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A Multi-Center, Phase II Trial of HLA-Mismatched Unrelated Donor Bone Marrow Transplantation with Post-Transplantation Cyclophosphamide for Patients with Hematologic Malignancies

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Resource for Clinical Investigation in Blood and Marrow Transplantation (RCI BMT) PROTOCOL 15-MMUD Version 4.0

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PROTOCOL SYNOPSIS – RCI BMT PROTOCOL 15-MMUD

A Multi-Center, Phase II Trial of Transplantation of HLA-Mismatched Unrelated Donor Bone Marrow Transplantation with Post-Transplantation Cyclophosphamide for Patients with Hematologic Malignancies

Sponsor: National Marrow Donor Program®

Principal Investigator(s): Javier Bolaños Meade, MD and Bronwen Shaw, MD, PhD

Study Design: This is a multi-center, single arm Phase II study of hematopoietic cell transplantation (HCT) using human leukocyte antigen (HLA)-mismatched unrelated bone marrow transplantation donors and post-transplantation cyclophosphamide (PTCy), sirolimus and mycophenolate mofetil (MMF) for graft versus host disease (GVHD) prophylaxis in patients with:

1. Acute lymphoblastic leukemia (ALL)/T lymphoblastic lymphoma (T-LBL), acute myelogenous leukemia (AML), acute biphenotypic leukemia (ABL), or acute undifferentiated leukemia (AUL) in 1st or subsequent complete remission (CR)
2. Myelodysplastic syndrome (MDS), fulfilling the following criteria:
    a. Subjects with de novo MDS who have or have previously had Intermediate-2 or High risk disease as determined by the International Prognostic Scoring System (IPSS). Current Intermediate-2 or High risk disease is <u>not</u> a requirement.
    b. Subjects must have < 20% bone marrow blasts, assessed within 60 days of informed consent.
    c. Subjects may have received prior therapy for the treatment of MDS prior to enrollment.
3. Chronic lymphocytic leukemia (CLL) in complete response (CR) (if reduced intensity conditioning [RIC] is to be used), or complete or partial response (PR) (if full intensity conditioning [FIC] is to be used).
4. Chronic myeloid leukemia (CML) in 1st or subsequent chronic phase characterized by <10% blasts in the blood or bone marrow.
5. Chemotherapy-sensitive lymphoma in status other than 1st CR.

Primary Objective: The primary objective is to determine overall survival (OS) 1-year after HLA-mismatched unrelated donor (MMUD) bone marrow transplantation using PTCy, sirolimus and MMF to prevent GVHD.

Secondary Objectives: Subjects enrolled in this study will also be followed for the following endpoints: progression-free survival (PFS) at Day+180 and 1-year post-HCT, cumulative incidence of neutrophil recovery, cumulative incidence of platelet recovery, cumulative incidence of primary graft failure, donor chimerism, cumulative incidences of acute GVHD (aGVHD) and chronic GVHD (cGVHD), cumulative incidences of viral reactivations and infections, transplant-related mortality (TRM) at Day+100, Day+180, and 1-year post-HCT, cumulative incidence of relapse/progression, cumulative incidences of thrombotic microangiopathy (TMA) and hepatic veno-occlusive disease (VOD)/ sinusoidal obstruction syndrome (SOS), and incidence of donor clonal hematopoiesis. The proportion of subjects

proceeding to HCT after informed consent will be described. The donor selection characteristics will also be described. Time to donor identification from the search request will be described. Analysis of long-term survivors will be performed for subjects who consent to the CIBMTR Research Database protocol using routine data collection forms and time points per CIBMTR requirements.

Eligibility Criteria: Subjects must be between the ages of 15 and 70 years with the diagnosis of a hematologic malignancy as described above and with a partially (4/8 – 7/8) HLA-MMUD defined by high resolution typing at HLA-A, -B, -C and –DRB1. Subjects must have adequate organ function defined as: 1) left ventricular ejection fraction (LVEF) at rest ≥ 35% (RIC cohort) or LVEF at rest ≥ 40% (FIC cohort), or shortening fraction (LVFS) ≥ 25%; 2) diffusing capacity of the lungs for carbon monoxide (DLCO), forced expiratory volume (FEV_1), forced vital capacity (FVC) ≥ 50% predicted; 3) total bilirubin ≤ 2.5 mg/dL, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) < 5 x upper limit of normal (ULN) (unless ALT, AST, and/or ALP are disease related); 4) serum creatinine (SCr) within normal range for age, or creatinine clearance (CrCl) > 40 mL/min/1.73m^2 if SCr outside normal range for age; 5) Karnofsky or Lansky performance score ≥ 60%; and 6) if applicable, > 3 months since a previous autologous HCT.

Treatment Descriptions:

The **conditioning regimen** will consist of one of the following:

Regimen A (RIC). Recommended schedule as below:

1. Fludarabine 30 mg/m^2/day intravenously (IV) on Days –6, –5, –4, –3, –2
2. Cy 14.5 mg/kg/day IV on Days –6, –5
3. Total body irradiation (TBI) 200 centigray (cGy) on Day –1
4. Day 0 will be the day of infusion of non-T-cell depleted bone marrow

Although a schedule is proposed above, the regimen can be given according to institutional standards as long as the prescribed doses are the same as in the recommended regimen above.

Regimen B (FIC). Recommended schedule as below:

1. Busulfan ≥ 9mg/kg total dose (IV or oral (PO)) on Days -6, -5, -4, -3
   (Pharmacokinetic (PK) monitoring required to achieve a daily area under the curve (AUC) target of 4800-5300 μM*min (see section 2.6.1))

2a. Cy 50mg/kg/day IV on Days -2, -1

OR

1. Busulfan ≥ 9mg/kg total dose (IV or oral (PO)) on Days -6, -5, -4, -3
   (Pharmacokinetic (PK) monitoring required to achieve a daily area under the curve (AUC) target of 4800-5300 μM*min (see section 2.6.1))

2b. Fludarabine 30 mg/m^2/day IV on Days –6, –5, –4, –3, –2

3. Day 0 will be the day of infusion of non-T-cell depleted bone marrow

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Although a schedule is proposed above, the regimen can be given according to institutional standards as long as the prescribed doses, and busulfan monitoring, are the same as in the recommended regimen above.

Regimen C (FIC). Recommended schedule as below:

1. Cy 50mg/kg/day IV on Days -5, -4
2. TBI 200cGy twice a day on Days -3, -2, -1
3. Day 0 will be the day of infusion of non-T-cell depleted bone marrow

Although a schedule is proposed above, the regimen can be given according to institutional standards as long as the prescribed doses are the same as in the recommended regimen above.

Post-Transplant Treatments

The **GVHD prophylaxis regimen** will consist of:

1. Cy 50 mg/kg/day IV on Days+3, +4
2. Mesna recommendation: Mesna IV dose ≥ 80% of the total daily dose of Cy, given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy Institutional standards for mesna use and dosing may be used when necessary.
3. Sirolimus (PO) beginning on Day+5 with dose adjusted to maintain a level of 5-15 ng/mL through Day+180
4. MMF 15 mg/kg PO TID beginning on Day+5, maximum dose 1g PO TID (maximum daily dose 3g/day) through Day+35

The **Hematopoietic Growth Factor regimen** will consist of:

1. Granulocyte-colony stimulating factor (G-CSF) or a biosimilar given at a dose of 5 mcg/kg/day (actual body weight) IV or subcutaneously (SC) (rounding to the nearest vial dose is allowed) beginning Day+5 until absolute neutrophil count (ANC) ≥ 1,000/mm^3 for 3 consecutive days

Accrual Objective: The target sample size is 80 subjects accrued into two strata: 40 subjects receiving FIC and 40 subjects receiving RIC.

Accrual Period: The estimated accrual period is 2 years.

Study Duration: Subjects will be followed for 1-year post-HCT.

Interim Analysis: There will be no formal interim analyses for efficacy. Monitoring of two key safety endpoints (overall mortality and grade III-IV aGVHD by Day+100) will be conducted weekly. If a stopping rule as described in table 5.4 is triggered, enrollment will be paused while the Data and Safety Monitoring Board (DSMB) conducts a review of the safety data.

Stopping Guidelines: See table 5.4.

STUDY SCHEMA

Aim: To determine OS at 1-year after HLA-MMUD bone marrow HCT using PTCy, sirolimus and MMF to prevent GVHD.

Recipient Inclusion Criteria:

1. Age ≥ 15 years and < 71 years at the time of signing the informed consent form. Note: HIV-negative subjects with MDS must be < 50 years of age at time of signing the informed consent form.
2. Partially HLA-MMUD: HLA typing will be performed at high resolution (allele level) for the HLA-A, -B, -C, and –DRB1 loci; a minimum match of 4/8 at HLA-A, -B, -C, and –DRB1 is required
3. Product planned for infusion is bone marrow
4. Disease and disease status:
    a. ALL/T-LBL; AML; ABL; AUL in 1st or subsequent CR
    b. MDS, fulfilling the following criteria:
        i. Subjects with de novo MDS who have or have previously had Intermediate-2 or High risk disease as determined by the IPSS. Current Intermediate-2 or High risk disease is not a requirement.
        ii. Subjects must have < 20% bone marrow blasts, assessed within 60 days of informed consent.
        iii. Subjects may have received prior therapy for the treatment of MDS prior to enrollment
    c. CLL in:
        i. CR if RIC is to be used OR
        ii. CR or PR if FIC is to be used
    d. CML in 1st or subsequent chronic phase characterized by <10% blasts in the blood or bone marrow
    e. Chemotherapy-sensitive lymphoma in status other than 1st CR
5. Performance status: Karnofsky or Lansky score ≥ 60% (Appendix A)
6. Adequate organ function defined as:
    a. Cardiac: LVEF at rest ≥ 35% (RIC cohort) or LVEF at rest ≥ 40% (FIC cohort), or LVFS ≥ 25%
    b. Pulmonary: DLCO, FEV_1, FVC ≥ 50% predicted by pulmonary function tests (PFTs). DLCO value may be corrected or uncorrected for hemoglobin.
    c. Hepatic: total bilirubin ≤ 2.5 mg/dL, and ALT, AST, and ALP < 5 x ULN (unless ALT, AST, and/or ALP are disease related)
    d. Renal: SCr within normal range for age (see table 2.3A). If SCr is outside normal range for age, CrCl > 40 mL/min/1.73m^2 must be obtained (measured by 24-hour (hr) urine specimen or nuclear glomerular filtration rate (GFR), or calculated GFR (by Cockcroft-Gault formula for those aged ≥ 18 years; by Original Schwartz estimate for those < 18 years) (see table 2.3B))
7. Subjects ≥ 18 years of age must have the ability to give informed consent according to applicable regulatory and local institutional requirements. Legal guardian permission must be obtained for subjects < 18 years of age. Pediatric subjects will be included in age appropriate discussion in order to obtain assent.
8. Subjects with documentation of confirmed HIV-1 infection (i.e. HIV-positive), and a hematologic malignancy who meets all other eligibility requirements must:

a. Receive only RIC regimen (i.e. Regimen A)
b. Be willing to comply with effective antiretroviral therapy (ARV)
c. Have achieved a sustained virologic response for 12 weeks after cessation of hepatitis C antiviral treatment (in HIV-positive subjects with hepatitis C)

Recipient Exclusion Criteria:

1. Suitable HLA-matched related or 8/8 allele matched (HLA-A, -B, -C, -DRB1) unrelated donor available. This exclusion does not apply to HIV-positive subjects who have a CCR5delta32 homozygous donor.
2. Autologous HCT < 3 months prior to the time of signing the informed consent form
3. Females who are breast-feeding or pregnant
4. HIV-positive subjects:
    a. Acquired immunodeficiency syndrome (AIDS) related syndromes or symptoms that may pose an excessive risk for transplantation-related morbidity as determined by the Treatment Review Committee (see Appendix D).
    b. Untreatable HIV infection due to multidrug ARV resistance. Subjects with a detectable or standard viral load > 750 copies/mL should be evaluated with an HIV drug resistance test (HIV-1 genotype). The results should be included as part of the ARV review (described in Appendix D).
    c. May not be currently prescribed ritonavir, cobacistat and/or zidovudine
5. Current uncontrolled bacterial, viral or fungal infection (currently taking medication with evidence of progression of clinical symptoms or radiologic findings)
6. Prior allogeneic HCT
7. Primary myelofibrosis or myelofibrosis secondary to essential thrombocythemia or polycythemia vera
8. HIV-negative subjects with MDS may not receive RIC and must be < 50 years of age at the time of signing the informed consent form.

