospital discharge. Blood chemistries performed weekly after hospital discharge until Day 84 post-transplant, then at Days 98, 180, 270 and 365 post-transplant.

[3]Infectious disease titers should be performed per institutional guidelines and may include: CMV, Hepatitis panel (HepA Ab, HepB SAb, HepB SAg, HepB Core Ab, HepC Ab), herpes simplex virus, syphilis, HIV and HTLV I/II antibody, and varicella zoster.

[4]Evaluation of disease: (A) For acute leukemia, CML, and MDS, evaluation for malignant disease includes a bone marrow aspirate and biopsy for pathology and cytogenetics. **A bone marrow biopsy must be performed no more than 44 days prior to the initiation of conditioning.** (B) For lymphomas, bone marrow biopsy and/or imaging studies are appropriate for disease evaluation and will be done according to institutional practices. Patients with lymphomas should undergo the same post-transplant testing as their pre-transplant evaluation for matter of subsequent comparison. **Imaging studies must be done no more than 60 days prior to patient randomization.**

[5]Pregnancy test must be performed ≤ 30 days before the start of the transplant conditioning regimen. Pregnancy test is required for females of child-bearing potential and may be performed per institutional practices.

[6]GVHD assessments performed weekly from Day 7 until Day 84 post-transplant, and then at Days 98, 180, 270 and 365. The GVHD assessment will include a review of **all** abnormalities experienced **during the entire assessment period** and the **highest grade** for each abnormality during the assessment period will be recorded on the Acute GVHD form and the Follow-up/Chronic GVHD form in eClinical. The Chronic GVHD Provider Survey will record GVHD symptoms present in the last week (*whether attributed to GVHD or not*) and must be completed by a clinician on the day of the assessment.

[7]The toxicity assessment will include a review of **all** toxicities experienced **during the entire assessment period** and the **highest grade** for each toxicity during the assessment period will be recorded on the Toxicity form in eClinical.

[8]Chimerism may be evaluated in bone marrow, whole blood or blood cell fractions, including CD3 and CD33 or CD15 fraction, according to institutional practice. The actual measurement dates may be within +/- 7 days of the recommended time points.

[9]The pre-conditioning baseline sample must be collected prior to the initiation of the transplant conditioning regimen and pre-antibiotic prophylaxis. For patients, stool samples at Pre-Conditioning, Day 0, Day 7, Day 14, Day 21, and Day 28 are **mandatory**. Starting day 35 through day 77, then at day 98, 180, 270, 1 year, and 2 years, the stool samples are **optional**. Weekly urine sample collection for microbiome assays starting Pre-conditioning through Day 270, then at 1 year, and 2 years are **ALL OPTIONAL.** Pre-transplant donor samples are to be collected from those donors who have signed the Mi-Immune consent.

[10]Height is only required at baseline. It is not required to be repeated at the other time points.

[11]The related donor's CBC needs to be collected at the same time as the scheduled Mi-Immune research samples and reported in Advantage eClinical from those donors who have signed the Mi-Immune consent.

4.6.2 Pre-transplant Evaluations

The following observations must be completed within 60 days prior to patient randomization, or 74 days prior to initiation of conditioning regimen unless otherwise indicated.

- History, physical examination, height and weight.
- Karnofsky performance status
- HCT-Specific Comorbidity Index score.
- CBC with differential and platelet count, serum creatinine, bilirubin, alkaline phosphatase, AST and ALT.
- Infectious disease titers should be performed per institutional guidelines and may include: CMV antibody, Hepatitis panel (HepA Ab, HepB SAb, HepB SAg, HepB Core Ab, HepC Ab), herpes simplex virus, syphilis, HIV and HTLV I/II antibody, and varicella zoster.
- EKG and LVEF – **can be performed within 90 days prior to patient randomization.**
- Pulmonary function tests, including DLCO and FEV1 - **can be performed within 90 days prior to patient randomization.**
- HLA typing of patient and donor. HLA typing can be performed at any time prior to randomization.
    - Sibling donors must be HLA typed for HLA-A and -B at intermediate (or higher) resolution, and -DRB1 at high resolution using DNA-based typing.
    - Unrelated donors must be HLA typed for HLA-A, -B, -C and -DRB1 at high resolution using DNA-based typing.
- Disease evaluation for patients with acute leukemia, CML or MDS includes a bone marrow aspirate and biopsy for pathology and cytogenetics. **A bone marrow biopsy must be performed no more than 44 days prior to the initiation of conditioning.**
- Disease evaluation for patients with lymphomas includes imaging studies for matters of comparison post-transplant, the types of which may be determined according to the center's institutional practices. **Imaging studies must be done no more than 60 days prior to patient randomization.**
- Chest X-ray or chest CT.
- Pregnancy test per institutional practices for females of child-bearing potential. **NOTE: pregnancy test must be performed no more than 30 days prior to randomization and must be repeated if not within 30 days prior to the initiation of conditioning.**
- Patient Reported Outcomes (PRO) to be completed by English or Spanish speaking study patients.
- Pre-transplant donor and recipient samples for post-transplant chimerism studies.
- Related donor and recipient blood, urine and stool samples for Mi-Immune study (Appendix J). CBCs need to be collected at the same time as the scheduled Mi-Immune research samples.

4.6.3 Post-transplant Evaluations

The following observations will be made according to Table 4.6B:

- History and physical exam to assess GVHD and other morbidity weekly through Day 84 post-transplant, then at Days 98, 180, 270 and 365 post-transplant. GVHD will be monitored in accordance with BMT CTN guidelines as specified in the BMT CTN Technical Manual of Procedures (BMT CTN MOP). GVHD assessments weekly from Day +7 through Day +84 post-transplant, and then at Days 98, 180, 270 and 365 post-transplant.
- Assessment for toxicities at Days 28, 56, 98, 180, 270 and 365 post-transplant.
- CBC with differential performed at least three times a week from Day 0 until ANC at least 500/μL for 3 consecutive measurements over 3 days and platelet count at least 20,000/μL for 3 days without platelet transfusion in the prior 7 days (while hospitalized only) after nadir is reached. Thereafter, CBC weekly until Day 84 post-transplant, then at Days 98, 180, 270, 365 and 730 post-transplant. CBCs need to be collected at the same time as the scheduled Mi-Immune research samples.
- Serum creatinine, bilirubin, alkaline phosphatase, ALT and AST, twice a week until hospital discharge and then weekly until Day 84 post-transplant, then at Days 98, 180, 270 and 365 post-transplant.
- Chimerism studies performed at Days 28 and 98 post-transplant. Chimerism may be evaluated in bone marrow, whole unfractionated blood or blood cell fractions, including CD3 and CD33 or CD15 fraction. The actual measurement dates may be within +/- 7 days of the recommended time points. (see Appendix B)
- Disease evaluation of the malignant disease at Days 98 and 365 post-transplant: For acute leukemia, CML and MDS this includes a bone marrow aspirate and biopsy for pathology and cytogenetics. For lymphomas this includes imaging studies, which will be done according to institutional practices and the same as prior to transplant, for matter of comparison.
- Data on occurrence of Grade 2 and 3 infections and recorded as per the BMT CTN Technical MOP.
- Blood, urine and stool samples for Mi-Immune study to be collected Days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 98, 180, 270, 365 and 730. (see Appendix J)
- Patient Reported Outcomes (PRO) to be completed by English or Spanish speaking study participant at Days 98, 180 and 365. The CIBMTR Survey Research Group will contact patients via email, phone or mail to collect the PRO instruments online, or on paper. The Survey Research Group will notify the transplant center if a patient's contact information has changed or if they find through follow-up that the patient has died.

4.6.4 Criteria for Forms Submission

Criteria for timeliness of submission for all study forms are detailed in the Data Management Handbook and User's Guide. Forms that are not entered into Advantage eClinical within the specified time will be considered delinquent. A missing form will continue to be requested either until the form is entered into the Advantage eClinical and integrated into the eClincal master database, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Data Management Handbook.

4.6.5 Reporting Patient Deaths

Recipient death information must be entered into Advantage eClinical within 24 business hours of knowledge of the patient's death. If the cause of death is unknown at that time, it need not be recorded at that time. However, once the cause of death is determined, the form must be updated in Advantage eClinical.

4.7 Adverse Event Reporting Requirements

4.7.1 Definitions

Adverse Event: An Adverse Event (AE) is defined as any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease that is temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure.

Expectedness: An adverse event can be Expected or Unexpected

- **Expected adverse events** are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.
- **Unexpected adverse events** are those that vary in nature, intensity or frequency from information in the current adverse event list, the Investigator's Brochure, the package insert, or when it is not included in the informed consent document as a potential risk.

Serious Adverse Event: A serious adverse event (SAE), as defined by per 21 CFR 312.32, is any adverse event that results in one of the following outcomes, regardless of causality and expectedness:

- **Results in death**
- **Is life-threatening.** Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- **Requires or prolongs inpatient hospitalization** (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not

deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).

- **Results in persistent or significant disability/incapacity.** Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- **Is a congenital anomaly or birth defect;** or
- **Is an important medical event** when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Medical and scientific judgment should be exercised in deciding whether expected reporting is also appropriate in situations other than those listed above. For example, important medical events may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above (eg, suspected transmission of an infectious agent by a medicinal product is considered a Serious Adverse Event). Any event is considered a Serious Adverse Event if it is associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

4.7.2 BMT CTN Adverse Event Reporting Guidelines

Adverse event reporting will be consistent with BMT CTN procedures (BMT CTN Administrative Manual of Procedures, Chapter 6). It is BMT CTN policy that AEs must be reported even if the investigator is unsure whether a relationship exists between the adverse event and the use of study treatment.

Unexpected, serious adverse events (SAEs) will be reported through an expedited AE reporting system via Advantage eClinical. **Unexpected, life-threatening and fatal SAEs must be reported within 24 hours of knowledge of the event. All other unexpected SAEs must be reported within three business days of knowledge of the event.** Events entered in Advantage eClinical will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.

Expected AEs will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 at regular intervals as defined on the Form Submission Schedule, including calendar-driven case report forms (e.g., Toxicity and GVHD) or event-driven case report forms (e.g., Relapse/Progression, Infection, and Death). **Any expected life-threatening SAE not collected on another study form must be reported through the expedited AE reporting system via Advantage eClinical.**

The Data and Safety Monitoring Board will receive summary reports of all unexpected SAEs on a semi-annual basis.

4.8 CIBMTR Data Reporting

Centers participating in BMT CTN trials must register pre- and post-transplant outcomes on all consecutive hematopoietic stem cell transplants done at their institution during their time of participation to the Center for International Blood and Marrow Transplant Research (CIBMTR).

Registration is done using procedures and forms of the Stem Cell Transplant Outcomes Database (SCTOD). (Note: Federal legislation requires submission of these forms for all US allotransplant recipients.) Enrollment in BMT CTN 1703 must be indicated on the SCTOD pre-transplant registration form. Additionally, CIBMTR pre- and post- transplant Comprehensive Report Forms must also be submitted for all patients enrolled on this trial. CIBMTR forms will be submitted directly to the CIBMTR at the times specified on the Form Submission Schedule.