Donor Inclusion Criteria:	**Donor Exclusion Criteria:**
1. Must be unrelated to the subject and matched at between 4/8 and 7/8 HLA alleles for HLA-A, -B, -C, and –DRB1 2. Donors must meet NMDP medical suitability requirements for bone marrow donation. 3. Donors must undergo eligibility screening according to current FDA requirements. Donors who do not meet one or more of the donor screening requirements may donate under urgent medical need. 4. Must have the ability to give informed consent according to applicable NMDP donor regulatory requirements 5. Must agree to donate bone marrow	1. Recipient positive anti-donor HLA antibodies against a mismatched allele in the selected donor determined by either: a. a positive crossmatch test of any titer (by complement-dependent cytotoxicity or flow cytometric testing) or b. the presence of anti-donor HLA antibody to any HLA locus (HLA-A, -B, -C, –DRB1, -DQB1, -DQA1, -DPB1, -DPA1) with mean fluorescence intensity (MFI) >1000 by solid phase immunoassay

Primary Endpoint:	**Secondary Endpoints:**
OS is defined as the time from HCT to death from any cause, loss to follow up or end of study, whichever comes first (through 1-year).	• PFS at Day+180 and 1-year post-HCT • TRM at Day+100, day+180 and 1-year post-HCT • Cumulative incidence of neutrophil recovery • Cumulative incidence of platelet recovery • Cumulative incidence of primary graft failure • Donor chimerism • Cumulative incidences of aGVHD and cGVHD • Cumulative incidences of viral reactivations and infections (Cytomegalovirus (CMV), Epstein–Barr virus (EBV), BK virus, Adenovirus (ADV), human herpesvirus 6 (HHV-6)) • Cumulative incidence of relapse/progression • Cumulative incidences of TMA and VOD/SOS • Incidence of development of donor clonal hematopoiesis • Proportion of subjects proceeding to HCT after informed consent • Donor selection characteristics • Time to donor identification from search request • Analysis of long-term survivors

Conditioning Regimens:	**Post-Transplant Treatments:**
Regimen A (RIC). Recommended schedule as below: 1. Fludarabine 30 mg/m^2/day intravenously (IV) on Days –6, –5, –4, –3, –2 2. Cy 14.5 mg/kg/day IV on Days –6, –5 3. Total body irradiation (TBI) 200 centigray (cGy) on Day –1 4. Day 0 will be the day of infusion of non-T-cell depleted bone marrow **Although a schedule is proposed above, the regimen can be given according to institutional standards as long as the prescribed doses are the same as in the recommended regimen above.**	**GVHD Prophylaxis regimen:** • Cy 50 mg/kg/day IV on Days+3, +4 • Mesna recommendation: Mesna IV dose ≥ 80% of the total daily dose of Cy, given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy. Institutional standards for mesna use and dosing may be used when necessary. • Sirolimus (PO) beginning on Day+5 with dose adjusted to maintain a level of 5-15 ng/mL through Day+180 • MMF 15 mg/kg PO TID beginning on Day+5, maximum dose 1g PO TID (maximum daily dose 3g/day) through Day+35

Regimen B (FIC). Recommended schedule as below: 1. Busulfan ≥ 9mg/kg total dose (IV or oral (PO)) on Days -6, -5, -4, -3 **(Pharmacokinetic (PK) monitoring required** to achieve a daily area under the curve (AUC) target of 4800-5300 μM*min (see section 2.6.1)) 2a. Cy 50mg/kg/day IV on Days -2,-1 **OR** 1. Busulfan ≥ 9mg/kg total dose (IV or oral (PO)) on Days -6, -5, -4, -3 **(Pharmacokinetic (PK) monitoring required** to achieve a daily area under the curve (AUC) target of 4800-5300 μM*min (see section 2.6.1)) 2b. Fludarabine 30 mg/m^2/day IV on Days –6, –5, –4, –3, –2 3. Day 0 will be the day of infusion of non T-cell depleted bone marrow **Although a schedule is proposed above, the regimen can be given according to institutional standards as long as the prescribed doses, and Busulfan monitoring, are the same as in the recommended regimen above.** **Regimen C (FIC). Recommended schedule as below:** 1. Cy 50mg/kg/day IV on Days -5, -4 2. TBI 200cGy twice a day on Days -3, -2, -1 3. Day 0 will be the day of infusion of non-T-cell depleted bone marrow **Although a schedule is proposed above, the regimen can be given according to institutional standards as long as the prescribed doses are the same as in the recommended regimen above.**	**Hematopoietic Growth Factor regimen:** • G-CSF or a biosimilar given at a dose of 5 mcg/kg/day IV or SC beginning on Day+5 until ANC ≥ 1,000/mm^3 for 3 consecutive days

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1 BACKGROUND AND RATIONALE

1.1 Background

Allogeneic HCT is a potentially curative therapy for a variety of hematologic diseases, including the acute and chronic leukemias, myelodysplasia, lymphoma, and multiple myeloma. Clinical data now confirm that T-cells contained within the donor graft are capable of exerting a "graft-versus-malignancy" effect (Horowitz et al., 1990). However, successful application of allogeneic HCT to patients with hematologic diseases is limited by the toxicity related to the allogeneic donor cells which result in GVHD, a life threatening complication. Development of GVHD is associated with lower OS and thus the prevention of GVHD is a cornerstone of allogeneic HCT therapy (Choi & Reddy, 2014).

Transplantation using cells from HLA-identical sibling donors results in the lowest incidence of GVHD, and such donors are prioritized; however, only approximately 30% of patients have an HLA-identical sibling donor. Alternative sources of stem cells for those without an HLA-identical sibling include unrelated donors, umbilical cord blood and HLA-mismatched (haploidentical) related donors (Kekre & Antin, 2014). When using these donor sources, more intensive strategies are required to reduce the incidence of rejection and/or GVHD resulting from greater differences in both major and minor histocompatibility antigens.

Several methods may overcome the drawbacks associated with HLA-mismatched transplantation. In vivo T-cell depletion using alemtuzumab or anti-thymocyte globulin (ATG) is well recognized to reduce the incidence of both acute and chronic GVHD and graft rejection but is associated with delayed immune reconstitution and subsequent problems with infection, particularly reactivation of viruses (Potter et al., 2014; Chakraverty et al., 2010; Walker et al., 2016; Bacigalupo et al., 2006).

Several studies indicate that administering high dose, PTCy decreases the incidence of acute and chronic GVHD after HLA-mismatched transplantation without an adverse effect on immune reconstitution. The immunobiologic rationale for administering Cy after HCT is that recently activated, alloreactive T-cells (the cells most responsible for GVHD) are selectively sensitive to the toxic effects of this drug (Mayumi, Umesue, & Nomoto, 1996).

This strategy was initially tested in HCT using cells from partially HLA-mismatched (HLA-haploidentical) relatives in two independent single center clinical trials with favorable results (Luznik et al., 2008). Eighty-eight consecutive patients of all ages with high-risk hematologic malignancies were accrued to these trials between 1999 and 2006. Transplantation conditioning consisted of Cy 14.5 mg/kg/day IV on Days –6 and –5, fludarabine 30 mg/m^2/day IV on Days –6 to –2, and 200 cGy of TBI on Day –1. On Day 0, patients received donor bone marrow, which was obtained in a targeted collection of 4 x 10^8 nucleated cells/kg recipient weight and depleted of red blood cells by processing on a Gambro Spectra apheresis instrument. On Day+3, 50 mg/kg Cy was administered. A subset of patients received an additional dose of Cy on Day+4. Pharmacologic prophylaxis of GVHD was initiated on the day following completion of PTCy. All patients received tacrolimus and MMF. Patients received filgrastim 5 mcg/kg daily starting at Day+5 and continuing until recovery of neutrophils to >1000/μL for three days. The outcomes of these studies showed: graft failure in 18% (73% of whom had measurable disease); complete donor chimerism (>95%) in patients with sustained engraftment by 2 months after HCT;

cumulative incidences of grades II-IV and III-IV aGVHD by Day+200 of 35% and 10%, respectively; cumulative incidences of overall and extensive cGVHD in the first year after HCT of 22% and 14%, respectively. The cumulative incidences of non-relapse mortality (NRM) at 180 days and 1-year after HCT were 13% and 19%, respectively. The cumulative incidences of relapse at 1 and 2 years after HCT were 50% and 57%, respectively. At a median follow-up of survivors of 817 days (range, 112-1808 days), the actuarial OS of the entire group at 1 and 2 years were 45% and 35%, respectively.

Based on these encouraging results, the BMT CTN initiated a Phase II study to test this approach in a multi-center setting (Brunstein, et al., 2011). Fifty patients (<70 years) with high-risk leukemia or lymphoma were included. Only one patient had primary graft failure and all engrafting patients had 100% donor chimerism at Day+56. The 100-day cumulative incidence of grade II-IV aGVHD was 32%.The 1-year cumulative incidences of NRM and relapse were 7% and 45%, respectively, with 1-year probabilities of OS and PFS of 62% and 48%, respectively.

Some disease states continue to have a higher non-engraftment rate in this setting. Unpublished data from Johns Hopkins University reveals that in the haploidentical non-myeloablative setting, patients with active CLL have a 7-fold increase risk of graft-failure. Other groups have found higher rates of graft failure in patients with CLL in the non-myeloablative setting (Delgado et al., 2006).

These studies, and others, show that use of PTCy with additional post-HCT immune suppression allows successful outcomes using very mismatched donors. Clinical practice has now evolved to include PTCy as standard of care GVHD prophylaxis for patients receiving cells from haploidentical donors (Bashey & Solomon, 2014). However, there are several drawbacks to use of haploidentical related donors. Related donors often fall outside the norms used for accepting unrelated donors (i.e. >60 or <18, or presence of comorbidities) with several important consequences for both the patient and the donor.

The use of older donors is associated with worse clinical outcomes for recipients. Kollman et al. (2001) showed, in a retrospective analysis of 6978 unrelated bone marrow transplants, that patients receiving cells from an unrelated donor under the age of 30 had significantly better survival than those with older donors ($p = 0.0002$). Younger donors were associated with a significantly lower risk of severe aGVHD among HLA-mismatched pairs. Incidences at Day+100 were 30%, 41%, and 40%, respectively, with donors aged 18 to 30 years, 31 to 45 years, and more than 45 years ($p < 0.0001$). A significantly lower risk of cGVHD was also found with younger donors ($p = 0.02$). These data were confirmed in a later study (Kollman et al., 2015) including 6349 unrelated donor transplant recipients, which again showed that using donors under the age of 32 conferred a significant survival advantage ($p <0.001$). As in the previous study, the incidence of GVHD was lower with a younger donor. This publication included a recent validation subset of 4690 HLA-matched (8/8 HLA-A, -B, -C, and –DRB1 loci) donor-recipient pairs which showed a linear impact of donor age on patient survival, with younger donor ages associated with higher survival ($p = .006$). Importantly, all three of these studies showed that the effect of donor age was present irrespective of the degree of HLA matching between the pair, suggesting that it is an independent risk factor for survival and GVHD.

Finally, a European Blood and Marrow Transplant group study (Kroger et al., 2012) of 719 MDS patients over the age of 50 years showed that patients transplanted from a younger unrelated donor (<30 years) had significantly higher 5-year OS in comparison with those transplanted from a related donor or an older unrelated donor (>30 years): 40% vs 33% vs 24% (p = 0.04). Other data do not confirm this finding of better outcomes with an unrelated compared to a related donor (Alousi et al., 2013).

Additionally, other non-HLA donor selection factors (such as ABO status, sex and parity), which are variably associated with differences in HCT outcomes, can often not be prioritized in the related donor setting (Kollman et al., 2015). Although there is controversy about the impact of ABO matching on HCT outcome, large recent CIBMTR studies suggest a survival advantage with ABO matching (Pidala, et al., 2014). Although the impact of ABO matching has not been studied in the haploidentical setting, it is an important logistical issue when transplanting bone marrow, as mismatching requires red cell depleting procedures, which may result in a loss of stem cells, potentially increasing the risk of poor engraftment. The ability to select amongst donors to avoid ABO mismatching is an attractive option, seldom available when choosing among related donors. Use of female donors is also associated with reduced engraftment and parous donors are associated with increased cGVHD (Shaw et al., 2005; Kollman et al., 2001). In the haploidentical PTCy setting, transplanting female donor bone marrow into a male recipient is associated with an increase in the probability of graft failure (Kongtim, Cao, & Ciurea, 2016).

There are also potential ethical and logistic considerations when using related donors. While any potential coercion and conflicts of interest are removed in the unrelated donor setting due to anonymity between patients and donor and the separation of patient and donor care, this is not always the case in the related donor setting. Additionally, older donors are much more likely to have co-existent health issues, which may increase the risks of adverse events (AEs) post donation or require peripheral blood rather than bone marrow harvest (Anthias, et al., 2015a), though most of the experience with PTCy is with HCT using bone marrow grafts. Recently, concerns have been raised about the psychologic impact of donation in pediatric donors (Bitan et al., 2015). Conflicts of interest may arise where the donor and recipient are both managed by the same team. Although health assessment and consent are not separated in many centers, this is almost always not the case with the bone marrow harvest, since only transplant teams are trained in this intervention (Anthias et al., 2015b). There is evidence that donor AEs are increased in centers where harvest numbers are low (Shaw et al., 2015) which may be an issue in centers performing only infrequent bone marrow harvest on related donors. In this protocol utilizing unrelated donors, bone marrow collection will be centralized using facilities performing large numbers of procedures for the NMDP. Operators are likely to have appropriate levels of expertise thus minimizing donor AEs.

A perceived restriction in the use of unrelated donors is the time necessary to find a donor, a delay which is thought to be associated with inferior patient outcomes. The ability to select a MMUD would remove this barrier by greatly increasing the appropriate donor pool for each patient, thereby increasing the likelihood of finding a donor available to donate in a timely manner. The ability to select a MMUD would also benefit patients from ethnic minorities who are also more likely to find a mismatched than a matched unrelated donor on international donor

registries. With currently over 20 million unrelated donors listed on the international donor registries, multiple mismatched donors are expected to be available for each patient; thus, donor selection can be optimized and expedited.

A recent single center feasibility study at Johns Hopkins University is evaluating the use of MMUD bone marrow transplantation using PTCy, sirolimus and MMF to prevent GVHD (Kasamon et al., 2015). The conditioning regimen used is non-myeloablative and includes fludarabine, Cy and low dose TBI with two doses of PTCy (Day+4 and +5). Sirolimus and MMF are started on Day+5. The primary objectives include estimating that 6 month probability of survival without grade III-IV GVHD or graft failure. The disease and age inclusion criteria are broad. Patients are eligible if they lack a first degree related donor or haploidentical donor. Potential donors in this protocol could be unrelated donors, first cousins and second-degree relatives. HLA matching at 6 HLA loci is considered. Patients with donor specific antibodies are excluded.