CHAPTER 5

5 STATISTICAL CONSIDERATIONS

5.1 Study Design

The study is designed as a Phase III randomized, open label, multicenter trial to compare PTCy/Tac/MMF versus Tac/MTX for GVHD prophylaxis in patients with controlled malignant disease receiving an allogeneic PBSC transplant after a RIC regimen. The primary endpoint is GRFS at 1 year. The target enrollment is 428 patients in total, with 214 patients on each of the two treatment arms.

Of note, all statistical considerations for the Mi-Immune study are addressed in Chapter 5 of Appendix J.

5.1.1 Accrual

It is estimated that 36 months of accrual will be necessary to enroll the targeted sample size with an accrual rate of approximately 12 patients per month. Both Core and Affiliate Centers will enroll patients on this study. Accrual will be reported by race, ethnicity, gender, and age.

5.1.2 Randomization

All patients will be randomized within 14 days prior to the initiation of conditioning therapy. Randomization will be performed in a 1:1 ratio using random block sizes for the two arms. Randomization will be stratified by centers and by disease risk using Disease Risk Index (DRI Low, Intermediate and High). The DRI level “High” will include patients classified as both “High” and “Very High”.

5.1.3 Primary Endpoint

The primary endpoint is GRFS as a time to event endpoint from the time of randomization. All transplanted patients will be followed for the primary endpoint for one year; however, the primary endpoint will be analyzed as a time to event endpoint. The primary analysis will be done using the intent-to-treat principle.

5.1.4 Primary Hypothesis

The primary null hypothesis is that the hazard ratio between PTCy/Tac/MMF vsTac/MTX for GRFS endpoint is equal to one versus an alternative hypothesis that the hazard ratio not equal to one. A hazard ratio equal to one indicates no difference between the two treatments, while a hazard ratio less than one implies that the hazard of GRFS is lower for patients receiving PTCy/Tac/MMF compared with those in the Tac/MTX patient group. A hazard ratio greater than one would indicate an opposite treatment effect. This null hypothesis will be tested using a two-sided significance level of 5%

5.2 Sample Size and Power Considerations

Sample size and power considerations are based on the comparison of PTCy/Tac/MMF to Tac/MTX using a Cox proportional hazards model. We assume an accrual period of 36 months and a 12-month follow-up period with a 5% drop-out rate. We further assume that the drop-out rate is exponentially distributed and that the GRFS endpoint matches the results of the BMT CTN 1203 trial control group. The survival probabilities for GRFS are based on data collected from the BMT CTN 1203 study. Therefore, a sample size of 428 patients (214 per arm) is required to sufficiently maintain a two-sided type I error of 5% while providing 90% statistical power for a two-sided test to detect a HR of 0.66.

5.3 Interim Analysis and Stopping Guidelines

The study will consist of one interim analysis for efficacy after the required total number of events is reached in all evaluable patients for the primary endpoint to be reviewed by the NHLBI-appointed Data and Safety Monitoring Board (DSMB). An interim analysis for efficacy will be conducted after reaching a total of 147 events, at a 60% information fraction. The final analysis will be conducted when the targeted number of events of 244 occurs, or 1 year after the last patient is randomized. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures.

5.3.1 Interim Analysis for Efficacy

Analyses will be performed as described below for the primary endpoint. At the interim analysis time point, a Z test for comparing the two treatments will be compared to the critical values shown in Table 5.3A. All patients with follow-up post randomization prior to the time of the interim analyses will be used to compute this statistic. If the test statistic is outside the continuation range, the DSMB will discuss the continuation of the trial. Should the DSMB stop the trial for efficacy, all patients receiving the inferior treatment arm will be switched to the superior treatment arm where the study will proceed until the targeted sample size for Mi-Immune is reached.

Efficacy stopping rules are based on Wang and Tsiatis boundary family with shape parameters P=1.44, A=0, R=0 and G=1.9618.[25] Higher values of P, with A and R fixed at zero, and G fixed at 1.9618 will cause the group sequential test to be increasingly conservative at the earliest analyses times. This boundary corresponds approximately to a hazard ratio less than 0.5918 or greater than 1.6898 and Z statistics less than -3.1710 or greater than 3.1710, respectively. A P-value less than 0.00152 at the interim analysis would indicate a statistically significant result.

Table 5.3A: Efficacy Stopping Thresholds with Type I Error 0.05, Power 90% and an Effect Size of 15% or Hazard Ratio 0.66

Analysis Time	Information Fraction	Total Cumulative Sample Size Under Alternative	Cumulative Events Under Alternative	Hazard Ratio Efficacy Boundary		Z-Statistic Efficacy Boundary		Cumulative Type I Error
				Lower	Upper	Lower	Upper	
Interim	0.60	334	147	0.5918	1.6898	-3.1710	3.1710	0.152%
Final	1.00	428	244	0.7777	1.2858	-1.9618	1.9618	5.000%

5.3.2 Operating Characteristics of the Design

The statistical power to reject the null hypothesis under various treatment effect sizes using the Z test for comparing treatments is shown in Table 5.3A. This table shows that the target sample size of 428 patients has 90% power to detect a 0.66 hazard ratio.

Table 5.3B: Summary of Operating Characteristics

Hazard Ratio	Corresponding Approximate Effect Size for GRFS Endpoint	Expected Number of GRFS Events	Overall Cumulative Power	Probability of Stopping Early at Interim Analysis
0.57	20%	186	99.24%	0.5897
0.66	15%	219	90.00%	0.2548
0.68	14%	224	85.28%	0.2005
0.72	12%	232	72.66%	0.1179
0.76	10%	237	57.17%	0.0652
1.00	0.0	243	5.00%	0.0015

5.3.3 Guidelines for Safety Monitoring

Monitoring of a key safety endpoint will be conducted monthly, and if rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures. The stopping guidelines serve as a trigger for consultation with the DSMB for additional review and are not formal "stopping rules" that would mandate automatic closure of study enrollment. Toxicity, adverse events, and other safety endpoints will be monitored regularly and reported to the DSMB at each meeting and sooner if there are concerns.

The key safety endpoint for this study is Day 100 mortality. The rate of mortality will be monitored up to Day 100 post-randomization separately in each of the two treatment arms. The expected probability of Day 100 mortality after a reduced intensity conditioning transplant is 10-15%, based on CIBMTR data. Each month, the null hypothesis that the Day 100 mortality rate is less than or equal to 15% is tested separately in each treatment arm using an extension of the sequential probability ratio test (SPRT) for censored exponential data—See Appendix C for more details on SPRT.

This sequential testing procedure conserves type I error at 5% across all of the monthly examinations for a treatment arm. The censored exponential SPRT can be represented graphically. At each monthly examination, the total patient time at risk is plotted against the cumulative number of events. The continuation region of the SPRT is defined by two parallel lines. Only the upper boundary will be used for monitoring to protect against excessive Day 100 mortality. If the cumulative number of events falls above the upper boundary, the SPRT rejects the null hypothesis, and concludes that there are more events than predicted by the total time at risk. Otherwise, the SPRT continues until enrollment reaches the target sample size of 214 patients per arm.

This procedure assumes an exponential distribution for the time until death during the first 100 days and censors follow-up time at Day 100. Only deaths that occur on or before the patient has been followed for 100 days are counted. Total time on study is computed as time from randomization to death or to Day 100, whichever comes first, summed for all patients on study. A SPRT contrasting 15% versus 25% Day 100 mortality rate results in decision boundaries with a common slope of 0.8005 and an upper intercept of 4.8015 with nominal type I and II errors of 5.8% and 10%, respectively. Because of truncation of the SPRT at a finite sample size the actual type I and II errors will usually be lower than nominal levels.

The actual operating characteristics of the truncated test were determined in a simulation study that assumed uniform accrual of 428 participants over a 36-month period. The actual error rates are shown in Table 5.3C.

Table 5.3C: Operating Characteristics of the Sequential Probability Ratio Test for Day 100 Mortality with 100,000 Simulation Replicates

True Day 100 Mortality Rate	15%	17%	20%	23%	25%
Probability Reject Null	0.050	0.152	0.474	0.813	0.935
Mean Month Stopped	36	34	27	19	15
Mean Number of Day 100 Mortality Events	31	33	31	25	20
Mean Number of Patients Enrolled	207	195	157	108	82

For example, the testing procedure rejects the null hypothesis in favor of the alternative 5.0% of the time when the true Day 100 mortality rate is 15%, and 93.5% of the time when the rate is 25%. This corresponds to a type I error rate of $\alpha = 0.050$ and a type II error rate of $\beta = 0.065$. When the true Day 100 mortality rate is 25%, on average, the DSMB will be consulted 15 months after opening, when 20 events have been observed in 82 patients.

5.4 Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, race/ethnicity, performance status, primary disease, disease-specific risk categories, DRI, hematopoietic cell transplant comorbidity index (HCT CI), donor type and HLA matching, donor/recipient CMV status, donor/recipient sex match, donor/recipient ABO match, and conditioning regimen. Between group comparisons will be performed for continuous variables via a Kruskal-Wallis test and for categorical variables, via the chi-square test.

5.5 Analysis Populations

5.5.1 Primary Population

The intention-to-treat population will serve as the population for the primary analysis. All randomized patients will be included in this population. Patients will be included in the treatment

group to which they are randomized. Intention-to-treat population consists of all randomized patients whether or not treatment was administered.

5.5.2 Safety Population

The safety analysis population in this study will comprise of all patients "as treated" in the study. This population will be used for the analysis of safety data. The "as treated" population consists of all randomized patients who received a HCT with one of the two randomized GVHD prophylaxis regimens. Patients will be included in the treatment group corresponding to the study treatment (GVHD prophylaxis) they actually received for the analysis of safety data using the "as treated" population. For most patients this will be the treatment group to which they are randomized.

5.5.3 Transplant Population

The transplant population will include all patients who have received a transplant after randomization.

5.6 Analysis of Primary Endpoint

Kaplan-Meier curves along with 95% confidence intervals will be constructed to estimate GRFS probabilities at 1 year for each treatment group. The primary analysis will consist of a comparison of GRFS in the ITT population by treatment arm based on a multivariate Cox regression model. The following covariates will be adjusted for in the regression model: age, DRI, planned RIC regimen, donor type/HLA matching, and planned use of post-transplant maintenance therapy. A significance level of 0.05 (two-sided) will be used to test the null hypothesis of no difference between the treatments. Ninety-five percent confidence intervals for the hazard ratio of the PTCy/Tac/MMF treatment will also be constructed.

5.7 Analysis of Secondary Endpoints

5.7.1 Acute GVHD

Incidence of acute GVHD grade II-IV and grade III-IV at Days 100, 180 and 1 year will be estimated with 95% confidence intervals for each treatment group - using the cumulative incidence estimate, treating death prior to aGVHD as a competing event. Comparison of cumulative incidence will be done using Gray's test. A multivariate Cox regression model for the cause-specific hazard of aGVHD will be used to compare the treatment groups, after adjustment for baseline characteristics as described for the primary endpoint.