Thus far 16 patients have been enrolled onto the protocol with a MMUD. Most (N=9) received the HCT for AML. The unrelated grafts had a median of 2 HLA mismatches and included three 7/10 matches, one 6/10 match, and one 5/10 match. HLA-C mismatch was present in 10 grafts (63%) and complete -DP mismatch in 6 (38%). By revised Disease Risk Index, 25% of patients were high risk, 69% intermediate risk, and 6% low risk; 69% were in CR at time of transplant. The median graft doses were 3.36×10^8 total nucleated cells/kg and 3.87×10^7 $CD3^+$ cells/kg. All patients engrafted, and there were no non-relapse deaths or prohibitive toxicities. By competing-risk analysis, the estimated cumulative incidence of neutrophil recovery was 100% by Day+29, with a median of 20 (range 14-29) days. Platelet recovery ≥ 20,000/μL was 100% by Day+46, with a median of 32 (range 12-46) days. By Day+60, 14/15 (93%) evaluable patients achieved full donor chimerism in either $CD3^+$ or unsorted cells. Notably, there were no cases of grade III-IV aGVHD. Grade ≥ II aGVHD was limited to 2 cases of skin-only grade II aGVHD and 1 case of grade II skin and ungradable visceral aGVHD. By competing-risk analysis, the estimated cumulative incidence of grade II aGVHD was 12% at Day+100 (90% confidence interval (CI), 0-27%) and 19% at Day+180 (90% CI, 2-37%). The cumulative incidence of any cGVHD at 1 and 2 years was 7% (90% CI, 0-18%). Critical illness prior to relapse was limited to 1 case of sepsis which resolved. With a 3-year median follow-up, the probability of PFS was 56% at 1-year and 50% at 3 years. The cumulative incidence of relapse was 44% at 1-year. Median OS has not been reached, with an estimated 1-year and 3-year OS of 68%.

In order to broaden the expanded access which this protocol will provide to patients without a matched donor to additional patients, we have included the option of myeloablative conditioning (Shabbir-Moosajee, Lombardi, & Ciurea, 2015). Although this PTCy approach has not been tested using mismatched unrelated donors, there is evidence for its efficacy in the haploidentical setting. Data published in abstract (Symons et al., 2015) from Johns Hopkins University is presented here (personal communication: Heather Symons). 96 patients have been transplanted using the PTCy, CNI and MMF approach using unmanipulated BM from haploidentical donors. 73 patients received Busulfan (Day -6, -5, -4 and -3, adjusted to AUC) and Cytoxan (day -2 and -1, 50mg/kg/day) (Symons et al., 2015); 23 received standard CyTBI conditioning (Thomas et al., 1977). Median patient age was 42 (range: 1-65), and transplants were done for diverse hematological malignancies. 80/88 patients were eligible for assessment of chimerism at day 60,

with 91% having >95% donor chimerism at that time. Rates of grade II-IV and III-IV acute GVHD were 17% and &% respectively. Chronic GVHD occurred in 15% of patients (5% moderate/severe). The TRM at 100 days and 1-year was 6% and 11%, and relapse at the 1-year and 3 years was 36% and 44%. Overall survival at 1-year is 72%. Based on these encouraging results we include the option of a myeloablative approach in this study.

Given that this is the only current experience with PTCy in the unrelated donor setting, and the lack of increased toxicity with PTCy and sirolimus as described above, we will use a harmonized approach to GVHD prophylaxis for all patients in this study irrespective of conditioning regimen. This approach is also supported by in vitro data showing synergism between PTCy and sirolimus (Fitzhugh et al., 2013). Additionally, sirolimus promotes Treg expansion, and may inhibit graft rejection and GVHD by promoting T-cell tolerance (Cutler & Antin, 2004). There may also be positive antiviral and graft versus leukemia effects (Kasamon et al., 2012).

Based upon the encouraging safety and efficacy data from the Johns Hopkins University study and the potential advantages of using HLA-mismatched unrelated versus related donors, we wish to test the safety and anti-tumor efficacy of HLA MMUD bone marrow transplantation with PTCy, sirolimus and MMF in a national, multi-center Phase II trial.

1.2 Rationale for Study

The proposed study will evaluate outcomes in MMUD bone marrow transplant recipients using either FIC or RIC regimens with PTCy, sirolimus and MMF for GVHD prophylaxis, where patients do not have an HLA-identical sibling or 8/8 matched unrelated donor available. This study will extend the single center approach used at Johns Hopkins University in the non-myeloablative setting to a multi-center setting. In addition, it will test the encouraging results of myeloablative conditioning in the haploidentical setting to that of MMUD.

2 STUDY DESIGN

2.1 Study Overview

This is a multi-center, single arm Phase II study to assess the safety and efficacy of MMUD bone marrow transplantation using PTCy, sirolimus and MMF for GVHD prophylaxis. The purpose is to determine whether results in a single center setting can be duplicated in a multi-center setting with survival outcomes similar to those achieved with haploidentical bone marrow HCT using a similar approach.

2.2 Hypotheses and Objectives

2.2.1 Primary Hypothesis

Our primary hypothesis is that 1-year survival after HLA MMUD bone marrow transplantation is 65% or higher, similar to the 1-year survival observed after haploidentical (related) donor bone marrow transplantation.

2.2.2 Secondary Hypotheses

1. Greater than 90% of subjects will engraft and more than 80% of engrafting subjects will achieve ≥ 95% donor chimerism by Day+56.

2. The incidence of grades III-IV GVHD will be less than 15% at Day+100.

2.2.3 Study Objectives

The primary objective is to determine OS through 1-year after HLA MMUD bone marrow HCT using PTCy.

Secondary objectives include estimating the following: PFS at Day+180 and 1-year post-HCT; TRM at Day+100, Day+180, and 1-year post-HCT; cumulative incidence of neutrophil recovery; cumulative incidence of platelet recovery; cumulative incidence of primary graft failure; donor chimerism; cumulative incidences of aGVHD and cGVHD; cumulative incidences of viral reactivations and infections; cumulative incidence of relapse/progression; and cumulative incidences of TMA and VOD/SOS. The proportion of subjects proceeding to HCT after informed consent will be described. The donor selection characteristics will also be described. Time to donor identification from the search request will be described. Incidence of development of donor clonal hematopoiesis will be described. Analysis of long-term survivors will be performed for subjects who consent to the CIBMTR Research Database protocol using routine data collection forms and time points per CIBMTR requirements.

2.3 Subject Eligibility

2.3.1 Inclusion Criteria

1. Age ≥ 15 years and < 71 years at the time of signing the informed consent form. Note: HIV-negative subjects with MDS must be <50 years of age at time of signing the informed consent form.
2. Partially HLA-MMUD: HLA typing will be performed at high resolution (allele level) for the HLA-A, -B, -C, and –DRB1 loci; a minimum match of 4/8 at HLA-A, -B, -C, and –DRB1 is required
3. Product planned for infusion is bone marrow
4. Disease and disease status:
   a. Acute leukemias or T-LBL in 1st or subsequent CR
      i. ALL or T-LBL defined by the following:
         - < 5% blasts in the bone marrow
         - Normal maturation of all cellular components in the bone marrow
         - No currently active extramedullary disease (EMD) (e.g., central nervous system (CNS), soft tissue disease)
         - ANC ≥ 1,000/mm^3
      ii. AML defined by the following:
         - < 5% blasts in the bone marrow
         - No blasts with Auer rods
         - Normal maturation of all cellular components in the bone marrow
         - No currently active EMD (e.g., CNS, soft tissue disease)
         - ANC ≥ 1,000/mm^3
      iii. ABL/AUL defined by the following:
         - < 5% blasts in the bone marrow

- Normal maturation of all cellular components in the bone marrow
- No currently active EMD (e.g., CNS, soft tissue disease)
- ANC ≥ 1,000/mm^3

b. MDS fulfilling the following criteria:
- Subjects with de novo MDS who have or have previously had Intermediate-2 or High risk disease as determined by the IPSS. Current Intermediate-2 or High risk disease is not a requirement.
- Subjects must have < 20% bone marrow blasts, assessed within 60 days of informed consent.
- Subjects may have received prior therapy for the treatment of MDS prior to enrollment

c. CLL in CR (if RIC is to be used), or CR or PR (if FIC is to be used)
CR is defined by the following:
- No evidence of lymphadenopathy
- No organomegaly
- Neutrophils ≥ 1.5 × 10^9/L
- Hemoglobin > 11 g/dL
- Lymphocytes < 4 × 10^9/L
- Bone marrow < 30% lymphocytes
- Absence of constitutional symptoms (including weight loss, fever, and night sweats)

PR is defined by the following:
- ≥ 50% decrease in peripheral blood lymphocyte count from pretreatment value
- ≥ 50% reduction in lymphadenopathy if present pretreatment
- ≥ 50% reduction in liver and/or spleen size if enlarged pretreatment

One or more of the following:
- Neutrophils ≥ 1.5 ×10^9/L or 50% improvement over baseline
- Platelets > 100 ×10^9/L or 50% improvement over baseline
- Hemoglobin > 11 g/dL or 50% improvement over baseline

d. CML in 1st or subsequent chronic phase characterized by <10% blasts in the blood or bone marrow
e. Chemotherapy-sensitive lymphoma in status other than 1st CR

5. Performance status: Karnofsky or Lansky score ≥ 60% (Appendix A)
6. Adequate organ function defined as:
   a. Cardiac: LVEF at rest ≥ 35% (RIC cohort) or LVEF at rest ≥ 40% (FIC cohort), or LVFS ≥ 25%
   b. Pulmonary: DLCO, FEV_1, FVC ≥ 50% predicted by PFTs. DLCO value may be corrected or uncorrected for hemoglobin.
   c. Hepatic: total bilirubin ≤ 2.5 mg/dL, and ALT, AST, and ALP < 5 x ULN (unless ALT, AST, and/or ALP are disease related)
   d. Renal: SCr within normal range for age (see table 2.3A). If SCr is outside normal range for age, CrCl > 40 mL/min/1.73m^2 must be obtained (measured by 24-hr urine specimen or nuclear GFR, or calculated GFR (by Cockcroft-Gault formula

for those aged ≥ 18 years; by Original Schwartz estimate for those < 18 years) (see table 2.3B))

TABLE 2.3A: SCr WITHIN NORMAL RANGE FOR AGE

Age (Years)	Maximum SCr (mg/dL)
15	1.2
> 15	1.5

TABLE 2.3B: CALCULATED GFR CrCl ESTIMATION METHOD BY AGE

Age	Calculated GFR CrCl estimation method	Formula
≥ 18 years	Cockcroft-Gault formula (based on IBW) IBW(men)=50kg+2.3kg∗(heightover60inches) IBW(women)=45.5kg+2.3kg∗(heightover60inches)	Cockcroft-Gault formula: CrCl (mL/min) = [(140 – Age) x (Weight in kg)] / (SCr x 72) (x 0.85 for females)
<18 years	Original Schwartz estimate	CrCl (mL/min/1.73m^2)= [length (cm) x k] / SCr k = 0.55 for adolescent females <18 years k = 0.7 for adolescent males <18 years

7. Subjects ≥ 18 years of age must have the ability to give informed consent according to applicable regulatory and local institutional requirements. Legal guardian permission must be obtained for subjects < 18 years of age. Pediatric subjects will be included in age appropriate discussion in order to obtain assent.
8. Subjects with documentation of confirmed HIV-1 infection (i.e. HIV-positive) and a hematologic malignancy who meets all other eligibility requirements must:
   a. Receive only RIC regimen (i.e. Regimen A)
   b. Be willing to comply with effective ARV
   c. Have achieved a sustained virologic response for 12 weeks after cessation of hepatitis C antiviral treatment (in HIV-positive subjects with hepatitis C)

2.3.2 Exclusion Criteria

1. Suitable HLA-matched related or 8/8 allele matched (HLA-A, -B, -C, -DRB1) unrelated donor available. This exclusion does not apply to HIV-positive subjects who have a CCR5delta32 homozygous donor.
2. Autologous HCT < 3 months prior to the time of signing the informed consent form
3. Females who are breast-feeding or pregnant
4. HIV-positive subjects:
   a. AIDS related syndromes or symptoms that may pose an excessive risk for transplantation-related morbidity as determined by the Treatment Review Committee (see Appendix D).
   b. Untreatable HIV infection due to multidrug ARV resistance. Subjects with a detectable or standard viral load > 750 copies/mL should be evaluated with an HIV drug resistance test (HIV-1 genotype). The results should be included as part of the ARV review (described in Appendix D).

c. Not currently prescribed ritonavir, cobacistat and/or zidovudine
5. Current uncontrolled bacterial, viral or fungal infection (currently taking medication with evidence of progression of clinical symptoms or radiologic findings)
6. Prior allogeneic HCT
7. Primary myelofibrosis or myelofibrosis secondary to essential thrombocythemia or polycythemia vera
8. HIV-negative subjects with MDS may not receive RIC and must be < 50 years of age at the time of signing the informed consent form

2.4 Donor Eligibility

2.4.1 Inclusion Criteria

1. Must be unrelated to the subject and matched at between 4/8 and 7/8 HLA alleles for HLA-A, -B, -C, and –DRB1
2. Must meet NMDP medical suitability requirements for bone marrow donation.
3. Must undergo eligibility screening according to current FDA requirements. Donors who do not meet one or more of the donor screening requirements may donate under urgent medical need.
4. Must have the ability to give informed consent according to applicable NMDP donor regulatory requirements
5. Must agree to donate bone marrow

2.4.2 Exclusion Criteria

1. Recipient positive anti-donor HLA antibodies against a mismatched allele in the selected donor determined by either:
    a. a positive crossmatch test of any titer (by complement-dependent cytotoxicity or flow cytometric testing) or
    b. the presence of anti-donor HLA antibody to any HLA locus (HLA-A, -B, -C, –DRB1, -DQB1, -DQA1, -DPB1, -DPA1) with MFI >1000 by solid phase immunoassay

2.5 Donor Prioritization Schema

The donor search will be coordinated centrally by the NMDP but the final donor selection will be made by the study site. The following donor selection characteristics must be considered to perform the selection: number of mismatches at HLA-A, -B, -C, -DRB1, -DQB1, -DPB1, donor age, donor-recipient CMV serostatus match, donor weight, donor-recipient sex match, and donor-recipient ABO group match. In addition, CCR5delta32 genotype should be assessed in donors being considered for HIV-positive patients.

The MMUD selection algorithm (Appendix J) is the *proposed* search strategy that prioritizes donor age, CMV serostatus, and ABO match over HLA match. This strategy should not be considered the *prescribed* search strategy for potential study subjects. Study sites may instead utilize institutional donor selection practices.

2.6 Treatment Plan

2.6.1 Protocol-specific treatment information

Regimen A (RIC). Recommended schedule as below:

1. **Fludarabine**
   - Fludarabine 30 mg/m^2/day (adjusted for renal function) is administered over a 30-60 minute IV infusion on Days –6 through –2 (maximum cumulative dose, 150 mg/m^2).
   - The body surface area (BSA) dosing is based on adjusted ideal body weight (IBW) (Appendix K), unless the subject weighs less than IBW, in which case dosing is based on actual body weight.
   - CrCl may change during the days fludarabine is given. Adjustment in dose due to creatinine changes during conditioning is permitted. Please see table 2.3B for calculated GFR CrCl estimation method by age.
     - For decreased CrCl, fludarabine dosage is reduced as follows:
       - CrCl 46-60 mL/min, fludarabine = 24 mg/m^2
       - CrCl 31-45 mL/min, fludarabine = 22.5 mg/m^2
       - CrCl 21-30 mL/min, fludarabine = 19.5 mg/m^2
       - CrCl < 20 mL/min, fludarabine = 15 mg/m^2
2. **Pre-HCT Cy**
   - Cy 14.5 mg/kg/day is administered as a 1-2 hour IV infusion on Days –6 and –5 after hydration.
   - Use of Mesna and dosing will be done according to institutional standards. A recommended approach is as follows: Mesna IV dose of ≥ 80% of the total daily dose of Cy and given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy.
   - Hydration prior to Cy may be given according to institutional guideline.
   - Cy and mesna are dosed according to adjusted IBW (Appendix K), unless the subject weighs less than IBW, in which case these drugs will be dosed according to actual weight.
3. **TBI**
   - 200 cGy TBI is administered in a single fraction on Day –1.
   - Radiation sources, dose rates, and shielding follow institutional practice.

Although a schedule is proposed above, the regimen can be given according to institutional standards as long as the prescribed doses are the same as in the recommended regimen above.

Regimen B (FIC). Recommended schedule as below:

1. **Busulfan**
   - Busulfan ≥ 9mg/kg total dose (IV or PO) on Days -6, -5, -4, -3 **(PK monitoring required** to achieve a daily AUC target of 4800-5300 μM*min (Perkins et al., 2012))
   - Busulfan dosing is based on adjusted IBW (Appendix K), unless the subject weighs less than IBW, in which case dosing is based on actual body weight.

2a. Pre-HCT Cy

- Cy 50mg/kg/day is administered as a 1-2 hour IV infusion on Days –2 and –1 after hydration.
- Use of Mesna and dosing will be done according to institutional standards. A recommended approach is as follows: Mesna IV dose of ≥ 80% of the total daily dose of Cy and given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy.
- Hydration prior to Cy may be given according to institutional guideline.
- Cy and mesna are dosed according to adjusted IBW (Appendix K), unless the subject weighs less than IBW, in which case these drugs will be dosed according to actual weight.

OR

2b. Fludarabine

- Fludarabine 30 mg/m^2/day (adjusted for renal function) is administered over a 30-60 minute IV infusion on Days –6 through –2 (maximum cumulative dose, 150 mg/m^2).
- The BSA dosing is based on adjusted IBW (Appendix K), unless the subject weighs less than IBW, in which case dosing is based on actual body weight.
- CrCl may change during the days fludarabine is given. Adjustment in fludarabine dose due to creatinine changes during conditioning is permitted. Please see table 2.3B for calculated GFR CrCl estimation method by age.
    - For decreased CrCl, fludarabine dosage is reduced as follows:
        - CrCl 46-60 mL/min, fludarabine = 24 mg/m^2
        - CrCl 31-45 mL/min, fludarabine = 22.5 mg/m^2
        - CrCl 21-30 mL/min, fludarabine = 19.5 mg/m^2
        - CrCl < 20 mL/min, fludarabine = 15 mg/m^2

Although a schedule is proposed above, the regimen can be given according to institutional standards as long as the prescribed doses, and Busulfan monitoring, are the same as in the recommended regimen above.

Regimen C (FIC). Recommended schedule as below:

1. **Pre-HCT Cy**
    - Cy 50mg/kg/day is administered as a 1-2 hour IV infusion on Days –5 and –4 after hydration.
    - Use of Mesna and dosing will be done according to institutional standards. A recommended approach is as follows: Mesna IV dose of ≥ 80% of the total daily dose of Cy and given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy.
    - Hydration prior to Cy may be given according to institutional guideline.
    - Cy and mesna are dosed according to adjusted IBW (Appendix K), unless the subject weighs less than IBW, in which case these drugs will be dosed according to actual weight.

2. **TBI**
   - 200cGy TBI is administered in twice daily on Days –3, -2, and -1.
   - Radiation sources, dose rates, and shielding follow institutional practice.

Although a schedule is proposed above, the regimen can be given according to institutional standards as long as the prescribed doses are the same as in the recommended regimen above.

Bone marrow transplantation

- On Day 0, the harvested bone marrow is infused.
- Donor bone marrow will be harvested with a target yield of 4×10^8 nucleated cells/kg recipient weight (actual, ideal, or adjusted ideal per institutional practice).
- The lowest acceptable nucleated cells yield is 1.5×10^8 cells/kg recipient weight (actual or adjusted per institutional practice).
  - Study sites should notify the 15-MMUD CIBMTR Protocol Coordinators if they are aware of a collection that yields lower total nucleated cells (TNC) than expected.
- TNC/kg (and CD34+ cells/kg if available) infused will be recorded.
- The graft will not be manipulated to deplete T-cells.
- Processing for ABO incompatibility follows institutional practices.
- Peripheral stem cell harvest is not permitted.
- Use of a cryopreserved graft is not permitted.

Post-Transplant Treatments

PTCy

- Cy 50mg/kg IV, over 1-2 hours (depending on volume), is given on Day+3 (ideally between 60 and 72 hours after bone marrow infusion) and on Day+4 (approximately 24 hours after Day+3 Cy).
- Hydration with Cy, management of volume status, and monitoring for hemorrhagic cystitis will follow institutional standards.
- Mesna recommendation: Mesna IV dose ≥ 80% of the total daily dose of Cy and given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy. Institutional standards for mesna use and dosing may be used when necessary.
- Cy and mesna are dosed according to IBW, unless the subject weighs less than IBW, in which case these drugs will be dosed according to actual body weight.

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

Sirolimus

For subjects **≥ 18 years**:

- Sirolimus dosing is based on adjusted IBW (Appendix K).

- A one-time sirolimus loading dose, 6 mg PO, is given on Day+5, at least 24 hours after Cy completion.
- Sirolimus is then continued at a maintenance dose (starting dose 2 mg PO QD), with dose adjustments to maintain a trough of 5 – 15 ng/mL as measured by high performance liquid chromatography (HPLC) or immunoassay.

For subjects **< 18 years**:

- Sirolimus dosing is based on adjusted IBW (Appendix K).
- A one-time sirolimus loading dose, 3 mg/m^2 PO with the dose not to exceed 6 mg, is given on Day+5, at least 24 hours after Cy completion.
- Sirolimus is then continued at a maintenance dose (starting dose 1 mg/m^2 PO QD, maximum 2 mg PO QD), with dose adjustments to maintain a trough of 5 – 15 ng/mL as measured by HPLC or immunoassay**.**

All subjects:

- Sirolimus prophylaxis is discontinued after the last dose on Day+180, or may be continued if there is GVHD.
- There is no planned taper. If subject experiences toxicity potentially attributable to sirolimus requiring dose adjustment or omission of 4 or more doses prior to Day+180, study site Principal Investigator must contact protocol chairs for guidance regarding sirolimus dosing.
- Study site Principal Investigator must contact protocol chairs for guidance regarding sirolimus dosing if considering stopping sirolimus before Day+56 (in the presence of suspected graft failure or for other clinical reasons)
- Sirolimus troughs should be checked at minimum weekly through Day+100 and then monitoring thereafter per institutional practices.
- More frequent monitoring is required if a new medication is initiated or if the subject develops renal insufficiency or hepatic dysfunction.

Drugs that may **increase** sirolimus blood concentrations include:

- Calcium channel blockers: diltiazem, nicardipine, verapamil.
- Calcineurin inhibitors (CNI): cyclosporine.
- Antifungal agents: ketoconazole, clotrimazole, fluconazole, itraconazole, voriconazole, posaconazole, isavuconazole.
- Macrolide antibiotics: clarithromycin, erythromycin, troleandomycin.
- Gastrointestinal prokinetic agents: cisapride, metoclopramide.
- Other drugs: bromocriptine, cimetidine, danazol, HIV protease inhibitors.

Drugs that may **decrease** sirolimus concentrations include:

- Anticonvulsants: carbamazepine, phenobarbital, phenytoin.
- Antibiotics: rifabutin, rifapentine, rifampin
- Herbal preparations: St. John's Wort (*Hypericum perforatum*)

Guidelines for HIV-positive subjects continuing ARV with various prophylaxis regimens are detailed below in Table 2.6A (Alvarnas & Ambinder, 2012).

TABLE 2.6A CONTINUATION OF ARV BASED ON GVHD PROPHYLAXIS REGIMEN[1]

GVHD Prophylaxis Agent	Raltegravir Based Regimen	NNRTI[2] Based Regimen	Other
Sirolimus	Continue ARV as tolerated	Continue ARV. Sirolimus dose adjustments will be required (Appendix F)	Consultation recommended, reviewed on a case by case basis
Cy	Continue ARV as tolerated	Continue ARV as tolerated with caution and frequent monitoring (Appendix F)	Consultation recommended, reviewed on a case by case basis[3]

[1]Subjects should be switched to raltegravir-based regimens whenever possible
[2]Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI)
[3]Restart ARV after Cy prophylaxis (Day+5) as soon as subject can tolerate oral medications

MMF
MMF begins on Day+5, at least 24 hours after completion of PTCy. The MMF dose is 15 mg/kg PO TID (according to adjusted IBW (Appendix K)) with total daily dose not to exceed 3 g (i.e. maximum 1 g PO TID). An equivalent IV dose (1:1 conversion) may instead be given. Dose modification guidelines are provided in section 2.6.4. MMF prophylaxis is discontinued after the last dose on Day+35, or may be continued if there is GVHD.

Growth factors
G-CSF (filgrastim) or a biosimilar begins on Day+5 at a dose of 5 mcg/kg/day (according to actual body weight) IV or SC (rounding to the nearest vial dose is allowed), until the ANC is $\geq 1{,}000/mm^3$ over the course of 3 consecutive days. Additional G-CSF may be administered as warranted. Pegfilgrastim (Neulasta®) and granulocyte-macrophage colony-stimulating factor (GM-CSF) or their biosimilars are not permitted.

Post-HCT donor cellular infusion (DCI)
Prophylactic post-HCT DCI (for persistent detectable malignancy, prophylaxis in the absence of detectable malignancy, or mixed donor chimerism) is not permitted before Day+100. Any use of DCI, and the indication for DCI must be recorded.

Other post-HCT therapy
Preemptive systemic cancer therapy is permitted post-HCT (e.g., deoxyribonucleic acid (DNA)-methyltransferase inhibitor, tyrosine kinase inhibitor, rituximab for CD20+ malignancy). Intrathecal chemotherapy and consolidative radiation therapy are permitted. The use of such post-HCT therapies will be recorded.

2.6.2 Supportive Care

Subjects should receive supportive care including transfusions, antiemetics, infection prophylaxis and nutritional support according to institutional guidelines. Infection prophylaxis should include, but is not limited to, agents or strategies to prevent herpes simplex, CMV, *Pneumocystis jirovecii*, and fungal infections.

Seizure prophylaxis must be given with busulfan containing regimens per institutional guidelines.

Herbal supplements and nutraceuticals are contraindicated.

HIV-positive subjects: ARV is only effective when administered in combination and with consistency. Single agent therapy or repeated interruptions in therapy lead to drug resistance. The ARV guidelines (Appendices D and E) are designed to minimize the possibility of drug interactions with high dose chemotherapy. ARV should be administered only when it is anticipated that the ARV regimen can be taken on a consistent basis (i.e., conditioning regimen toxicities such as nausea and mucositis will not interfere with dosing schedules). In cases where the subject receives an HCT using a CCR5delta32 homozygous donor, ARV should be continued through at least Day+100.

- *Subjects on RIC Regimens*: Subjects will continue on ARV therapy.

Table 2.6B (Alvarnas & Ambinder, 2012) outlines continuation guidelines for ARV regimens based on the conditioning regimen used.

TABLE 2.6B: CONTINUATION OF ARV BASED ON CONDITIONING REGIMEN

	Raltegravir Based Regimen	**NNRTI Based Regimen**	**Other**
RIC			
Regimen A: Fludarabine Cy	Continue throughout conditioning as tolerated	Continue throughout conditioning as tolerated	Consultation recommended, reviewed on a case by case basis

See Appendix F (Table F-1) for detailed information on each interaction.