5.7.2 Chronic GVHD

Incidence of chronic GVHD at 1 year will be estimated with 95% confidence intervals for each treatment group using the cumulative incidence estimate, treating death prior to chronic GVHD as a competing event. Comparison of cumulative incidence will be done using Gray's test. A multivariate Cox regression model for the cause-specific hazard of chronic GVHD will be used to compare the treatment groups, after adjustment for baseline characteristics as described for the primary endpoint.

5.7.3 Systemic Immunosuppression-Free Survival

Proportions of patients alive, relapse free, and off immune suppression at 1 year will be described for each treatment group, along with 95% confidence intervals. If there is substantial censoring prior to one year, multistate models will be constructed to estimate these probabilities. Agreement between this endpoint and the primary endpoint of GRFS will be described using cross-tabulation frequencies and assessed using the Kappa statistic.

5.7.4 Hematologic Recovery

Probabilities of neutrophil recovery by Day 28 and Day 100 will be described with 95% confidence intervals for each treatment group using the cumulative incidence estimate, treating death as a competing event. Similarly, probabilities of platelet recovery by Day 60 and Day 100 will be described with 95% confidence intervals for each treatment group using the cumulative incidence estimate, treating death as a competing event. These cumulative incidence curves will be compared using Gray's test.

5.7.5 Donor Cell Engraftment

Donor chimerism at Day 28 and Day 100 after transplantation in each of the randomized treatment arms will be described numerically as median and range for those evaluable as well as according to proportions with full (>95% donor cell), mixed (5.0-94.9% donor cells), graft rejection (<5%), or death prior to assessment of donor chimerism. Incidence of secondary graft failure (chimerism <5% after initial donor cell engraftment) will be described for each arm using frequencies. Comparisons between quantitative donor chimerism will be done using Wilcoxon rank sum test, while comparisons between categorical donor chimerism groups will be done using chi-square tests.

5.7.6 Disease Relapse or Progression

Incidence of disease relapse or progression at 1 year will be estimated with 95% confidence intervals for each treatment group using the cumulative incidence estimate, treating death prior to disease relapse as a competing event. A multivariate Cox regression model for the cause-specific hazard of relapse or progression will be used to compare the treatment groups with the control group, after adjustment for baseline characteristics as described for the primary endpoint.

5.7.7 Transplant-related Mortality

Incidence of transplant-related mortality (TRM) at Days 100, 180 and 1 year will be estimated for each treatment group using the cumulative incidence estimate, treating disease relapse or progression as a competing event. A multivariate Cox regression model for the cause-specific hazard of TRM will be used to compare the treatment groups with the control group, after adjustment for baseline characteristics as described for the primary endpoint.

5.7.8 Toxicity

All Grade 3-5 toxicities will be tabulated by grade for each randomized treatment arm, by type of toxicity as well as the peak grade overall. Toxicity frequencies up to 1 year will be described for each time interval as well as cumulative over time.

5.7.9 Infections

The number of infections and the number of patients experiencing infections will be tabulated for each randomized treatment arm by type of infection, severity, and time period after transplant.

5.7.10 Disease-free Survival

Kaplan-Meier curves will be constructed to estimate 1 year disease free survival probabilities for each treatment group. A multivariate Cox regression model for the risk of death or relapse/progression will be used to compare the treatment groups, after adjustment for baseline characteristics as described for the primary endpoint.

5.7.11 Overall Survival

Kaplan-Meier curves will be constructed to estimate 1 year overall survival probabilities for each treatment group. A multivariate Cox regression model for the risk of death will be used to compare the treatment group, after adjustment for baseline characteristics as described for the primary endpoint.

5.7.12 Post-Transplant Lymphoproliferative Disease (PTLD)

Probabilities of PTLD at 1-year post transplant will be described with 95% confidence intervals for each treatment group using the cumulative incidence estimate, treating death as a competing event. These estimates will be compared between groups using Gray's test.

5.7.13 Patient-Reported Outcomes (PRO)

Patient-Reported Outcomes will be measured at baseline then at Days 100, 180 and one year post HCT. Using a repeated measures model, we will compute PRO composite scores and compare treatments after adjustment for baseline characteristics as described for the primary endpoint.

All patients with at least one post HCT PRO assessment will be incorporated within the relevant repeated measures analysis, whether they completed or discontinued the study. Missing assessments will not be imputed, as we assume these data are missing at random.

5.8 Safety Analysis

Adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events Version 5.0.

APPENDIX A

HUMAN SUBJECTS

1. Subject Consent

Candidates for the study will be identified as described in Chapter 4 of the protocol. The Principal Investigator or his/her designee at each transplant center will contact the candidates, provide them with information about the purpose of the study and obtain voluntary consent if the candidates agree to participate. The BMT CTN will provide a template of the consent form to each center. Each center will add their NMDP IRB-approved boiler-plate language to the consent and submit it for review by the NMDP Internal Review Board (IRB). The DCC will verify the adequacy of the consent forms prior to submission to the IRB. The NMDP IRB will provide evidence of IRB approval.

2. Confidentiality

Confidentiality will be maintained by individual names being masked and assigned a patient identifier code. The code relaying the patient's identity with the ID code will be kept separately at the center. The ID code will be transmitted to the network.

3. Participation of Women and Minorities

Women and ethnic minorities and other populations will be included in this study. Accrual of women and minorities at each center will be monitored to determine whether their rates of enrollment are reflective of the distribution of potentially eligible women and minorities expected from data reported to the CIBMTR and from published data on incidence of leukemia and lymphoma in these groups. Centers will be notified if their rates differ significantly from those expected and asked to develop appropriate recruitment reports.

Patients under the age of 18 years will not be eligible to enroll on this study. Reduced Intensity Conditioning (RIC) is not a standard therapy for patients in this age group. PBSC grafts are not used standardly in this age group for allogeneic transplants either.

APPENDIX B

CHIMERISM ASSESSMENT

We will study the proportion of patients with full (at least 95% or more) or mixed (5.0-94.9%) total donor chimerism or graft rejection (less than 5% total donor chimerism).

Chimerism analysis will be performed according to institutional practice, with the following as prioritization for analysis:

1. Sorted peripheral blood lymphocyte and myeloid populations
2. Unsorted peripheral blood mononuclear cell populations
3. Whole (unsorted) bone marrow

APPENDIX C

DERIVATION OF A SEQUENTIAL TEST STATISTIC FOR CENSORED EXPONENTIAL DATA

Background – The Sequential Probability Ratio Test

Let $f(.,\theta)$ be the density function for random variable X. According to Neyman and Pearson, the most powerful test of $H_0:\theta=\theta_o$ versus $H_1:\theta=\theta_1$ decides in favor of H_1 or H_0 if $L_n > c_\alpha$ or $L_n < c_\alpha$, respectively, where $L_n=\prod_i^n f(x_i;\theta_1)/f(x_i;\theta_0)$ is the likelihood ratio, and c_α is determined to have the size . When the sample size is not fixed in advance, further improvement is possible by using Wald's Sequential Probability Ratio Test (SPRT). The SPRT continues to sample as long as $B<L_n<A$ for some constant $B<1<A$, stops sampling and decides in favor of H_1 as soon as $L_n > A$, and stops sampling and decides in favor of H_0 as soon as $L_n < B$.

The usual measures of performance of such a procedure are the error probabilities and of rejecting H_0 when $\theta=\theta_0$, and of accepting H_0 when $\theta=\theta_1$, respectively, and the expected sample size $E(N\mid\theta_j)\equiv E_j(N)$. Wald and Wolfowitz showed that among all tests, sequential or not, for which $\Pr_0(\text{reject } H_0)\le\alpha$ and $\Pr_1(\text{reject } H_0)\le\beta$, and for which $E_j(N)$ are finite, j=0,1, the SPRT with error probabilities α and minimizes $E_0(N)$ and $E_1(N)$. If, in addition, the $x1, x2,...$ are independent and identically distributed (i.i.d.) with density function $f(x,\theta)$, with monotone likelihood ratio in $\tau(x)$, then any SPRT for testing θ_0 against $\theta_1(>\theta_0)$ has non-decreasing power function.

For the SPRT with error probabilities and , the SPRT boundaries are given approximately by $A=(1-\beta)/\alpha$ and $B=\beta/(1-\alpha)$. The operating characteristics of the SPRT are given by $O(\theta,\alpha,\beta,\theta_0,\theta_1)=(A^{h(\theta)}-1)/(A^{h(\theta)}-B^{h(\theta)})$ where $h(\theta)$ is the non-trivial solution to the equation $\int(f(x;\theta_1)/f(x,\theta_2))^{h(\theta)}f(x;\theta)dx=1$.

The formula $E(N;\theta)=[[(1-O(\theta)]\log A+O(\theta)\log B]/E(z;\theta)$ provides the average sample number for an arbitrary θ. The sample size distribution is very highly skewed, $Var(N)\approx[E(N)]^2$. Thus we will consider a truncated test with maximum sample size of N_0 and simulate to obtain the operating characteristics of the test.

Derivation of the SPRT for Uncensored Exponential Survival Times

For example, we wish to construct a sequential test for the composite null hypothesis that the rate of TRM at 180 days is less than or equal to 5% versus the alternative hypothesis that it is greater than or equal to 5%. For the derivation of the uncensored SPRT, we will require that the type I error of the test be less than 10%, and that the test provide 90% power to reject the null hypothesis under a specified alternative that the true rate is 10%. A maximum sample size of 250 patients will be permitted.

Let us assume that the survival times, $T_1, T_2, ..., T_n$, are completely observed (uncensored) and are i.i.d. with exponential density function $f(T,\theta) = \theta e^{-\theta T}$. These assumptions will be relaxed to incompletely observed data subsequently. In the exponential parameterization, a 180-day survival rate of 95% translates into a mean survival of 9.747 years ($\theta_0 = .1026$), and 90% translates into a mean survival of 4.746 years ($\theta_1 = .2107$).

The SPRT is derived with reference to a simple null and alternative hypothesis, in this case, $H_0 : \theta = \theta_o = .1026$ versus $H_1 : \theta = \theta_1 = .2107$. However, since the log-likelihood ratio for the exponential, $\log \prod_i^n f(x_i;\theta_1) - \log \prod_i^n f(x_i,\theta_0) = n(\log(\theta_1) - \log(\theta_0)) - (\theta_1 - \theta_0)\sum_i^n T_i$, is a monotone function of $\sum_i^n T_i$, the power of the test is non-decreasing in θ. Thus the SPRT is a one-sided level .10 test of a composite null ($H_0 : \theta \le \theta_o = .1026$) versus a composite alternative ($H_1 : \theta \ge \theta_o = .2107$), with power of $1 - \beta = .90$ at the selected alternative $\theta = \theta_1 = .2107$.

The SPRT can be represented graphically. The continuation region is bounded by two parallel lines with common slope $(\theta_1 - \theta_0)/(\log \theta_1 - \log \theta_0) = 0.150$, and intercepts $\log A/(\log \theta_1 - \log \theta_0) = 3.05$ and $\log B/(\log \theta_1 - \log \theta_0) = -3.05$ for the lower and upper bounds, respectively. As each individual unit is put on trial and observed to fail, the current sample size, *n*, is plotted against the cumulative sum of failure times. When this graph crosses the upper boundary, the null hypothesis is rejected.