2.6.3 Tests, Procedures, Observations

Pre-conditioning evaluations (must be completed within 4 weeks prior to start of conditioning)*:

- Complete history and physical (H&P) with height and weight
- HCT-Specific Co-Morbidity Index (HCT-CI) score
- Complete blood count (CBC) with differential
- HLA typing (recipient and donor)†
- Disease evaluation:
    - Acute leukemias, MDS, CLL, CML, and lymphomas with bone marrow involvement pre-HCT: Bone marrow aspiration with or without biopsy for

morphology, flow cytometry and cytogenetic analysis (fluorescence in situ hybridization (FISH) as indicated).
    - For lymphoma and CLL subjects: pre-HCT imaging required; computed tomography (CT)/positron emission tomography (PET)/ magnetic resonance imaging (MRI) scans of neck, chest, abdomen and pelvis as appropriate per center policy
- Performance status (Karnofsky if ≥ 16 years, Lansky score if < 16 years)
- Echocardiogram (echo) or multiple-gated acquisition scan (MUGA) with measurement of LVEF or LVFS
- PFT with measurements of FEV_1, FVC and DLCO. DLCO value may be corrected or uncorrected for hemoglobin.
- Serum chemistries including creatinine, total bilirubin, ALT, AST, and ALP. If SCr is outside normal range for age, CrCl (measured by 24-hr urine specimen or nuclear GFR, or calculated GFR (by Cockcroft-Gault formula for those aged ≥ 18 years; by Original Schwartz estimate for those < 18 years)) must be obtained
- β-HCG serum pregnancy test for females of childbearing potential (FCBP)
    - FCBP is defined as a female with evidence of menarche who has not been surgically sterilized, and is not post-menopausal (menopausal: > 45 years of age with no menstrual period for ≥ 12 months)
- Patient and donor CIBMTR Biorepository sample collection†

*HIV-positive subjects: See Appendix D for the Treatment Review Committee approval process for HIV-positive subject conditioning regimen; Appendix G for additional evaluations for HIV-positive subjects; and Appendix I for additional research laboratory samples.

†Pre-conditioning evaluations that do not need to be completed within 4 weeks prior to the start of conditioning.

Conditioning evaluations (Days -6 through -1):

- Busulfan PK monitoring for subjects receiving Regimen B (AUC target of 4800-5300 μM*min per day). Timing of sample collection for Busulfan PK determination may be done according to institutional standards.

Post-HCT evaluations:

- Day+7 (+/- 5 days)
    - H&P
    - CBC with differential
    - Serum chemistries
    - GVHD assessment
    - Toxicity assessment
    - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6
- Day+14 (+/- 5 days)
    - H&P

  - CBC with differential
  - Serum chemistries
  - GVHD assessment
  - Toxicity assessment
  - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6

- Day+21 (+/-5 days)
  - H&P
  - CBC with differential
  - Serum chemistries
  - Peripheral blood collection
    - Research: gene expression profile
  - GVHD assessment
  - Toxicity assessment
  - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6

- Day+28 (+/- 5 days)
  - H&P
  - CBC with differential
  - Serum chemistries
  - GVHD assessment
  - Toxicity assessment
  - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6
  - Peripheral blood collection
    - Routine: chimerism in whole blood (unsorted); CD3 and CD33 subsets if available

- Day+35 (+/- 5 days)
  - H&P
  - CBC with differential
  - Serum chemistries
  - GVHD assessment
  - Toxicity assessment
  - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6

- Day+42 (+/- 5 days)
  - H&P
  - CBC with differential
  - Serum chemistries
  - GVHD assessment
  - Toxicity assessment
  - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6

- Day+49 (+/- 5 days)

  - H&P
  - CBC with differential
  - Serum chemistries
  - GVHD assessment
  - Toxicity assessment
  - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6

- Day+56 (+/- 5 days)
  - H&P
  - CBC with differential
  - Serum chemistries
  - GVHD assessment
  - Toxicity assessment
  - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6
  - Peripheral blood collection
    - Routine: chimerism in whole blood (unsorted); CD3 and CD33 subsets if available

- Day+63 (+/- 5 days)
  - H&P
  - CBC with differential
  - Serum chemistries
  - GVHD assessment
  - Toxicity assessment
  - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6

- Day+100 (+/- 7 days)
  - H&P
  - CBC with differential
  - Serum chemistries
  - GVHD assessment
  - Toxicity assessment
  - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6
  - Peripheral blood collection
    - Routine: chimerism in whole blood (unsorted); CD3 and CD33 subsets if available
    - Research: gene expression profile; immune reconstitution
  - Bone marrow collection
    - Routine: disease evaluation (see below)
    - Research: donor clonal hematopoiesis assessment (all subjects – regardless of disease)
  - Disease evaluation
    - Acute leukemias, MDS, CLL, CML, lymphomas with bone marrow involvement pre-HCT: Bone marrow aspiration with or

without biopsy for morphology, flow cytometry and cytogenetic analysis (FISH as indicated)
        - Lymphoma/CLL: Imaging studies as indicated (e.g. CT, PET, MRI)
    - See Appendix G for additional Day+100 evaluations for HIV-positive subjects

- Day+150 (+/- 7 days)
    - H&P
    - CBC with differential
    - Serum chemistries
    - GVHD assessment
    - Toxicity assessment
    - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6

- Day+180 (+/- 28 days)
    - H&P
    - CBC with differential
    - Serum chemistries
    - GVHD assessment
    - Toxicity assessment
    - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6
    - Peripheral blood collection
        - Routine: chimerism in whole blood (unsorted); CD3 and CD33 subsets if available
        - Research: gene expression profile
    - Bone marrow collection
        - Routine: disease evaluation (see below)
    - Disease evaluation
        - Acute leukemias, MDS, CLL, CML, lymphomas with bone marrow involvement pre-HCT: Bone marrow aspiration with or without biopsy for morphology, flow cytometry and cytogenetic analysis (FISH as indicated).
        - Lymphoma/CLL: Imaging studies as indicated (e.g. CT, PET, MRI)
    - Survival status
    - See Appendices G and I for additional Day+180 evaluations for HIV-positive subjects

- Day+270 (+/- 28 days)
    - H&P
    - CBC with differential
    - Serum chemistries
    - GVHD assessment
    - Toxicity assessment

  - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6
  - See Appendix G for additional Day+270 evaluations for HIV-positive subjects

- 1-year (Day+365 (+/- 28 days))
  - H&P
  - CBC with differential
  - Serum chemistries
  - GVHD assessment
  - Toxicity assessment
  - Viral infections/reactivations assessment of CMV, EBV, BK, ADV, and HHV-6
  - Peripheral blood collection
    - Routine: chimerism in whole blood (unsorted); CD3 and CD33 subsets if available
    - Research: immune reconstitution
  - Bone marrow collection
    - Routine: disease evaluation (see below)
    - Research: donor clonal hematopoiesis assessment (all subjects – regardless of disease)
  - Disease evaluation
    - Acute leukemias, MDS, CLL, CML, lymphomas with bone marrow involvement pre-HCT: Bone marrow aspiration with or without biopsy for morphology, flow cytometry and cytogenetic analysis (FISH as indicated).
    - Lymphoma/CLL: Imaging studies as indicated (e.g. CT, PET, MRI)
  - Survival status
  - See Appendices G and I for additional Day+365 evaluations for HIV-positive subjects

2.6.3.1 Treatment Schemas

Regimen A (RIC): Flu/Cy/TBI

		Days pre-HCT			Day 0	Days post-HCT															
Evaluation/Event	**Eligibility assessments***	–6 → –5	–4 → –2	–1		3→ 4	5	7	14	21	28	35	42	49	56	63	100	150	180	270	365
Informed Consent and Study Enrollment	X																				
Complete H&P	X							X	X	X	X	X	X	X	X	X	X	X	X	X	X
HCT-CI score	X																				
HLA typing (recipient and donor)	X																				
Bone marrow collection: disease evaluation[1]	X																X		X		X
CBC w/differential	X							X	X	X	X	X	X	X	X	X	X	X	X	X	X
Imaging[2,3]	X																X		X		X
Performance Status	X																				
Echo or MUGA (LVEF or LVFS)	X																				
PFT (FEV_1, FVC & DLCO)	X																				
Serum chemistries[4]	X							X	X	X	X	X	X	X	X	X	X	X	X	X	X
β-HCG serum pregnancy test (FCBP)	X																				
Fludarabine 30 mg/m^2/day IV[5]		X	X																		
Cy 14.5 mg/kg/day IV[5]		X																			
Mesna[6]		X				X															
Begin antibiotic prophylaxis[7]		X																			
TBI 200cGy[5]				X																	
Infuse non-T-cell-depleted bone marrow					X																

Cy 50 mg/kg/day IV[8]						X															
Begin sirolimus (PO) dose adjusted to maintain a level of 5-15 ng/mL							X														
MMF 15 mg/kg PO TID (maximum dose 1g PO TID, maximum dose 3g/day)							X														
Begin G-CSF 5 mcg/kg/day SC or IV, continue until ANC ≥ 1000/mm^3 x 3 consecutive days							X														
Assess chimerism in peripheral blood[9]											X				X		X		X		X
Discontinue MMF (optional if GVHD is active)												X									
Discontinue sirolimus (optional if GVHD is active)																			X		
GVHD assessment								X	X	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity assessment								X	X	X	X	X	X	X	X	X	X	X	X	X	X
Viral infections/reactivations assessment								X	X	X	X	X	X	X	X	X	X	X	X	X	X
Survival status																			X		X
Research sample collection	X[10]									X							X		X		X

Regimen B – 2a (FIC): Bu/Cy

		Days pre-HCT		Day 0	Days post-HCT															
Evaluation/Event	**Eligibility assessments***	–6 → –3	–2 → –1		3→ 4	5	7	14	21	28	35	42	49	56	63	100	150	180	270	365
Informed Consent and Study Enrollment	X																			
Complete H&P	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X
HCT-CI score	X																			
HLA typing (recipient and donor)	X																			
Bone marrow collection: disease evaluation[1]	X															X		X		X
CBC w/differential	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X
Imaging [2,3]	X															X		X		X
Performance Status	X																			
Echo or MUGA (LVEF or LVFS)	X																			
PFT (FEV_1, FVC & DLCO)	X																			
Serum chemistries[4]	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X
β-HCG serum pregnancy test (FCBP)	X																			
Busulfan ≥ 9mg/kg total dose (IV or PO)[11]		X																		
Busulfan PK monitoring[12]		X	X																	
Begin antibiotic prophylaxis[7]		X																		
Mesna[6]			X		X															
Cy 50mg/kg/day IV[11]			X		X[8]															
Infuse non-T-cell-depleted bone marrow				X																

Begin sirolimus (PO) dose adjusted to maintain a level of 5-15 ng/mL						X														
MMF 15 mg/kg PO TID (maximum dose 1g PO TID, maximum dose 3 g/day)						X														
Begin G-CSF 5 mcg/kg/day SC or IV, continue until ANC ≥ 1000/mm^3 x 3 consecutive days						X														
Assess chimerism in peripheral blood[9]										X				X		X		X		X
Discontinue MMF (optional if GVHD is active)											X									
Discontinue sirolimus (optional if GVHD is active)																		X		
GVHD assessment							X	X	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity assessment							X	X	X	X	X	X	X	X	X	X	X	X	X	X
Viral infections/reactivations assessment							X	X	X	X	X	X	X	X	X	X	X	X	X	X
Survival status																		X		X
Research sample collection	X[10]								X							X		X		X

Regimen B – 2b (FIC): Bu/Flu

		Days pre-HCT			Day 0	Days post-HCT															
Evaluation/Event	**Eligibility assessments***	–6 → –3	–2	-1		3→ 4	5	7	14	21	28	35	42	49	56	63	100	150	180	270	365
Informed Consent and Study Enrollment	X																				
Complete H&P	X							X	X	X	X	X	X	X	X	X	X	X	X	X	X
HCT-CI score	X																				
HLA typing (recipient and donor)	X																				
Bone marrow collection: disease evaluation[1]	X																X		X		X
CBC w/differential	X							X	X	X	X	X	X	X	X	X	X	X	X	X	X
Imaging [2,3]	X																X		X		X
Performance Status	X																				
Echo or MUGA (LVEF or LVFS)	X																				
PFT (FEV_1, FVC & DLCO)	X																				
Serum chemistries[4]	X							X	X	X	X	X	X	X	X	X	X	X	X	X	X
β-HCG serum pregnancy test (FCBP)	X																				
Busulfan ≥ 9mg/kg total dose (IV or PO)[11]		X																			
Busulfan PK monitoring[12]		X	X	X																	
Fludarabine 30 mg/m^2/day IV[11]		X	X																		
Begin antibiotic prophylaxis[7]		X																			
Infuse non-T-cell-depleted bone marrow					X																
Mesna[6]						X															
Cy 50 mg/kg/day IV[8]						X															

Begin sirolimus (PO) dose adjusted to maintain a level of 5-15 ng/mL							X														
MMF 15 mg/kg PO TID (maximum dose 1g PO TID, maximum dose 3 g/day)							X														
Begin G-CSF 5 mcg/kg/day SC or IV, continue until ANC ≥ 1000/mm^3 x 3 consecutive days							X														
Assess chimerism in peripheral blood[9]											X				X		X		X		X
Discontinue MMF (optional if GVHD is active)												X									
Discontinue sirolimus (optional if GVHD is active)																			X		
GVHD assessment								X	X	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity assessment								X	X	X	X	X	X	X	X	X	X	X	X	X	X
Viral infections/reactivations assessment								X	X	X	X	X	X	X	X	X	X	X	X	X	X
Survival status																			X		X
Research sample collection	X[10]									X							X		X		X

Regimen C (FIC): Cy/TBI

		Days pre-HCT		Day 0	Days post-HCT															
Evaluation/Event	**Eligibility assessments***	–5 → –4	–3 → –1		3→ 4	5	7	14	21	28	35	42	49	56	63	100	150	180	270	365
Informed Consent and Study Enrollment	X																			
Complete H&P	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X
HCT-CI score	X																			
HLA typing (recipient and donor)	X																			
Bone marrow collection: disease evaluation[1]	X															X		X		X
CBC w/differential	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X
Imaging [2,3]	X															X		X		X
Performance Status	X																			
Echo or MUGA (LVEF or LVFS)	X																			
PFT (FEV_1, FVC & DLCO)	X																			
Serum chemistries[4]	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X
β-HCG serum pregnancy test (FCBP)	X																			
Begin antibiotic prophylaxis[7]		X																		
Mesna[6]		X			X															
Cy 50mg/kg/day IV [5]		X			X[8]															
TBI 200cGy twice a day [5]			X																	
Infuse non-T-cell-depleted bone marrow				X																
Begin sirolimus (PO) dose adjusted to maintain a level of 5-15 ng/mL						X														

MMF 15 mg/kg PO TID (maximum dose 1g PO TID, maximum dose 3 g/day)						X														
Begin G-CSF 5 mcg/kg/day SC or IV, continue until ANC ≥ 1000/mm^3 x 3 consecutive days						X														
Assess chimerism in peripheral blood[9]										X				X		X		X		X
Discontinue MMF (optional if GVHD is active)											X									
Discontinue sirolimus (optional if GVHD is active)																		X		
GVHD assessment							X	X	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity assessment							X	X	X	X	X	X	X	X	X	X	X	X	X	X
Viral infections/reactivations assessment							X	X	X	X	X	X	X	X	X	X	X	X	X	X
Survival status																		X		X
Research sample collection	X[10]								X							X		X		X

[1] Acute leukemias, MDS, CLL, CML, and lymphomas with bone marrow involvement pre-HCT: Bone marrow aspiration with or without biopsy for morphology, flow cytometry and cytogenetic analysis (FISH as indicated).
[2] Lymphoma/CLL subjects: Pre-HCT imaging required. CT/PET/MRI scans of neck, chest, abdomen and pelvis as appropriate per center policy.
[3] Lymphoma/CLL subjects: Post-HCT imaging studies as indicated (e.g. CT, PET, MRI).
[4] Serum chemistries include creatinine, total bilirubin, ALT, AST, and ALP. If SCr is outside normal range for age, CrCl (measured by 24-hr urine specimen or nuclear GFR, or calculated GFR (by Cockcroft-Gault formula for those aged ≥ 18 years; by Original Schwartz estimate for those < 18 years)) must be obtained.
[5] Although a schedule is proposed, the regimen can be given according to institutional standards as long as the prescribed doses are the same as in the recommended regimen.
[6] Mesna recommendation: Mesna IV dose ≥ 80% of the total daily dose of Cy, given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy. Institutional standards for mesna use and dosing may be used when necessary.
[7] Start of antibiotic prophylaxis may be altered based on institutional standard
[8] First dose of PTCy must be administered 60-72 hrs after infusion of bone marrow
[9] Peripheral blood chimerism in whole blood (unsorted); CD3 and CD33 subsets if available
[10] HIV-positive patients only: pre-conditioning sample collection for Latent HIV Cellular Reservoir Analysis
[11] Although a schedule is proposed, the regimen can be given according to institutional standards as long as the prescribed doses, and Busulfan monitoring, are the same as in the recommended regimen.
[12] Timing of sample collection for Busulfan PK determination may be done according to institutional standards.

* HIV-positive subjects: See Appendix D for the Treatment Review Committee approval process for HIV-positive subject conditioning regimen; Appendix G for additional evaluations for HIV-positive subjects; and Appendix I for additional research laboratory samples.

2.6.4 Risks and Toxicities

Below is a list of the most common toxicities for therapies used in this study. See the U.S. Food and Drug Administration (FDA)-approved package insert for each drug for a comprehensive list of AEs.

Busulfan (Busulfex®)

Busulfan (1, 4-dimethanesulfonoxybutane) is an alkylating agent. The drug is extensively metabolized by the liver and its metabolites are eventually excreted in the urine.

Busulfan toxicities include:

- Gastrointestinal: nausea, vomiting, constipation, diarrhea, abdominal discomfort, anorexia, dyspepsia, mucositis, stomatitis
- Hepatobiliary: VOD/SOS
- Neurologic: headache, insomnia, seizures, dizziness
- Cardiovascular: hypertension, hypotension and tachycardia
- Pulmonary: dyspnea, lung fibrosis, cough
- Endocrine and metabolic: electrolyte imbalance
- Genitourinary: amenorrhea, dysmenorrhea, infertility
- Dermatologic: rash, urticarial
- Miscellaneous: rhinorrhea, cataracts, fluid retention

PK monitoring of Busulfan is required to achieve a daily AUC target of 4800-5300 μM*min (Perkins et al., 2012). Busulfan dosing is based on adjusted IBW (Appendix K), unless the subject weighs less than IBW, in which case dosing is based on actual body weight.

Fludarabine (Fludara®)

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

Fludarabine toxicities include:

- Hematologic: hematopoietic suppression including neutropenia with increased risk of infection and immunosuppression
- Neurologic: peripheral neuropathy and encephalopathy manifested by fatigue, weakness, paresthesia, visual disturbances, somnolence and coma
- Gastrointestinal: nausea, vomiting, diarrhea and stomatitis
- Dermatologic: rash, urticarial
- Pulmonary: cough, idiopathic pneumonitis, dyspnea
- Miscellaneous: fever

Dose adjustments of fludarabine are required for renal insufficiency if the estimated CrCl is < 60 mL/min (per section 2.6.1). Fludarabine BSA dosing is based on adjusted ideal body weight (IBW) (Appendix K), unless the subject weighs less than IBW, in which case dosing is based on actual body weight.

Cyclophosphamide (Cytoxan®)

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

Cy toxicities include:

- Gastrointestinal: nausea, vomiting, anorexia, stomatitis, diarrhea, abdominal pain
- Hematologic: myelosuppression, thrombocytopenia, carcinogenesis, anemia
- Cardiovascular: severe chronic heart failure characterized by cardiomegaly, pericardial effusions, diffuse voltage decrease on ECG and decreased LVEF
- Genitourinary: hemorrhagic cystitis (prevented by hydration and mesna therapy or bladder irrigation) and gonadal function impairment, amenorrhea, dysmenorrhea, infertility, damage to unborn baby
- Pulmonary: rare pulmonary toxicity, lung fibrosis
- Dermatologic: alopecia, rash, urticarial
- Miscellaneous: fluid retention, allergic reaction

Dose adjustments for Cy will not be made.

Pre-HCT Cy: dosed according to adjusted IBW (Appendix K), unless the subject weighs less than IBW, in which case Pre-HCT Cy will be dosed according to actual weight.

PTCy: dosed according to IBW, unless the subject weighs less than IBW, in which case PTCy will be dosed according to actual body weight.

Mesna (Mesnex®)

Mesna (sodium-2-mercaptoethanesulphonate) is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxazophosphorines (Cy and ifosfamide). It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxazophosphorines, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxazophosphorines.

Mesna toxicities include:

- Cardiovascular: hypotension
- Dermatologic: rash, urticarial
- Gastrointestinal: nausea and vomiting, diarrhea, abdominal pain, altered taste
- Neurologic: headache, joint or limb pain
- Miscellaneous: fatigue

Pre-HCT Mesna: pre-transplant use of mesna and dosing will be done according to institutional standards. A recommended approach is as follows: mesna IV dose ≥ 80% of the total daily dose of Cy and given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy. Mesna is dosed according to adjusted IBW (Appendix K), unless the subject weighs less than IBW, in which case mesna will be dosed according to actual weight.

Post-HCT Mesna: the recommended approach is mesna IV dose ≥ 80% of the total daily dose of Cy, given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy. Institutional standards for mesna use and dosing may be used when necessary. Mesna is dosed according to IBW, unless the subject weighs less than IBW, in which case mesna will be dosed according to actual body weight.

MMF (Cellcept®)

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

MMF toxicities include:

- Hematologic: pancytopenia with increased risk of infection and immunosuppression, anemia
- Cardiovascular: hypertension, hypotension, tachycardia
- Dermatologic: rash, urticarial
- Gastrointestinal: nausea and vomiting, diarrhea, abdominal pain, altered taste, hematemesis, hematochezia
- Neurologic: headache, joint or limb pain, dizziness, insomnia, Progressive Multifocal Leukoencephalopathy (PML), tremors
- Genitourinary: miscarriage, birth defects, damage to unborn baby, limited effectiveness of birth control
- Endocrine and metabolic: hyperlipidemia, hyperglycemia, electrolyte imbalances
- Pulmonary: dyspnea
- Miscellaneous: fatigue, changes in vision, edema, unusual contusions, allergic reaction, leg cramps

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

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

The MMF dose is 15 mg/kg PO TID (adjusted IBW (Appendix K)) with total daily dose not to exceed 3 g (i.e. maximum 1 g PO TID). An equivalent IV dose (1:1 conversion) may instead be given.

Sirolimus (rapamycin, Rapamune®)

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

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

a. Sirolimus oral solution: sirolimus oral solution (1 mg/mL) should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). For dilution, the appropriate dose should be measured using an amber oral syringe, then added to a glass or plastic container that holds at least 60 mL. Before taking the dose, it should be diluted with water or orange juice then taken immediately; it should not be diluted with grapefruit juice. The syringe should be discarded after one use. Sirolimus oral solution provided in bottles may develop a slight haze when refrigerated, which does not affect product quality; allow the product to stand at room temperature and shake gently until the haze disappears.
b. Sirolimus tablets: sirolimus tablets are available in 1 mg and 2 mg tablets that cannot be crushed or broken. Sirolimus tablets should be stored at 20° to 25° C (68°–77°F), protected from light.

Sirolimus toxicities include:

- Gastrointestinal: constipation, abdominal pain, nausea, diarrhea, dyspepsia, vomiting, anorexia, stomatitis, ascites
- Pulmonary: dyspnea, asthma, cough, upper respiratory infection (URI), pleural effusion
- Dermatologic: acne, rash, urticarial, pruritus, delayed wound healing
- Hematologic: thrombocytopenia, anemia, thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), TMA, immunosuppression, leukopenia, lymphoproliferative disorders
- Cardiovascular: hypertension, hypotension, chest pain, tachycardia, congestive heart failure, pericardial effusion
- Genitourinary: urinary tract infection (UTI), renal dysfunction, proteinuria, kidney disease
- Neurologic: headache, arthralgia, back pain, tremors, insomnia
- Endocrine and metabolic: hypertriglyceridemia, hypercholesterolemia, hyperglycemia, electrolyte imbalance
- Musculoskeletal: arthrosis, bone necrosis, osteoporosis, myalgias
- Miscellaneous: peripheral edema, fever, flu-like syndrome

Sirolimus toxicities are summarized in Table 2.6C (Pidala, Alousi, & MacMillan, 2015) below.

TABLE 2.6C: SIROLIMUS TOXICITIES

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

(L): Toxicity may also occur later.
a Significant transaminitis, generally without sequellae, may occur. Sirolimus has been associated with higher rates of VOD after FIC.
b Incidence 3% to < 20% in a trial of kidney transplantation. In allogeneic HCT, increase in TMA from 4.2% with tacrolimus or cyclosporine alone, versus 10.8% with tacrolimus/sirolimus combination was noted.
c Lipid-lowering agent may be required; consider if fasting serum triglycerides are > 2.5 x ULN, and recommend starting if > 800 mg/dL.

Drug interactions:

- Sirolimus is known to be a substrate for both cytochrome CYP3A4 and Pglycoprotein.

- Agents that may increase sirolimus levels include tri-azole drugs (especially voriconazole and posaconazole*), amiodarone, calcium channel blockers, macrolide antibiotics (but not azithromycin), micafungin, gastrointestinal prokinetic agents (cisapride, metoclopramide), cimetidine, cyclosporine, grapefruit juice, and HIV protease inhibitors.
- Agents that may decrease sirolimus levels include anticonvulsants (carbamazepine, phenobarbital, phenytoin), rifamycins, St. John's Wort.

Dose adjustments:

- The sirolimus dose is adjusted to maintain a serum trough level of 5-15 ng/mL.
- Changes in levels due to altered bioavailability should be apparent within 24-48 hours.
- For sirolimus without CNI as in this study, a 20-25% reduction of sirolimus dose is recommended for trough levels >12 – 18 ng/mL, and a 20-25% increase is recommended for trough levels < 5 ng/mL.
- Renal failure does not affect the excretion of sirolimus.
- Excretion is reduced in liver failure; impaired hepatic function should prompt consideration of reduction in sirolimus maintenance doses but no dose adjustment of the loading dose is necessary.

Due to extreme interactions with triazole antifungal agents (fluconazole, itraconazole, clotrimazole, posaconazole, voriconazole, ketoconazole) **sirolimus should be administered cautiously together with fluconazole, itraconazole, or posaconazole, and with extreme caution if administered together with voriconazole**. If co-administration is unavoidable, then the dose of sirolimus should be greatly reduced when used in combination with an azole antifungal medication as recommended in Table 2.6D (Pidala, Alousi, & MacMillan, 2015) and that there should be very frequent monitoring of trough concentrations of sirolimus in whole blood. Sirolimus concentrations should be measured upon initiation, during co-administration, and at discontinuation of antifungal treatment, with sirolimus doses adjusted accordingly.