The maximum sample size of 250 patients requires that the SPRT be truncated. We choose to truncate the SPRT by declaring that if the test has failed to terminate after 250 patients, that the null hypothesis will be accepted. Since the probability that the untruncated SPRT would reject the null at a sample size of 250 is negligible, it makes little difference how the final boundary value is selected, and this rule is chosen for simplicity.

Derivation of a Modified SPRT for Censored Exponential Data

The assumption of uncensored exponential survival times is flawed. However, we consider it reasonable to assume the hazard for TRM is constant over the first 180 days post-transplant, and we will restrict our attention to this time interval. Furthermore, it is not practical to conduct a

clinical study by putting each individual on trial, and waiting until that individual is observed to fail. We relax our assumptions as follows. Firstly, each individual's time on study will be computed as time from transplant to failure, or to the 180 day time point, whichever comes first. Secondly, we will put individuals on trial as soon as they become available, without waiting for the previous individual to fail.

Let us consider the impact of relaxing these assumptions one at a time. In a fixed sample size trial with uncensored exponential failure times, mean survival time is estimated by the sample mean of the failure times, or total time on study divided by the number of individuals enrolled. When censoring is introduced, the estimate becomes the total time on study divided by the number of observed (non-censored) failures. This suggests that in an exponential SPRT test modified to incorporate censoring, we replace the observed failure times, $T_1, T_2, ..., T_n$, with censored failures times, $x_1, x_2, ..., x_n$, and the current sample size, n, with the number of observed failures, d.

Now we relax the second assumption, and put individuals on trial as soon as they become available, without waiting for the previous individual to fail. Assume that three years are required for accrual of 250 patients to the study, and that the final analysis takes place 180 days after the last patient is entered. Putting all of this together, we propose a modified truncated SPRT, where at any interim time point, s, ranging from 0 to 3 years 180 days, the number of observed failures, *d(s)*, is plotted against the sum of observed time on study, $\sum_i^n X_i(s)$. In practice, monitoring will be scheduled monthly after the start of enrollment to the study. A further modification to the SPRT was to only use the upper boundary for stopping since the primary focus of the monitoring is to protect against unacceptable 180-day TRM rates.

Operating Characteristics of the Modified SPRT Test for Censored Exponential Data

Recall that the uncensored SPRT targeted a drop in TRM-free survival at Day 180 from 95% to 90%, with type I and II errors of 10% and 10%. Since only the upper boundary is used for monitoring, the continuation region of the test was bounded above by a line with a slope of 0.150 and intercept of 3.05. In our example, the sample size is large enough that the reduction in power due to truncation of the test is negligible compared to the increase in power because the modified SPRT, lacking a lower boundary, cannot stop early to "accept" the null hypothesis. In order to maintain type I error, we raise the upper boundary to make it harder to cross. Under the further assumption of uniform accrual over a three year period, and monthly interim analyses over the course of the study, the operating characteristics of the modified SPRT were obtained from a simulation study. These simulation show that an intercept of 4.02, corresponding to setting parameters and to 10% and 10%, result in empirical type I and II error rates of 10% and 10%.

Table C.1: Operating Characteristics of Sequential Testing Procedures from a Simulation Study with 100,000 Replications

True 180-Day Rate	**5%**	**10%**
Probability Reject Null	0.095	0.903
Mean Month Stopped	41.0	20.2
Mean # Endpoints in 180 days	11.8	11.6
Mean # Patients Enrolled	240.8	135.4

While the motivation for this testing procedure is largely heuristic rather than theoretical, the simulation results validate the approach. When the true rate of TRM on or before Day 180 was 5%, the test crossed the lower boundary in 9484 of 100,000 replications, for an estimated type I error rate of 9.5%. When the true rate of TRM on or before Day 180 was 10%, the test failed to cross the boundary in the in 9742 of 100,000 replications, for an estimated type II error rate of 9.7%. In this setting, on average, the boundary will be crossed at 20.2 months.

It is interesting to note that the SPRT derived above for exponential failure times with censoring at 180 days, has operating characteristics which are similar to those of a more traditional SPRT, derived for binomial variates with success probability equal to the 180 day failure rate. Using time to failure rather than a simple binary indicator of failure, leads to little improvement in power when failure times are censored relatively soon after entry on study. We speculate that if the constant hazard rate over the first 180 days were high, the exponential test would reject faster than the binomial test, but have not conducted simulation studies to demonstrate this.

APPENDIX D

KARNOFSKY PERFORMANCE STATUS SCALE

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

APPENDIX E

HCT-SPECIFIC COMORBIDITY INDEX SCORE

Comorbidities	Definition	Score
Migraine/headache		0
Osteoporosis		0
Osteoarthritis		0
Hypertension		0
Gastrointestinal	Including inflammatory bowel disease	0
Mild pulmonary	DLCo and/or FEV_1 >80% or Dyspnea on moderate activity	0
Mild renal	Serum creatinine 1.2-2 mg/dl	0
Endocrine		0
Bleeding		0
Coagulopathy	Deep venous thrombosis or pulmonary embolism	0
Asthma		0
Arrhythmia		1
Myocardial	Coronary artery disease, congestive HF, history of medically documented MI, EF≤50%	1
Mild hepatic	Chronic hepatitis, Bilirubin >ULN- 1.5 X ULN, or AST/ALT >ULN-2.5XULN	1
Cerebro-vascular accident	History of transient ischemic attack or cerebro-vascular accident	1
Morbid obesity		1
Diabetes	Requiring treatment	1
Depression/anxiety		1
Infection	Requiring continuation of treatment after Day 0	1
Rheumatologic	SLE, RA, polymyositis, mixed CTD, polymyalgia rheumatica	2
Moderate pulmonary	DLCo and/or FEV_1 66-80% or Dyspnea on slight activity	2
Peptic ulcer	Patients who have required treatment	2
Moderate-severe renal	Serum creatinine >2 mg/dl, on dialysis, or prior renal transplantation	2
Valvular heart disease	Except mitral valve prolapse	3
Prior solid tumor	Requiring treatment with chemotherapy	3
Moderate-severe hepatic	Liver cirrhosis, Bilirubin >1.5 X ULN, or AST/ALT >2.5XULN	3
Severe pulmonary	DLCo and/or FEV_1 ≤65% or Dyspnea at rest or requiring oxygen	3

Total score is the sum of all comorbidities present at time of transplantation.

APPENDIX F

2014 REFINED DISEASE RISK INDEX[24]

Participating sites to use column labeled as "New DRI Group" to report DRI of enrolled patient.

Disease	Stage	No. of patients	HR*	Original DRI	Percentage of patients	New DRI Group	2-y OS (%)	95% CI
Hodgkin lymphoma CR		126	0.36	Int	14	Low	66	63-68
CLL CR		81	0.47	Low		Low		
Mantle cell lymphoma CR		160	0.51	Int		Low		
Indolent NHL CR		183	0.53	Low		Low		
AML favorable cytogenetics CR		190	0.64	Low		Low		
Indolent NHL PR		276	0.71	Low		Low		
CLL PR		400	0.78	Low		Low		
CML chronic phase 1/2		390	0.82	Low		Low		
CML advanced phase		69	0.92	Int	63	Int	51	50-52
Mantle cell lymphoma PR		149	0.95	Int		Int		
Myeloproliferative neoplasm	Any	426	0.98	Int		Int		
AML intermediate cytogenetics CR		3611	Ref	Int		Int		
ALL CR1		1023	1.00	Int		Int		
T-cell NHL CR		171	1.00	Int		Int		
Multiple myeloma CR/VGPR/PR		339	1.03	Int		Int		
Aggressive NHL CR		181	1.05	Int		Int		
Low-risk MDS adverse cytogenetics	Early†	103	1.06	High		Int		
T-cell NHL PR		164	1.06	Int		Int		
Low-risk MDS intermediate cytogenetics	Early†	516	1.09	Int		Int		
HL PR		225	1.09	Int		Int		
Low-risk MDS intermediate cytogenetics	Advanced†	235	1.18	Int		Int		
Indolent NHL	Advanced†	128	1.21	Int		Int		
CLL	Advanced	265	1.22	Int		Int		
High-risk MDS intermediate cytogenetics	Early	364	1.24	Int		Int		
Aggressive NHL PR		205	1.26	Int		Int		
T-cell NHL	Advanced†	93	1.41	High	20	High	33	31-35
AML favorable cytogenetics	Advanced†	34	1.42	Int		High		
HL	Advanced†	85	1.48	High		High		
High-risk MDS intermediate cytogenetics	Advanced†	179	1.56	Int		High		
High-risk MDS adverse cytogenetics	Early	80	1.58	High		High		
ALL CR2		407	1.58	Int		High		
AML adverse cytogenetics CR		175	1.59	High		High		
Mantle cell lymphoma	Advanced†	46	1.59	High		High		
High-risk MDS adverse cytogenetics	Advanced†	30	1.59	Very high		High		
BL‡ CR		23	1.65	NA		High		
Multiple myeloma	Advanced†	150	1.65	High		High		
ALL CR3		61	1.70	Int		High		
Low-risk MDS adverse cytogenetics	Advanced†	32	1.86	Very high		High		
AML intermediate cytogenetics	Advanced	1227	1.89	High		High		
CML blast phase		52	2.02	Int	4	Very high	23	20-27
ALL	Advanced†	235	2.23	High		Very high		
Aggressive NHL	Advanced†	154	2.54	High		Very high		
AML adverse cytogenetics	Advanced †	76	2.83	Very high		Very high		
BL‡ PR	Advanced †	12	5.21	NA		Very high		

Int, intermediate.
*Hazard ratio for mortality compared with AML intermediate cytogenetics in CR1.
†Advanced stage refers to induction failure or active relapse, including stable or progressive disease for NHL, HL, and CLL.
‡Those categories were not included in the original DRI.

APPENDIX G

DIAGNOSIS AND SEVERITY SCORING FOR ACUTE AND CHRONIC GVHD

1. Acute GVHD organ staging and grading[22]

Stage	Skin (Active Erythema Only)	Liver (Bilirubin)	Upper GI	Lower GI (stool output/day)
0	No active (erythematous) GVHD rash	<2 mg/dL	No or intermittent nausea, vomiting, or anorexia	Adult: <500 mL/day or <3 episodes/day Child: <10 mL/kg/day or <4 episodes/day
1	Maculopapular rash <25% BSA	2-3 mg/dL	Persistent nausea, vomiting or anorexia	Adult: 500-999 mL/day or 3-4 episodes/day Child: 10-19.9 mL/kg/day or 4-6 episodes/day
2	Maculopapular rash 25-50% BSA	3.1-6 mg/dL		Adult: 1000-1500 mL/day or 5-7 episodes/day Child: 20-30 mL/kg/day or 7-10 episodes/day
3	Maculopapular rash >50% BSA	6.1-15 mg/dL		Adult: >1500 mL/day or >7 episodes/day Child: >30 mL/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) *plus* bullous formation and desquamation >5% BSA	>15 mg/dL		Severe abdominal pain with or without ileus or grossly bloody stool (regardless of stool volume).