TABLE 2.6D: PRE-EMPTIVE DOSE REDUCTION OF SIROLIMUS WHEN USED IN COMBINATION WITH AZOLE

Antifungal	**Sirolimus**
	Dose ↓
Voriconazole	90%
Posaconazole	90%
Isavuconazole	90%
Itraconazole	90%
Fluconazole	50%

TBI

TBI toxicities include:

- Gastrointestinal: nausea, vomiting, diarrhea, anorexia
- Hematologic: hematopoietic suppression including neutropenia with increased risk of infection, bleeding and immunosuppression, carcinogenesis, anemia
- Dermatologic: reversible skin pigmentation and alopecia
- Genitourinary: infertility
- Pulmonary: lung fibrosis
- Miscellaneous: fever, parotiditis, fatigue, growth retardation, cataract formation

3 SUBJECT ENROLLMENT

3.1 Screening and Enrollment Procedures

3.1.1 Screening Procedures

Eligibility assessments must be performed within 4 weeks prior to the start of conditioning*:

- H&P
- HCT-CI score
- CBC with differential
- HLA typing (recipient and donor)†
- Performance status
- Echo or MUGA with measurement of LVEF or LVFS
- PFTs with measurements of FEV_1, FVC and DLCO. DLCO value may be corrected or uncorrected for hemoglobin.
- Serum chemistries (AST, ALT, ALP, total bilirubin, SCr (with CrCl measured by 24-hr urine specimen or nuclear GFR, or calculated GFR (by Cockcroft-Gault formula for those aged ≥ 18 years; by Original Schwartz estimate for those < 18 years) for subjects where SCr is outside normal range for age))
- β-HCG pregnancy test for FCBP
- Acute leukemias, MDS, CLL, CML, and lymphomas with bone marrow involvement pre-HCT: Bone marrow aspiration with or without biopsy for morphology, flow cytometry and cytogenetic analysis (fluorescence in situ hybridization (FISH) as indicated).
- Patient and donor CIBMTR Biorepository sample collection†
- Lymphoma/CLL subjects: pre-HCT imaging required. CT/PET/MRI scans of neck, chest, abdomen and pelvis as appropriate per center policy.

*See Appendix G for additional evaluations for HIV-positive subjects; Appendix D for the Treatment Review Committee approval process for the HIV-positive subject's conditioning regimen; and Appendix I for additional research laboratory samples.

†Pre-conditioning evaluations that do not need to be completed within 4 weeks prior to the start of conditioning.

3.1.2 Enrollment Procedures

Subjects must be consented prior to study-specific evaluations/procedures/tests/interventions (i.e. evaluations/procedures/tests/interventions that are beyond standard of care and directly required per protocol). Subjects having documented informed consent and meeting all eligibility criteria for the study should be enrolled at least 7 days prior to the start of conditioning.

- Note: HIV-positive subjects must be enrolled prior to collection of the pre-conditioning Latent HIV Reservoir sample (sample collection to take place within 8 weeks prior to the start of conditioning).

Subjects are to be registered using the following procedures:

- If not yet established, generate a CIBMTR Research Identification Number (CRID) using the tool in the FormsNet3 data entry system.
- An authorized user at the center completes the 15-MMUD Demographics and Inclusion/Exclusion forms in the Rave module.

3.2 Withdrawal Procedures

Subjects have the right to withdraw consent for study participation at any time and for any reason. If a subject withdraws prematurely after HCT, all data normally collected at the 1-year study time point should be gathered at the time of premature discontinuation and reported in the forms in the Rave module.

An explanation of why the subject is withdrawing from the study must be documented in the medical record and the Study Exit form in the Rave module. All subjects who withdraw from the study with an ongoing AE must be followed until resolution of the event, death, or until the investigator concludes that the event is stable with no further improvement anticipated.

Subjects lost to follow-up must be reported on the Study Exit form in the Rave module.

In the case that the subject decides to prematurely discontinue study treatment ("refuses treatment"), the subject must be asked if he or she can be contacted for further information. The outcome of that discussion must be documented in the medical record.

4 STUDY ENDPOINTS

4.1 Primary Endpoint

The primary endpoint for the study is OS at 1-year post-HCT. Subjects who are lost to follow-up prior to 1-year will be censored at the time of the last observation, and the OS proportion will be estimated using the Kaplan-Meier method.

4.2 Secondary Endpoints

Secondary endpoints include:

<u>Progression-free survival</u>
PFS is defined as the time from HCT until the documentation of disease progression/relapse or death due to any cause, whichever occurs first. PFS will be evaluated at Day+180 and +365.

<u>Transplant-related mortality</u>

TRM is defined as death without evidence of disease progression or recurrence. TRM will be evaluated at Day+100, +180, and +365.

Cumulative incidence of neutrophil recovery
Neutrophil recovery is defined as achieving a donor derived ANC $\geq 500/mm^3$ for 3 consecutive laboratory values on different days. The first day of the 3 laboratory values will be designated as the day of neutrophil engraftment. Death prior to neutrophil recovery will be considered a competing risk.

Cumulative incidence of platelet recovery
Platelet recovery is defined as achieving a platelet count $\geq$ 20,000/μL for 3 consecutive laboratory values on different days (with no platelet transfusions in the preceding seven days). The first day of the 3 laboratory values will be designated as the day of platelet recovery. Death prior to platelet recovery will be considered a competing risk.

Cumulative incidence of primary graft failure
Primary graft failure is defined as lack of donor-derived neutrophil engraftment (i.e. <5% donor chimerism on all laboratory values) by Day+56 in subjects surviving a minimum of 14 days post-HCT. Death prior to primary graft failure will be considered a competing risk.

Donor Chimerism
Peripheral blood chimerism (% of donor chimerism) in whole blood (unsorted) will be described on Day +28, +56, +100, +180, and +365.

Peripheral blood chimerism at Day+56
The percentage of subjects with peripheral blood chimerism in whole blood (unsorted) >95% by Day+56.

Cumulative incidences of aGVHD and cGVHD
aGVHD is defined as any skin, gastrointestinal or liver abnormalities fulfilling the BMT CTN Manual of Operations (MOP) criteria of grades II-IV or grades III-IV (Appendix H).

cGVHD is defined per National Institutes of Health (NIH) Consensus Criteria and includes organ involvement and severity, and overall global composite score (mild/moderate/severe) (Appendix H).

Cumulative incidences of viral reactivations and infections
We will assess the incidence of CMV, EBV, BK, ADV, and HHV-6 viral activations and infections at **Day+100, +180 and +365.** Severity grading of infections is defined in table 4.2 (BMT CTN MOP, 2013).

TABLE 4.2: INCIDENCE OF VIRAL REACTIVATIONS AND INFECTIONS

Type of Infection/ Severity Grade	Grade 1	Grade 2	Grade 3
CMV	Asymptomatic CMV viremia untreated or a CMV viremia with viral load decline by at least 2/3 of the baseline value after 2 weeks of therapy	Clinically active CMV infection (e.g. symptoms, cytopenias) or CMV Viremia not decreasing by at least 2/3 of the baseline value after 2 weeks of therapy	CMV end-organ involvement (pneumonitis, enteritis, retinitis)
EBV	EBV reactivation not treated with rituximab	EBV reactivation requiring institution of therapy with rituximab	EBV post-transplant lymphoproliferative disorder (PTLD)
ADV	Adenoviral conjunctivitis asymptomatic viruria, asymptomatic stool shedding and viremia not requiring treatment	Adenoviral URI, viremia, or symptomatic viruria requiring treatment	ADV with end-organ involvement (except conjunctivitis and upper respiratory tract)
HHV-6	Asymptomatic HHV-6 viremia untreated or an HHV-6 viremia with a viral load decline by at least 0.5 log after 2 weeks of therapy	Clinically active HHV-6 infection (e.g. symptoms, cytopenias) or HHV-6 viremia without viral load decline 0.5 log after 2 weeks of therapy	
BK	BK viremia or viruria with cystitis not requiring intervention	BK viremia or viruria with clinical consequence requiring prolonged therapy and/or surgical intervention	

Cumulative incidence of relapse/progression

Testing for recurrent malignancy in the blood, bone marrow or other sites will be used to assess relapse and progression post-HCT. For the purpose of this study, relapse is defined by either morphological, cytogenetic/molecular or radiological evidence of disease consistent with pre-HCT features. The event for this endpoint is the time interval from HCT to relapse/recurrence of disease or to last follow-up through 1-year post-HCT. Death in remission is considered a competing risk.

Acute leukemias (including ABL and AUL) and T-LBL relapse is defined as the recurrence of disease after CR, meeting the following criteria:

- ≥ 5% blasts in the bone marrow or peripheral blood
- EMD

MDS relapse/progression is defined by at least one of the following:

- Satisfying criteria for evolution into acute leukemia; or,

- Reappearance of pre-transplant morphologic abnormalities, detected in bone marrow specimens; or,
- Reappearance of pre-transplant cytogenetic abnormality in at least one metaphase on each of two separate consecutive examinations at least one month apart, regardless of the number of metaphases analyzed.
- Institution of any therapy to treat relapsed disease (institution of any therapy not meant for maintenance or prevention), including withdrawal of immunosuppressive therapy or DCI, will be considered evidence of relapse regardless of whether the criteria described above are met.

CLL disease relapse or progression is defined by at least one of the following:

- Reappearance of any manifestation of disease after achieving CR
- ≥ 50% increase in the sum of the products of ≥ 2 lymph nodes (≥ 1 lymph node must be ≥ 2 cm) or new nodes
- ≥ 50% increase in liver or spleen size, or new hepatomegaly or splenomegaly
- ≥ 50% increase in absolute lymphocyte count to ≥ 5×10^9/L
- Transformation to a more aggressive histology

CML disease relapse is defined as recurrence of disease after complete hematologic remission. Disease progression is defined as any of the following changes in disease status:

- Advancement from chronic phase to accelerated phase.
- Advancement from chronic phase to blast phase.

Accelerated phase
One or more of the following must be present:

- 10%-19% blasts in blood or marrow
- ≥ 20% basophils in peripheral blood
- Clonal marrow cytogenetic abnormalities in addition to the single Philadelphia chromosome (clonal evolution)
- Increasing spleen size, unresponsive to therapy
- Increasing WBC, unresponsive to therapy
- Thrombocytopenia (platelets < 100,000), unrelated to therapy
- Thrombocytosis (platelets > 1,000,000), unresponsive to therapy

Blast phase
Characterized by having ≥ 20% blasts in the peripheral blood or bone marrow. Having extramedullary blastic infiltrates (i.e., myeloid sarcoma, granulocytic sarcoma, or chloroma) also qualifies as blast phase.

Lymphoma disease relapse or progression is defined as any new lesion and/or > 50% increase in the least diameter of previously involved sites:

- *Spleen/Liver*
  > 50% increase from nadir of any previous lesions
- *Bone Marrow*
  New or recurrent involvement

Cumulative incidences of TMA and VOD/SOS

The cumulative incidences of TMA and VOD/SOS will be described in transplanted subjects, treating death as a competing risk.

VOD/SOS is defined as:

Modified Seattle Criteria
In the first 20 days after HCT, the presence of ≥2 of the following: • Bilirubin >2 mg/dL • Hepatomegaly or pain in right upper quadrant • Weight gain (>2% basal weight)

(Dalle, J.H., & Giralt, S., 2015)

TMA is defined as (Ho et al., 2005):

- RBC fragmentation and >2 schistocytes per high-power field on peripheral smear
- Concurrent increased serum LDH above institutional baseline
- Concurrent renal and/or neurologic dysfunction without other explanation
    - Renal dysfunction defined as either:
        - Doubling of SCr from baseline (baseline = creatinine before hydration and just before conditioning initiation) OR
        - 50% decrease in CrCl from baseline
- Negative direct and indirect Coombs test results

BMT CTN Consensus for TMA severity scoring:

Grade I	Evidence of RBC destruction (schistocytosis) without clinical consequences
Grade II	Evidence of RBC destruction with increased creatinine ≤ 3 x ULN
Grade III	Evidence of RBC destruction with creatinine > 3 x ULN not requiring dialysis
Grade IV	Evidence of RBC destruction with renal failure requiring dialysis, and/or encephalopathy

Proportion of subjects proceeding to transplant

The proportion of subjects proceeding to HCT will be described, along with a breakdown of the reasons for not proceeding to HCT and their frequencies.

Donor Selection Characteristics

The donor selection characteristics will be described. Donor selection characteristics include: number of mismatches at HLA-A, -B, -C, -DRB1, -DQB1, -DPB1, donor age, donor-recipient CMV serostatus match, donor weight, donor-recipient sex match and donor-recipient ABO group match.

Time from search to donor identification

Time from preliminary search and time from formal search to donor identification (i.e. the day the donor is selected by the study site) will be described.

Donor clonal hematopoiesis
The proportion of subjects developing donor clonal hematopoiesis will be described at Day+ 100 and +365.

4.3 Definition of CR by Disease Type

4.3.1 Acute Leukemias/T-LBL

4.3.1.1 ALL/T-LBL:

- < 5% blasts in the bone marrow
- Normal maturation of all cellular components in the bone marrow
- No currently active EMD (e.g., CNS, soft tissue disease)
- ANC ≥ 1,000/mm^3

4.3.1.2 AML:

- < 5% blasts in the bone marrow
- No blasts with Auer rods
- Normal maturation of all cellular components in the bone marrow
- No currently active EMD (e.g., CNS, soft tissue disease)
- Neutrophils ANC ≥ 1,000/mm^3

4.3.1.3 ABL/AUL:

- < 5% blasts in the bone marrow
- Normal maturation of all cellular components in the bone marrow
- No currently active EMD (e.g., CNS, soft tissue disease)
- Neutrophils ANC ≥ 1,000/mm^3

4.3.2 MDS (maintained for a minimum of 4 weeks):

- Bone marrow evaluation: < 5% myeloblasts with normal maturation of all cell lines
- Peripheral blood evaluation:
  - Hemoglobin ≥ 11 g/dL untransfused without erythropoietic support
  - ANC ≥ 1000/mm^3 without myeloid growth factor support
  - Platelets ≥ 100,000/mm^3 without thrombopoietic support
  - 0% blasts in blood

4.3.3 CLL:

- No evidence of lymphadenopathy
- No organomegaly
- Neutrophils ≥ 1.5 × 10^9/L
- Hemoglobin > 11 g/dL
- Lymphocytes < 4 × 10^9/L
- Bone marrow < 30% lymphocytes
- Absence of constitutional symptoms (including weight loss, fever, and night sweats)

4.3.4 CML (a treatment response where all of the following criteria are met):

- White blood count is < 10 × 10^9/L, without immature granulocytes and with < 5% basophils
- Platelet count < 450 × 10^9/L

- Non-palpable spleen

4.3.5 Lymphoma:

- Complete disappearance of all known disease.
- For typically PET-avid lymphoma, a post-treatment residual mass of any size is permitted as long as it is PET negative.
- For variably, PET-avid lymphoma, all lymph nodes and nodal masses must have regressed as measured by CT to < 1.5 cm (for nodes > 1.5 cm before therapy) or < 1 cm (for nodes 1.1 cm to 1.5 cm before therapy)
- *Spleen/Liver*: Not palpable; nodules disappeared
- *Bone Marrow*: Infiltrate cleared on repeat biopsy. If indeterminate by morphology, immunohistochemistry should be negative

5 STATISTICAL CONSIDERATIONS

5.1 General Considerations

All available data will be used and missing data will not be estimated or carried forward in any statistical summary or analyses. Descriptive summary statistics include the number of subjects, mean, median, percent coefficient of variation (if required), standard deviation, and minimum and maximum values (quantitative variables) or the number of subjects and percentages by category (qualitative variables). Baseline is defined as the last non-missing value prior to the receipt of study regimen.