Overall clinical grade (based on most severe target organ involvement):
Grade 0: No stage 1-4 of any organ.
Grade I: Stage 1-2 skin without liver, upper GI, or lower GI involvement.
Grade II: Stage 3 rash and/or stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI.
Grade III: Stage 2-3 liver and/or stage 2-3 lower GI, with stage 0-3 skin and/or stage 0-1 upper GI.
Grade IV: Stage 4 skin, liver, or lower GI involvement, with stage 0-1 upper GI.

2. Grading of Chronic GVHD (NIH Criteria)[23]

	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† **SCORE % BSA** ☐ *GVHD features to be scored by BSA:* **Check all that apply:** ☐ Maculopapular rash/erythema ☐ Lichen planus-like features ☐ Sclerotic features ☐ Papulosquamous lesions or ichthyosis ☐ Keratosis pilaris-like GVHD	☐ No BSA involved	☐ 1-18% BSA	☐ 19-50% BSA	☐ >50% BSA
SKIN FEATURES SCORE:	☐ No sclerotic features		☐ Superficial sclerotic features "not hidebound" (able to pinch)	**Check all that apply:** ☐ Deep sclerotic features ☐ "Hidebound" (unable to pinch) ☐ Impaired mobility ☐ Ulceration
Other skin GVHD features (NOT scored by BSA) **Check all that apply:** ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Poikiloderma ☐ Severe or generalized pruritus ☐ Hair involvement ☐ Nail involvement ☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
MOUTH *Lichen planus-like features present:* ☐ **Yes** ☐ **No**	☐ 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
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				

Organ scoring of chronic GVHD. ECOG indicates 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; ULN, normal upper limit. *Weight loss within 3 months. Skin scoring should use both percentage of BSA involved by disease signs and the cutaneous features scales. When a discrepancy exists between the percentage of total body surface (BSA) score and the skin feature score, OR if superficial sclerotic features are present (Score 2), but there is impaired mobility or ulceration (Score 3), the higher level should be used for the final skin scoring. To be completed by specialist or trained medical providers. **Lung scoring should be performed using both the symptoms and FEV1 scores whenever possible. FEV1 should be used in the final lung scoring where there is discrepancy between symptoms and FEV1 scores.

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist:* ☐ **Yes** ☐ **No** ☐ **Not examined**	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	☐ Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
GI Tract ***Check all that apply:*** ☐ Esophageal web/ proximal stricture or ring ☐ Dysphagia ☐ Anorexia ☐ Nausea ☐ Vomiting ☐ Diarrhea ☐ Weight loss ≥5%* ☐ Failure to thrive	☐ No symptoms	☐ Symptoms without significant weight loss* (<5%)	☐ Symptoms associated with mild to moderate weight loss* (5-15%) **OR** moderate diarrhea without significant interference with daily living	☐ Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
LIVER	☐ Normal total bilirubin and ALT or AP < 3 x ULN	☐ Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥ 3 x ULN	☐ Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	☐ Elevated total bilirubin > 3 mg/dL
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
LUNGS** **Symptom score**:	☐ 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)
Lung score: % FEV1 ☐	☐ FEV1≥80%	☐ FEV1 60-79%	☐ FEV1 40-59%	☐ FEV1 ≤39%
Pulmonary function tests ☐ Not performed				
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				

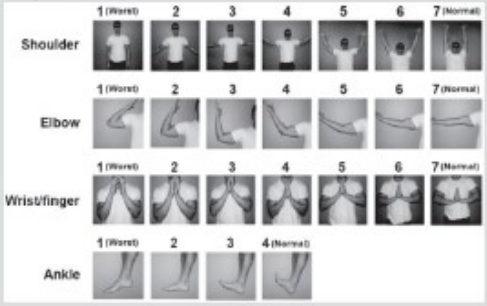
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **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.)
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GENITAL TRACT (*See Supplemental figure*‡) ☐ Not examined *Currently sexually active* ☐ Yes ☐ No	☐ No signs	☐ Mild signs‡ and females with or without discomfort on exam	☐ Moderate signs‡ and may have symptoms with discomfort on exam	☐ Severe signs‡ with or without symptoms
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

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

☐ Ascites (serositis)___	☐ Myasthenia Gravis___	
☐ Pericardial Effusion___	☐ Peripheral Neuropathy___	☐ Eosinophilia > 500/µl___
☐ Pleural Effusion(s)___	☐ Polymyositis___	☐ Platelets <100,000/µl ___
☐ Nephrotic syndrome___	☐ Weight loss>5%* without GI symptoms___	☐ Others (specify):_____

Overall GVHD Severity *(Opinion of the evaluator)* ☐ **No GVHD** ☐ **Mild** ☐ **Moderate** ☐ **Severe**

Photographic Range of Motion (P-ROM)



3. Categories of Acute and Chronic GVHD

Categories of Acute and Chronic GVHD			
Category	**Time of Symptoms after HCT**	**Presence of Acute GVHD Features**	**Presence of Chronic GVHD Features***
Acute GVHD			
Classic acute GVHD	≤100 d	Yes	No
Late-onset acute GVHD	>100 d	Yes	No
Chronic GVHD			
Classic chronic GVHD	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes

*As defined in section 4 (below)

4. Signs and Symptoms of Chronic GVHD[23]

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of chronic GVHD)	Distinctive* (Seen in chronic GVHD, but Insufficient Alone to Establish a Diagnosis)	Other Features or Unclassified Entities†	Common‡ (Seen with Both Acute and chronic GVHD)
Skin	Poikiloderma Lichen planus–like features Sclerotic features Morphea-like features Lichen sclerosus–like features	Depigmentation Papulosquamous lesions	Sweat impairment Ichthyosis Keratosis pilaris Hypopigmentation Hyperpigmentation	Erythema Maculopapular rash Pruritus
Nails		Dystrophy Longitudinal ridging, splitting or brittle features Onycholysis Pterygium unguis Nail loss (usually symmetric, affects most nails)		
Scalp and body hair		New onset of scarring or nonscarring scalp alopecia (after recovery from chemoradiotherapy) Loss of body hair Scaling	Thinning scalp hair, typically patchy, coarse or dull (not explained by endocrine or other causes) Premature gray hair	
Mouth	Lichen planus–like changes	Xerostomia Mucoceles Mucosal atrophy Ulcers Pseudomembranes		Gingivitis Mucositis Erythema Pain
Eyes		New onset dry, gritty, or painful eyes Cicatricial conjunctivitis KCS Confluent areas of punctate keratopathy	Photophobia Periorbital hyperpigmentation Blepharitis (erythema of the eyelids with edema)	
Genitalia	Lichen planus–like features Lichen sclerosus–like features	Erosions Fissures		
Females	Vaginal scarring or clitoral/labial agglutination	Ulcers		
Males	Phimosis or urethral/meatus scarring or stenosis			
GI Tract	Esophageal web Strictures or stenosis in the upper to mid third of the esophagus		Exocrine pancreatic insufficiency	Anorexia Nausea Vomiting Diarrhea Weight loss Failure to thrive (infants and children
Liver				Total bilirubin, alkaline phosphatase > 2 × upper limit of normal ALT > 2 × upper limit of normal
Lung	Bronchiolitis obliterans diagnosed with lung biopsy BOS§	Air trapping and bronchiectasis on chest CT	Cryptogenic organizing pneumonia Restrictive lung disease‖	
Muscles, fascia, joints	Fasciitis Joint stiffness or contractures secondary to fasciitis or sclerosis	Myositis or polymyositis¶	Edema Muscle cramps Arthralgia or arthritis	
Hematopoietic and Immune			Thrombocytopenia Eosinophilia Lymphopenia Hypo- or hyper-gammaglobulinemia Autoantibodies (AIHA, ITP) Raynaud's phenomenon	
Other			Pericardial or pleural effusions Ascites Peripheral neuropathy Nephrotic syndrome Myasthenia gravis Cardiac conduction abnormality or cardiomyopathy	

ALT indicates alanine aminotransferase; AIHA, autoimmune hemolytic anemia; ITP, idiopathic thrombocytopenic purpura.
* In all cases, infection, drug effect, malignancy, or other causes must be excluded.
† Can be acknowledged as part of the chronic GVHD manifestations if diagnosis is confirmed.
‡ Common refers to shared features by both acute and chronic GVHD.
§ BOS can be diagnostic for lung chronic GVHD only if distinctive sign or symptom present in another organ (see text).
‖ Pulmonary entities under investigation or unclassified.
¶ Diagnosis of chronic GVHD requires biopsy.

APPENDIX H

PATIENT REPORTED OUTCOME QUESTIONS

BMT CTN 1703 Hemorrhagic Cystitis questions

During the past 7 days, how many days did you see blood in your urine?

1. No days
2. 1 day
3. 2 days
4. 3-5 days
5. 6-7 days

How often did you feel like you needed to empty your bladder right away or else you would have an accident?

1. Never
2. One time during the past 7 days
3. 2-6 times during the past 7 days
4. Often once a day
5. More than once a day

PROMIS questions

[GISX38] During the past 7 days, how many days did you have loose or watery stools?

1. No days
2. 1 day
3. 2 days
4. 3-5 days
5. 6-7 days

[GISX42] How often did you feel like you needed to empty your bowels right away or else you would have an accident?

1. Never
2. One time during the past 7 days
3. 2-6 times during the past 7 days
4. Often once a day
5. More than once a day

[PFA6] Does your health now limit you in bathing or dressing yourself?

1. Not at all
2. Very little
3. Somewhat
4. Quite a lot
5. Cannot do

[PFA53] Are you able to run errands and shop?

1. Without any difficulty
2. With a little difficulty
3. With some difficulty
4. With much difficulty
5. Unable to do

[PFA21] Are you able to go up and down stairs at a normal pace?

1. Without any difficulty
2. With a little difficulty
3. With some difficulty
4. With much difficulty
5. Unable to do

PSRPSAT06r1] I am satisfied with my ability to do things for my family.

1. Not at all
2. A little bit
3. Somewhat
4. Quite a bit
5. Very much

[SRPSAT49r1] I am satisfied with my ability to perform my daily routines.

1. Not at all
2. A little bit
3. Somewhat
4. Quite a bit
5. Very much

[SRPSAT09r1] I am satisfied with my ability to do the work that is really important to me (include work at home).

1. Not at all
2. A little bit
3. Somewhat
4. Quite a bit
5. Very much
6.

LEE CHRONIC GVHD SYMPTOM SCALE questions

On a scale of 0 to 4, indicate how much you have been bothered by the following problems <u>in the past month</u>:

Skin:

- Abnormal skin color
- Rashes
- Thickened skin
- Sores on skin
- Itchy skin

Eyes and Mouth:

- Dry eyes
- Need to use eye drops frequently
- Difficulty seeing clearly
- Need to avoid certain foods due to mouth pain
- Ulcers in mouth
- Receiving nutrition from an intravenous line or feeding tube

Breathing:

- Frequent cough
- Colored sputum
- Shortness of breath with exercise
- Shortness of breath at rest
- Need to use oxygen

Eating and Digestion:

- Difficulty swallowing solid foods
- Difficult swallowing liquids
- Vomiting
- Weight loss

Muscles and Joints:

- Joint and muscle aches
- Limited joint movement
- Muscle cramps
- Weak muscles

Energy:

- Loss of energy
- Need to sleep more/take naps
- Fevers

Mental and Emotional:

- Depression
- Anxiety
- Difficulty sleeping

APPENDIX I

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APPENDIX J

Mi-Immune Study

BLOOD AND MARROW TRANSPLANT CLINICAL TRIALS NETWORK

Microbiome and Immune Reconstitution in Cellular Therapies and Hematopoietic Stem Cell Transplantation (Mi-Immune)

BMT CTN Protocol 1801

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CHAPTER 1

1 BACKGROUND AND SIGNIFICANCE

Allogeneic HCT is an effective treatment for patients with malignant and non-malignant hematologic diseases. However, this treatment is complicated by high rates of morbidity and mortality limiting its broader application. The leading causes of post-transplant morbidity and mortality include acute and chronic GVHD, relapse and infectious disease. The goal of the trial is to apply a systems biology approach to understanding the mechanisms driving these complications, such that evidence-based treatment strategies can be devised. Results of this study may also inform the development of accurate and predictive surrogate markers of clinically relevant endpoints that may be informative for future BMT clinical trial design.

The sample repository and molecular and clinical databases established through the trial will be made available to the biomedical community and are expected to facilitate studies that will establish the utility of molecular biomarkers for risk assessment, diagnosis and monitoring, and allow more rational treatment strategies to be developed for patients undergoing allogeneic HCT. These studies are also likely to provide mechanistic insights and to identify new therapeutic targets leading to development of more targeted and effective therapies.

The goal of this protocol is several-fold: First, it is to test the primary hypothesis that the engraftment stool microbiome diversity (determined by 16s rRNA sequencing analysis of the sample closest after, but within 14 days of neutrophil engraftment) predicts 1-year non-relapse mortality in patients undergoing reduced-intensity allogeneic HCT. Second, it is to perform additional analyses on patient samples to answer key questions concerning: a) the impact of the stool microbiome on transplant outcome as well as the impact of transplant on the microbiome and the associated downstream impact on patient health; and b) reconstitution of the T cell repertoire after transplant. Third, it is to establish a cohort of biologic samples collected prospectively from patients treated on BMT CTN 1703 that will be a shared biospecimen resource for conducting future allogeneic HCT correlative studies. The resulting dataset will become an additional resource generated by the trial and will be made available to the biomedical community. Mi-Immune is designed to link molecular data and biospecimens with high quality clinical phenotype and outcomes data to identify risk factors for development and severity of key complications after allogeneic HCT.

To achieve this goal, patients and donors will be recruited and consent will be obtained at the time that they enroll on BMT CTN protocol 1703. Samples will be collected: (1) from patients and donors pre-transplant; and, (2) from patients post-transplant on a calendar-driven schedule through the first year post-HCT. Shared clinical data will be collected in the context of the primary transplant protocol, BMT CTN 1703, and Mi-Immune-specific data will be collected using Case Report Forms (CRFs). Additional clinical data will be available from data submitted to the Center for International Blood and Marrow Transplant Research (CIBMTR) using the CIBMTR CRFs.

CHAPTER 2

2 STUDY DESIGN

2.1 Study Overview

In this study, we will collect stool, urine, and blood biospecimens, as well as detailed clinical data on infections and antibiotic exposures in an attempt to understand the microbial and immune recovery predictors of HCT outcomes. This study will form the correlative science arm of the BMT CTN 1703 study (PROGRESS III), which will compare two acute graft-versus-host disease (aGVHD) prophylaxis regimens: tacrolimus/methotrexate (Tac/MTX) versus post-transplant cyclophosphamide/tacrolimus/mycophenolate mofetil (PTCy/Tac/MMF) in the setting of reduced intensity conditioning (RIC) allogeneic peripheral blood stem cell (PBSC) transplantation.

2.2 Hypothesis and Objectives

2.2.1 Hypothesis

The engraftment stool microbiome diversity (determined within 14 days after neutrophil engraftment) predicts 1-year non-relapse mortality.

2.2.2 Objectives

Primary Objective:

The goal of this protocol is to test the primary hypothesis that the engraftment stool microbiome diversity (determined by 16s rRNA sequencing analysis of the sample closest after, but within 14 days of neutrophil engraftment) predicts 1-year non-relapse mortality in patients undergoing reduced-intensity allogeneic HCT.

Secondary Objectives:

Microbiome, Infection, and Antimicrobial Exposure:

1) Association of engraftment microbiome diversity with development of aGVHD, cGVHD, and overall survival (corrected for GVHD prophylaxis).

2) Association of baseline microbiome diversity with development of aGVHD, cGVHD, and overall survival (corrected for GVHD prophylaxis).

3) Effect of GVHD prophylaxis on microbiome diversity: Difference in the level of microbiome diversity detected at 6, 12, and 24 months in the 2 arms.

4) Association of microbiome oligodomination at the time of engraftment (>30% of microbiome being a single operational taxonomic unit) with increased subsequent risk of bloodstream infection.

5) Association of volume of antimicrobial exposure (number and duration of drugs) in first 30 days after transplant with rates of aGVHD, cGVHD, and overall survival.

6) Association between urine metabolites (such as indoxyl sulfate) at the time of engraftment and rate of acute GVHD.

Immune Repertoire:

1) Effect of GVHD prophylaxis on TCR diversity: Difference in the level of TCR diversity detected at 3, 6, 12 and 24 months in the 2 arms.

2) Effect of TCR diversity at 3 months, 6 months, 12 months and 24 months on subsequent non-relapse mortality, overall survival, and relapse, using landmark analyses and time-dependent covariates.

Biorepository:

1) To establish a cohort of biologic samples and a linked clinical and molecular dataset from patients treated on BMT CTN 1703. The product of this study will be a shared biospecimen and data resource for conducting future allogeneic HCT mechanistic studies. Examples of future exploratory objectives that can be addressed with this biorepository are the following:

Exploratory Objectives:

1) Effect of antibiotic use (length of exposure, anaerobic sparing vs. not) on level of microbiome diversity at 6, 12, and 24 months.

2) Effect of aGVHD and/or its treatment on microbiome at 6, 12 and 24 months.

3) Effect of cGVHD and/or its treatment on microbiome at 12 and 24 months.

4) Effect of CMV and BK reactivation on TCR diversity at 3, 6, 12 and 24 months.

5) Effect of patient age on TCR diversity at 3, 6, 12, and 24 months.

6) Effect of donor age on TCR diversity at 3, 6, 12, and 24 months.

7) Effect of aGVHD and/or its treatment on TCR diversity at 3, 6, 12 and 24 months.

8) Effect of cGVHD and/or its treatment on TCR diversity at 12 and 24 months.

9) Correlation of microbiome diversity and TCR diversity at 3, 6, 12, and 24 months.

2.3 Eligibility Criteria for Enrollment

The primary intention of the Mi-Immune protocol is to co-enroll patients on BMT CTN 1703. Patient inclusion and exclusion criteria are those of BMT CTN 1703. Related and unrelated donors must be at least 18 years of age.

2.4 Treatment Plan

The conditioning, GVHD prophylaxis, and supportive care are described in BMT CTN 1703.

CHAPTER 3

3 STUDY ENDPOINTS AND DEFINITIONS

3.1 Primary Endpoint

The primary endpoint is non-relapse mortality within one year. This endpoint is defined according to the BMT CTN 1703 protocol. This primary endpoint will be associated with the engraftment stool microbiome diversity (grouped by tertiles) for the primary analysis of this study.

3.2 Secondary Endpoints

3.2.1 Clinical Endpoints

Standard transplant endpoints and clinical outcomes, with the exception of infections and antimicrobial use, will be collected according to BMT CTN 1703, including long-term follow-up data collection through CIBMTR. The endpoints collected on BMT CTN 1703 include acute GVHD, chronic GVHD, non-relapse mortality, relapse, death and cause of death. In addition, a feasibility endpoint (i.e., proportion of samples successfully collected on schedule), and exploratory analyses of donor/recipient pairs will be included.

3.2.2 Infection

Data on infections and antimicrobial agent administration occurring through Day 365 will be collected specifically for patients with Mi-Immune samples collected. The incidence of definite and probable viral, fungal and bacterial infections will be tabulated. The infections will be reported using the BMT CTN Advantage eClinical data reporting system.

3.2.3 Correlative endpoints

3.2.3.1 Microbiome

The microbiome will be analyzed in two ways:

First, the microbiome taxonomic diversity will be evaluated using 16S ribosomal RNA amplicon sequencing. Data will be generated per standard protocols, as previously described.[5] Resultant sequencing data will be quality filtered, adapter sequences will be trimmed, and sequences will be analyzed using the QIIME pipeline.[6] Shannon diversity will be calculated, as previously described.[7]

Second, urinary indoxyl sulfate has been used as a biomarker of microbiome composition.[8] We will measure urinary indoxyl sulfate using liquid chromatography followed by mass spectrometry, as previously described.[9]

3.2.3.2 T cell Repertoire

T cell repertoire will be analyzed using next generation sequencing on the Adaptive Biotechnologies ImmunoSEQ platform. PBMCs from patients will be collected at 3, 6, 12 and 24 months post HCT. TCR diversity will be assessed using the inverse Simpson's diversity index (1/Ds), which sums the frequency of each clonotype[1].

CHAPTER 4

4 PATIENT EVALUATION

4.1 Pre-transplant Evaluations

All pre-transplant evaluations occur as dictated by BMT CTN 1703. The data will be captured in the BMT CTN 1703 forms and not re-entered for Mi-Immune.

Additional Pre-transplant requirements for Mi-Immune to be collected no sooner than 7 days prior to the start of conditioning regimen:

1. Specimen Collection: Urine, stool, and blood will be collected pre-conditioning (Day -14 to Day -7) and pre-infusion (Day -1 to Day 0). Please note that stool sample collection at pre-conditioning is MANDATORY and urine sample collection is OPTIONAL.
2. A CBC will be performed pre-conditioning (Day -14 to Day -7) and pre-infusion (Day -1 to Day 0).
3. Infection data collection at baseline.

4.2 Post-transplant Evaluations

1. For all enrolled HCT patients, the empty bag or syringe in which the stem cell product was infused will be retrieved by the study team and sent to the Central Reference Laboratory at ambient temperature where it will be washed to obtain donor cells for analysis.
2. Toxicities associated with specimen collection for Mi-Immune will be collected and reported on days 28, 56, 98, 180, 270, and 365 post-transplant. All other toxicities will be reported for BMT CTN 1703.
3. Specimen collection (blood, urine, and stool) for microbiome assays will occur weekly starting on day 0 through day 77 (through day 84 for blood), then at day 98, day 180, day 270, 1 year, and 2 years.
    - Weekly stool sample collection for microbiome assays starting Day 0 weekly through Day 28 is MANDATORY.
    - Weekly stool sample collection for microbiome assays starting day 35 through day 77, then at day 98, 180, 270, 1 year, and 2 years are OPTIONAL.
    - Weekly urine sample collection for microbiome assays starting Pre-conditioning through Day 270, then at 1 year, and 2 years are ALL OPTIONAL.
4. A CBC will be performed weekly from day 0 through day 77 (through day 84 for blood), then day 98, 180, 270, 365, And 730.
5. Infection data: This will occur every 14 days from day 0 until day 98, then on day 180, 270, and 365 post-transplant.
6. Antimicrobial medications: Specific medications including route of administration, start date, and stop date will be collected every 14 days from day 0 to day 98. Medications prior to start of conditioning and on day 180, 270, and 365 post-transplant will be listed with route of administration for the 7 days prior to the form date.

Table 4.2: Patient and Donor Evaluations

		Pre-conditioning (Day -14 to -7)	Pre-infusion (Day -1 to 0)	0	7 ± 3	14 ± 3	21 ± 3	28 ± 3	35 ± 3	42 ± 3	49 ± 3	56 ± 3	63 ± 3	70 ± 3	77 ± 3	84 ± 3	98 ± 7	180 ± 28	270 ± 28	365 ± 28	730 ± 60
Patient Data	**CBC**	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Infections	X		X		X		X		X		X		X		X	X	X	X	X	
	Antimicrobial medications	X		X		X		X		X		X		X		X	X	X	X	X	
	Toxicities							X				X					X	X	X	X	
Patient Samples	**Peripheral Blood**	X***	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Urine[1]	X***	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X
	Stool[2]	X***	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X
Related Donor Samples	**CBC**		X*																		
	Peripheral Blood		X*																		
	Urine		X*																		
	Stool		X*																		
	Hematopoietic Stem Cell Product Cells			X**																	
Unrelated Donor Samples	**Hematopoietic Stem Cell Product Cells**			X**																	

*Pre-donation.

**Empty bag or syringe in which the stem cell product was infused.

***Samples should be collected Pre-conditioning, pre-antibiotic prophylaxis on Day -14 to Day -7.

[1] Weekly urine sample collection for microbiome assays starting Pre-conditioning through Day 270, then at 1 year, and 2 years are ALL OPTIONAL

[2] Weekly stool sample collection for microbiome assays at Pre-conditioning, Pre-infusion/Day 0 weekly through Day 28 is MANDATORY. Weekly stool sample collection for microbiome assays starting day 35 through day 77, then at day 98, 180, 270, 1 year, and 2 years are OPTIONAL.

4.3 Collection of Research Samples

Blood, stool, urine and biopsy research samples will be collected in this study. The majority of study samples will be collected on a calendar driven schedule. Based on historical experience with BMT CTN 1202, we anticipate high compliance (1202 calendar-driven samples were collected at a rate of 96% completeness).

4.3.1 Study Blood Draws

For transplant patients, the blood sample collections will occur at baseline (defined as pre-conditioning, pre-antibiotic prophylaxis) at day -7 to day -14 prior to start of conditioning. Samples will then be collected weekly from day 0 (pre-infusion) through day 84 (+/- 3 days), then on day +98 +/- 7 days, day +180 +/- 28 days, day +270 +/- 28 days, day +365 +/- 28 days, day +730 +/- 60 days as outlined in Table 4.2. Related donor samples will be drawn prior to the peripheral blood stem cell collection.

4.3.2 Stool Samples

Stool samples will be collected according to standard operating procedures modified from the NIH Human Microbiome Project protocols. Briefly, samples will be stored at 4^{o}C within 30 minutes of collection and then will be shipped to NMDP within 24-48 hours. At NMDP, the samples will be aliquoted and stored at -80^{o}C. Samples will be collected with and without preservative solutions to enable downstream DNA, RNA and mass spectrometry (metabolite and metaproteome) analysis. Selected centers with substantial microbiome sampling experience will collect and process samples for additional downstream transcriptome and volatile metabolite sampling, which requires rapid processing. For transplant patients, the stool sample collections will occur at baseline (defined as pre-conditioning, pre-antibiotic prophylaxis) at day -7 to day -14 prior to start of conditioning. Samples will then be collected weekly from day 0 through day 77 (+/- 3 days), then on day +98 +/- 7 days, day +180 +/- 28 days, day +270 +/- 28 days, day +365 +/- 28 days, day +730 +/- 60 days as outlined in Table 4.2.1 (see Laboratory Procedures Table for inpatient and outpatient stool collection schedule). 5-10ml of stool will be obtained at each timepoint and placed into a sterile container. Stool sample will then be placed on ice (4^{o}C) and transferred to the lab.

4.3.3 Urine Samples

Urine metabolites such as indoxyl sulfate are associated with GVHD and specific intestinal microbiome compositions. Urine collections will be performed according to the standard operating procedures outlined in the Laboratory Procedures Table. For transplant patients, the urine sample collections will occur at baseline (defined as pre-conditioning, pre-antibiotic prophylaxis) at day -7 to day -14 prior to start of conditioning. Samples will then be collected weekly from day 0 through day 77 (+/- 3 days), then on day +98 +/- 7 days, day +180 +/- 28 days, day +270 +/- 28 days, day +365 +/- 28 days, day +730 +/- 60 days as outlined in Table 4.2. Samples will be placed at 4^{o}C within 30 minutes of collection and for no more than 48 hours prior to aliquoting and deep freeze storage.

CHAPTER 5

5 STATISTICAL CONSIDERATIONS

5.1 Study Overview

This study is designed as a companion study to the BMT CTN 1703 Phase III randomized, open label, multicenter trial to compare PTCy/Tac/MMF versus Tac/MTX for GVHD prophylaxis in patients with controlled malignant disease receiving an allogeneic PBSC transplant after a RIC regimen. Patients enrolled on this study will be asked to provide additional study samples which will be linked with the clinical data collected on the parent protocol, in order to address specific research questions associated with those study samples and to provide banked samples for future studies.

5.1.1 Accrual

Accrual will occur in conjunction with the BMT CTN 1703 trial. The target enrollment of the parent trial is 428 patients; we plan to enroll approximately 70%, or n=300 patients, on this companion protocol. It is estimated that 36 months of accrual will be necessary to enroll the targeted sample size. The study will remain open until the last recipient enrolled has been followed for two years post-transplant.

5.1.2 Randomization

Patients on the BMT CTN 1703 trial will be randomized at a ratio of 1:1 between the treatment arms; no further randomization will be done on this companion protocol.

5.1.3 Primary Endpoint

The primary endpoint for this study is non-relapse mortality within 1 year of transplant.

5.1.4 Primary Objective

The primary objective of Mi-Immune is to compare NRM within 1 year between groups defined by engraftment stool microbiome diversity. Tertiles will be used to define 3 approximately equal sized groups.

5.1.5 Sample Size and Power Considerations

The primary analysis will be done using a Fine and Gray model comparing the non-relapse mortality within 1 year between the three groups. Non-relapse mortality by 1 year is expected to be approximately 15% overall based on BMT CTN 1203 trial data. Using the method of Latouche (2004), we computed power to detect differences of 20% in the 1 year NRM between any two pairs of groups, using a Bonferroni adjusted type I error of 0.05/3 to account for pairwise comparisons[2]. The table below shows various comparisons of this difference in NRM over an expected range of NRM for this protocol; the targeted sample size of 300 evaluable patients would have at least 85% power to detect a 20% difference in 1 year NRM between any two microbiome diversity groups.

Table: Power to detect 20% differences in 1 year NRM between any two microbiome diversity groups:

NRM difference	Power
5% vs. 25%	99%
10% vs. 30%	93%
15% vs. 35%	85%

5.2 Interim Analysis and Pausing Guidelines

As this is a non-interventional correlative study, no interim analyses will be included specifically as part of this protocol, separate from those used in BMT CTN 1703.

5.3 Demographic and Baseline Characteristics

Demographics and baseline characteristics will be described for all patients and separately for each cohort. Characteristics to be examined are: age, gender, race/ethnicity, performance status, primary disease, disease-specific risk categories, DRI, hematopoietic cell transplant comorbidity index (HCT CI), donor type and HLA matching, donor/recipient CMV status, donor/recipient sex match, donor/recipient ABO match, and conditioning regimen. Between group comparisons will be performed for continuous variables via a Kruskal-Wallis test and for categorical variables, via the chi-square test. Additionally, we will compare the distributions of these characteristics between patients with Mi-Immune samples collected vs. those enrolled on BMT CTN 1703 but who did not provide Mi-Immune samples, to assess whether the Mi-Immune cohort appears representative of the larger trial.

5.4 Analysis Populations

5.4.1 Engraftment stool Microbiome Population

All enrolled patients with an available engraftment stool microbiome diversity measure will be included in this population. The primary analysis as well as secondary analyses focusing on the impact of the engraftment stool microbiome diversity on clinical outcomes will use this population. Outcomes are determined from the time of the engraftment stool sample collection.

5.4.2 Baseline Microbiome Population

All enrolled patients with an available baseline stool microbiome diversity measure will be included in this population. This population will be used for secondary analyses looking at the impact of baseline microbiome diversity on subsequent clinical outcomes. Outcomes will be determined from the time of transplant.

5.4.3 Landmark Microbiome Populations

Various landmark microbiome populations will be used to examine the impact of microbiome diversity measures at that timepoint on subsequent clinical outcomes, or to compare microbiome

diversity measures at that timepoint between groups such as randomized GVHD prophylaxis strategy. In each case, patients will be included if they are still at risk for the clinical outcome at the landmark time, and have an available microbiome diversity measure at that timepoint. Clinical outcomes will be assessed starting at that landmark timepoint.

5.4.4 Antimicrobial exposure population

All enrolled patients with complete data collection on antimicrobial exposure within the first 30 days and who are still at risk for the clinical outcome at day 30 will be included in this landmark antimicrobial exposure cohort. The antimicrobial exposure population will be used to examine the impact of antimicrobial exposure within 30 days on subsequent clinical outcomes. Clinical outcomes will be assessed starting at 30 days.

5.4.5 Landmark TCR diversity Populations.

Various landmark TCR diversity populations will be used to examine the impact of TCR diversity measures at that timepoint on subsequent clinical outcomes, or to compare TCR diversity measures at that timepoint between groups such as randomized GVHD prophylaxis strategy. In each case, patients will be included if they are still at risk for the clinical outcome at the landmark time, and have an available TCR diversity measure at that timepoint. Clinical outcomes will be assessed starting at that landmark timepoint.

5.5 Analysis Plan

5.5.1 Analysis of the Primary Objective: Compare NRM within 1 year between groups defined by engraftment stool microbiome diversity.

This analysis will use the engraftment stool microbiome cohort. Microbiome diversity will be separated into three groups based on tertiles for the primary analysis. Non-relapse mortality will be summarized for each group using cumulative incidence with relapse as a competing event and compared in a univariate manner between tertiles using Gray's test. A Fine and Gray model will be used to compare microbiome diversity groups after adjustment for baseline covariates; microbiome diversity as well as GVHD prophylaxis received will be forced into the model and stepwise selection will be used to identify additional variables associated with outcome. Interactions between GVHD prophylaxis received and microbiome diversity will be assessed. A secondary analysis will further examine the functional form of the relationship between quantitative values of microbiome diversity and the subdistribution hazard for NRM using splines, and if appropriate, an optimal cutpoint will be determined.

5.5.2 Analysis of the Secondary Objectives

Microbiome, Infection, and Antimicrobial Exposure:

1) Association of engraftment microbiome diversity with development of aGVHD, cGVHD, and overall survival (corrected for GVHD prophylaxis).

   This analysis will use the engraftment stool microbiome cohort; diversity will be grouped by tertiles or if a more appropriate classification is identified in the primary analysis, that

may be used instead. aGVHD (grade 2-4 or 3-4) and chronic GVHD (any or moderate-severe) will be described in each group using cumulative incidence with death or relapse as a competing event, and compared between groups using Gray's test. Overall survival will be described using the Kaplan-Meier estimator and compared between groups using the log-rank test. Multivariate models will be constructed in a similar manner as for the primary endpoint using Fine and Gray models for aGVHD and cGVHD, and using Cox models for OS.

2) Association of baseline microbiome diversity with development of aGVHD, cGVHD, and overall survival (corrected for GVHD prophylaxis).

   This analysis will use the baseline stool microbiome cohort; diversity at baseline will be separated into three groups based on tertiles for initial analysis. aGVHD (grade 2-4 or 3-4) and chronic GVHD (any or moderate-severe) will be described in each group using cumulative incidence with death or relapse as a competing event, and compared between groups using Gray's test. Overall survival will be described using the Kaplan-Meier estimator and compared between groups using the log-rank test. Multivariate models will be constructed in a similar manner as for the primary endpoint using Fine and Gray models for aGVHD and cGVHD, and using Cox models for OS. We will also consider further examination of the functional form of the relationship between quantitative values of baseline microbiome diversity and the risk of aGVHD, cGVHD, or death using splines, and if appropriate, an optimal cutpoint will be determined.

3) Effect of GVHD prophylaxis on microbiome diversity: Difference in the level of microbiome diversity detected at 6, 12, and 24 months in the 2 arms.

   This analysis will use a series of landmark microbiome populations at each time point. Microbiome diversity will be compared between GVHD prophylaxis groups at each time point using Mann-Whitney tests; randomized GVHD prophylaxis groups will be used rather than GVHD prophylaxis received to maintain the ITT principle, however a secondary analysis using the GVHD prophylaxis received may also be performed. Transformation of diversity will be considered to induce normality. If a normalizing transformation can be identified, analysis of covariance will be used to compare diversity at each time point, after adjustment for baseline diversity and other covariates; stepwise selection will be used to identify additional covariates associated with diversity.

4) Association of microbiome oligodomination at the time of engraftment (>30% of microbiome being a single operational taxonomic unit) with increased subsequent risk of bloodstream infection.

   This analysis will use the engraftment stool microbiome population. The number of bloodstream infections and the number of patients experiencing a bloodstream infection between engraftment and day 100 will be described by presence of microbiome oligodomination group. Prevalence of active bloodstream infection will be described at subsequent visit times by microbiome oligodomination group, and compared using the chi-

square test. Additional details on organisms will also be summarized. Bloodstream infection density in the first 100 days will be computed as infection frequencies over 100 patient days at risk. If there are sufficient numbers of bloodstream infection events in the first 100 days, multivariate regression analyses will be performed to examine the association between microbiome oligodomination and infection density, while adjusting for other relevant covariates. Proportional rates/means models will be used to model the infection density, with a robust variance estimator to account for within patient correlation; microbiome oligodomination and GVHD prophylaxis received will be forced into the model, and additional variables will be added using stepwise regression[3]. Interaction between microbiome oligodomination and GVHD prophylaxis received will be assessed.

5) Association of volume of antimicrobial exposure (number and duration of drugs) in first 30 days after transplant with rates of aGVHD, cGVHD, and overall survival.

   This analysis will use the antimicrobial exposure population. Volume of antimicrobial exposure will be computed by measuring the "daily defined dose" exposure for each individual. This value is calculated on a per antimicrobial basis. Each day that a patient is given a specific antimicrobial agent counts as one daily defined dose. Thus, if a patient receives two different antimicrobials for one day each, the patient has received 2 daily defined doses. Alternatively, if a patient receives one antimicrobial for three days, the patient has received 3 daily defined doses. For combination drugs, such as trimethoprim/sulfamethoxazole, the daily defined doses will be calculated individually for each of the two active antimicrobial components of the combination therapy[4]. Volume of antimicrobial exposure will be dichotomized at the median. aGVHD (grade 2-4 or 3-4) and chronic GVHD (any or moderate-severe) will be described in each group using cumulative incidence with death or relapse as a competing event, and compared between groups using Gray's test. Overall survival will be described using the Kaplan-Meier estimator and compared between groups using the log-rank test. Multivariate models will be constructed in a similar manner as for the primary endpoint using Fine and Gray models for aGVHD and cGVHD, and using Cox models for OS.

6) Association between urine metabolites (such as indoxyl sulfate) at the time of engraftment and rate of acute GVHD.

   This analysis will use the engraftment stool microbiome cohort; urine metabolites at the time of engraftment will be dichotomized at the median. aGVHD (grade 2-4 or 3-4) will be described in each group using cumulative incidence with death or relapse as a competing event, and compared between groups using Gray's test. Multivariate models will be constructed in a similar manner as for the primary endpoint using Fine and Gray models for aGVHD.

Immune Repertoire:

1) Effect of GVHD prophylaxis on TCR diversity: Difference in the level of TCR diversity detected at 3, 6, 12 and 24 months in the 2 arms.

This analysis will use a series of landmark TCR diversity populations at each time point. TCR diversity will be compared between GVHD prophylaxis groups at each time point using Mann-Whitney tests; randomized GVHD prophylaxis groups will be used rather than GVHD prophylaxis received to maintain the ITT principle, however a secondary analysis using the GVHD prophylaxis received may also be performed. Transformation of diversity will be considered to induce normality. If a normalizing transformation can be identified, analysis of covariance will be used to compare diversity at each time point, after adjustment for baseline diversity and other covariates; stepwise selection will be used to identify additional covariates associated with diversity.

2) Effect of TCR diversity at 3 months, 6 months, 12 months and 24 months on subsequent non-relapse mortality, overall survival, and relapse, using landmark analyses and time-dependent covariates

   This analysis will use a landmark TCR diversity population at each time point. TCR diversity will be dichotomized as above or below the median at each corresponding landmark time point, and cumulative incidence of non-relapse mortality and relapse will be estimated in each group, with relapse and death in remission respectively as competing risks. Survival will be estimated in each group using Kaplan-Meier method. We will examine the impact of TCR diversity over time as a time-dependent covariate in Cox models for the cause specific hazard for NRM or relapse, or for the hazard of death; GVHD prophylaxis received will be forced into the models, and other covariates will be considered using stepwise variable selection. We will also explore the functional form of the relationship between TCR diversity and outcome using splines and if appropriate will identify an optimal cutpoint.

LABORATORY PROCEDURES TABLE

Study Subject	Research Sample Type	Time Points	Sample Quantity	Shipping Temperature to BMT CTN Biorepository	Sample Collection or Shipping Container	Processed Research Sample Material	Research Sample Material Aliquots	Storage Temperature
Patient (N = 300)	***Peripheral Blood***	Pre-conditioning, Day 14, 28, 98, 180, 365, 730	**39 mL**	Insulated Ambient	5 mL Clot Tube	Serum	5 - 0.5 mL aliquots	-80^0 C
					2 mL EDTA Tube	Whole Blood	2 - 1.0 mL aliquots	
					32 mL in Sodium Heparin Tubes	Heparin Plasma	15 - 0.5 mL aliquots	
						PBMC	~3 - 5x10^6 / 1 mL aliqouts	LN
		Day 7, 21, 35, 42, 49, 56, 63, 70, 77, 84, 270	**31 mL**	Insulated Ambient	5 mL Clot Tube	Serum	5 - 0.5 mL aliquots	-80^0 C
					2 mL EDTA Tube	Whole Blood	2 - 1.0 mL aliquots	
					24 mL in Sodium Heparin Tubes	Heparin Plasma	15 - 0.5 mL aliquots	
						PBMC	~2 - 5x10^6 / 1 mL aliqouts	LN
		Pre-infusion	**24 mL**	Insulated Ambient	5 mL Clot Tube	Serum	5 - 0.5 mL aliquots	-80^0 C
					24 mL in Sodium Heparin Tubes	Heparin Plasma	15 - 0.5 mL aliquots	
						PBMC	~2 - 5x10^6 / 1 mL aliqouts	LN
	Urine	Pre-conditioning, Weekly Day 0 to Day 77, Day 98, 180, 270, 365, 730	**10-12 mL**	2-8^0 C	Sterile Specimen Container	Urine - No Preservative	10 - 1.0 mL aliquots	-80^0 C
	Stool	Pre-conditioning, Weekly Day 0 to Day 77, Day 98, 180, 270, 365, 730	**10-12 mL**	2-8^0 C	Sterile Specimen Container	Stool - No Preservative	4 - 1 to 1.5 mL aliquots	-80^0 C
						Stool in RNAlater	2 - 1.5 mL processed aliquots	

Study Subject	Research Sample Type	Time Points	Sample Quantity	Shipping Temperature to BMT CTN Biorepository	Shipping Temperature to Leslie Kean Lab	Sample Collection or Shipping Container	Processed Research Sample Material	Research Sample Material Aliquots	Storage Temperature
Related Donor (N= ~120)	***Peripheral Blood***	Pre-donation	**41 mL**	Insulated Ambient		5 mL Clot Tube	Serum	5 - 0.5 mL aliquots	-80^0 C
						4 mL EDTA Tube	Whole Blood	4 - 1.0 mL aliquots	
						32 mL Sodium Heparin Tubes	Heparin Plasma	15 - 0.5 mL aliquots	
							PBMC	~3 - $5x10^6$ / 1 mL aliqouts	LN
	Urine	Pre-donation	**10-12 mL**	$2\text{-}8^0$ C		Sterile Specimen Container	Urine - No Preservative	10 - 1.0 mL aliquots	-80^0 C
	Stool	Pre-donation	**10-20 mL**	$2\text{-}8^0$ C		Sterile Specimen Container	Stool - No Preservative	4 - 1 to 1.5 mL aliquots	-80^0 C
							Stool in RNAlater	2 - 1.5 mL processed aliquots	
All Donors (*Will need protocol-specific donor consent*)	***Hematopoietic Stem Cell Product Cells***	Day 0	**Product Bag or Syringe**		$2\text{-}8^0$ C	Empty bag or syringe in which the stem cell product was infused	Viably Cryopreserved WBC	2-4 - $10x10^6$ / 1 mL aliqouts	LN

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