5.2 Study Design

This study is a single arm Phase II, multi-center trial. It is designed to assess OS 1-year after HLA-MMUD bone marrow HCT and PTCy. The sample size is 80 subjects split into two strata of 40 subjects each for separate analysis. One stratum will include subjects receiving FIC, and the other will include subjects receiving RIC.

5.2.1 Accrual

The target sample size is 80 subjects accrued into two strata: 40 subjects receiving FIC and 40 subjects receiving RIC. It is estimated that two years of accrual will be necessary to enroll the targeted sample size. Accrual will be reported by race, ethnicity, sex, and age. If accrual to one of the strata occurs faster than for the other stratum, we will conduct the analysis for the stratum which is accrued faster without waiting for the other stratum to close and complete follow up.

5.3 Sample Size and Power Considerations

The sample size is 80 subjects for this trial, split into two strata of 40 subjects each (subjects receiving FIC and subjects receiving RIC will be analyzed separately). Ninety percent CIs were calculated for varying probabilities based on the sample size. Table 5.3A provides CIs for a variety of true underlying proportions. Of particular interest is where the OS probability is 65%, which is the targeted 1-year survival probability. For this setting, the CI length is 27%. The percentages above and below this range are meant to represent other plausible PFS percentages.

The precision of the estimates alternatively could be viewed as a lower bound on the probability of OS. The probability to rule out OS percentages of a certain size is known as "power." Table 5.3B provides the probability (or power) that the lower bound of a 90% two-sided CI for the OS probability will be greater than various OS thresholds between 40% and 55%. In particular, when the true OS percentage is 65%, there is 80% power to rule out an OS percentage of ≤ 45%. This can equivalently be viewed as testing the following hypothesis: H_o: p = 0.45 versus H_1: p ≠ 0.45. Based on table 5.3B below, there is 80% power at α= .10 (two-sided) to reject the null hypothesis if the true OS percentage is 65%.

TABLE 5.3A: CI LENGTHS AND POSSIBLE CIs FOR VARIOUS UNDERLYING OVERALL SURVIVAL PROBABILITIES

N/stratum	OS%	Length of 90% CI	Possible CIs	
40	80	22.8	66.8	89.6
40	70	25.7	56.0	81.7
40	65	26.6	50.8	77.4
40	60	27.3	45.8	73.1
40	50	27.8	36.1	63.9

The OS probability estimate will be based on the Kaplan-Meier product limit estimator using Greenwood's formula as the variance estimate. In the absence of censoring, the Kaplan-Meier estimate reduces to the simple binomial proportion.

TABLE 5.3B: PROBABILITY OF RULING OUT A THRESHOLD OF SIZE T OR LARGER FOR VARIOUS TRUE UNDERLYING OVERALL SURVIVAL PERCENTAGES, WITH N=40 PER STRATUM

	Probability of ruling out OS Percentages of size T or smaller			
True OS	T=0.55	0.50	0.45	0.40
0.70	0.58	0.81	0.94	0.99
0.68	0.47	0.72	0.89	0.97
0.65	0.31	0.57	**0.80**	0.93
0.60	0.13	0.32	0.57	0.79

5.4 Interim Analysis and Stopping Guidelines

There will be no formal interim analyses for efficacy. Monitoring of two key safety endpoints (overall mortality and grade III-IV aGVHD by Day+100) will be conducted weekly. If a stopping rule as described below is triggered, enrollment will be paused while the DSMB conducts a review of the safety data. Both grade III-IV aGVHD and overall mortality within 100 days are expected to occur no more than 15% of the time; therefore, the same stopping rules will be applied to each safety endpoint. The detailed stopping rule is shown in the table below.

The DSMB has regularly scheduled meetings at least annually and as frequently as quarterly. Emergency meetings may be called at any time by either Monitoring Board Chairperson, Vice-Chairperson or appropriate CIBMTR representative.

TABLE 5.4: SAFETY STOPPING RULE (APPLIES TO BOTH GRADE III-IV AGVHD AND OVERALL MORTALITY WITHIN 100 DAYS). STOP IF ≥X EVENTS IN N SUBJECTS EVALUABLE.

N	5	9	13	17	21	26	31	35	40
# of events, x	3	4	5	6	7	8	9	10	11

This toxicity pausing rule has a 10% chance of being triggered if the true toxicity rate is 15%, and a 92% chance of being triggered if the true toxicity rate is 35%. If the true toxicity rate is 35%, the stopping rule will be triggered on average after approximately 16 subjects are evaluable for the safety endpoint. Note that the pausing 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.

5.5 Analysis of Study Endpoints

5.5.1 Analysis of Primary Endpoint

The primary analysis will consist of estimating the 1-year OS probability based on the Kaplan-Meier product limit estimator, separately in the two strata (subjects receiving FIC; subjects receiving RIC). The event is death from any cause. The time to this event is the time from HCT to death, loss to follow-up, or end of study (whichever comes first). The 1-year OS probability and 90% CI will be calculated. All transplanted subjects will be included in this analysis.

5.5.2 Analysis of Secondary Endpoints

Progression-free survival

Death or relapse/progression will be considered events, and the PFS distribution will be estimated by the Kaplan-Meier curve, starting from the time of HCT. A 90% CI will be constructed at Day+180 and +365.

Transplant-related mortality

TRM is death occurring in subjects in the absence of disease progression or relapse. A cumulative incidence curve will be computed along with a 90% CI at Day+100, +180, and +365. Progression/relapse will be considered as a competing risk.

Cumulative incidence of neutrophil recovery

To assess the incidence of neutrophil recovery from day of HCT, a cumulative incidence curve will be computed along with a 90% CI. Death prior to neutrophil recovery will be considered a competing risk.

Cumulative incidence of platelet recovery

To assess the incidence of platelet recovery from day of HCT, a cumulative incidence curve will be computed along with a 90% CI. Death prior to platelet recovery will be considered a competing risk.

Cumulative incidence of primary graft failure
The frequency and proportion of subjects experiencing primary graft failure by Day+56 will be described with a 90% CI. Death prior to primary graft failure will be considered a competing risk.

Donor Chimerism
Peripheral blood donor chimerism will be measured at Day+28, +56, +100, +180, and +365. The degree of donor chimerism will be summarized at each time point using descriptive statistics.

Peripheral blood chimerism at Day+56
The percentage of patients with peripheral blood chimerism >95% by Day+56 will be summarized.

Cumulative incidence of aGVHD
We will assess the incidence of grades II-IV and grades III-IV aGVHD from day of HCT. The first day of aGVHD onset at a certain grade will be used to calculate a cumulative incidence curve for that aGVHD grade. An overall cumulative incidence curve will be computed along with a 90% CI at Day+100 with death and relapse considered as competing risks.

Cumulative incidence of cGVHD
To assess the incidence and severity of cGVHD from day of HCT, a cumulative incidence curve will be computed along with a 90% CI at Day+180 and 365. Death and relapse prior to occurrence of cGVHD will be considered as competing risks.

Cumulative incidences of viral reactivations and infections
To assess the incidence of CMV, EBV, BK, ADV, and HHV-6 viral activations and infections, a cumulative incidence curve will be computed along with a 90% CI at Day+100, +180 and +365.

Cumulative incidence of relapse/progression
To assess the incidence of progression/relapse from day of HCT, a cumulative incidence curve will be computed along with a 90% CI at Day+180 and +365. Death prior to progression/relapse will be considered as a competing risk.

Cumulative incidences of TMA and VOD/SOS
The cumulative incidence of TMA and VOD/SOS will be described in transplanted subjects, treating death as a competing risk.

Proportion of subjects proceeding to transplant
The proportion of subjects proceeding to HCT will be described, along with a breakdown of the reasons for not proceeding to HCT and their frequencies.

Donor Selection Characteristics
The donor selection characteristics will be described. Donor selection characteristics include: number of mismatches at HLA-A, -B, -C, -DRB1, -DQB1, -DPB1, donor age, donor-recipient CMV serostatus match, donor weight, donor-recipient sex-match and donor-recipient ABO group match.

Time from search to donor identification
Time from preliminary search to and time from formal search to donor identification (i.e. the day the donor is selected by the study site) will be described.

Subgroup analysis of HIV-positive subjects
If CCR5delta32 homozygous donors are successfully found and used for one or more HIV-positive subjects, a descriptive analysis of baseline characteristics and outcomes for those HIV-positive subjects will be conducted, including the viral load detected over time obtained from collected samples.

Donor clonal hematopoiesis
The proportion of subjects developing donor clonal hematopoiesis will be described at Day+100 and +365.

5.6 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized for all subjects. Characteristics to be described are: recipient age, recipient race/ethnicity, recipient performance status, pre-HCT comorbidity index (HCT-CI), HLA match, recipient disease type and stage, recipient remission status, number of prior treatments, time from diagnosis to HCT, and conditioning regimen. Donor/recipient sex match, donor/recipient CMV serostatus, donor/recipient ABO group, donor weight and donor age will also be described.

5.7 Disposition of Subjects

The number of subjects enrolled in the study and the disposition of all subjects will be summarized. Subjects who discontinue study participation will be listed according to the off-study criteria that applies.

Off-Study Criteria

All subjects who have received their HCT will be followed on-study through 1-year post-HCT. Subjects will be removed from the study for the following reasons:

1. Withdrawal of consent
2. Subject determined ineligible by the study team after initial enrollment
3. Discontinued per medical discretion of the P.I. or Medical Monitor
4. Donor cannot provide bone marrow as planned
5. Transplant canceled, insufficient dose of bone marrow obtained
6. Transplant canceled, donor reason
7. Transplant canceled, subject (recipient) reason
8. Lost to Follow-Up

9. Death

Study sites must complete the Study Exit form in Rave for any subjects meeting one of these criteria. The Study Exit form is also completed to indicate that a subject has completed the study per protocol.

6 DATA REPORTING

6.1 Data Capture Methods

The data collection forms for the subjects enrolled on this study include the standard CIBMTR data collection forms in the FormsNet3 Recipient module, and study-specific CIBMTR data collection forms in Rave.

CIBMTR data reporting time points used in this study:

- Baseline
- Infusion data
- 100 days post-HCT
- 6 months post-HCT
- 1-year post-HCT
- Subject death (if applicable)

Many important data elements for the study are collected on the standard reporting forms and therefore timely and accurate completion of these forms is essential. Centers must continue to do standard CIBMTR follow-up reporting on these subjects beyond the study time point of 1-year post-HCT, per CIBMTR reporting requirements.

The following forms will be completed by study site personnel within the timeframes specified:

Study-specific forms in Rave module	**Submission timeframes**
Demographics	At least 7 days prior to the start of conditioning
Inclusion/Exclusion	At least 7 days prior to the start of conditioning
Medical history	At least 7 days prior to the start of conditioning
Conditioning regimen (with regimen-specific form completion)	Within 7 days of HCT date
Infusion	Within 7 days of HCT date
Follow-up	Within 7 days of evaluation date for study time point
Post-transplant treatments	Within 7 days of evaluation date for study time point
Sirolimus	Within 7 days of evaluation date for study time point
MMF	Within 7 days of evaluation date for study time point
Hematopoietic recovery	Within 7 days of evaluation date for study time point
Protocol-specified sample collections	Within 7 days of evaluation date for study time point
Peripheral blood chimerism	Within 7 days of evaluation date for study time point
DCI	Within 7 days of evaluation date on corresponding Follow-up form (when DCI is indicated on the Follow-up form completed for the study time point)

Disease evaluation	Within 7 days of evaluation date for study time point (Day+100; Day+180; Day+365; or when relapse is reported)
Acute GVHD assessment	Within 7 days of evaluation date on corresponding Follow-up form (when aGVHD is indicated on the Follow-up form completed for the study time point)
Chronic or Overlap GVHD • Assessment • Organ involvement at diagnosis • Status • Therapy	Within 7 days of evaluation date on corresponding Follow-up form (when chronic or overlap GVHD is indicated on Follow-up form for time point)
Viral reactivations or infections	Within 7 days of evaluation date on corresponding Follow-up form (when viral reactivations or infections is indicated on Follow-up form for time point)
TMA assessment	Within 7 days of evaluation date on corresponding Follow-up form (when TMA is indicated on Follow-up form for time point)
VOD/SOS • Assessment • Evaluations at diagnosis • Therapy • Maximum severity • Current status	Within 7 days of evaluation date on corresponding Follow-up form (when VOD/SOS is indicated on Follow-up form for time point)
HIV-positive pre-conditioning	At least 7 days prior to the start of conditioning (for HIV-positive subjects only)
HIV-positive post-transplant	Within 7 days of evaluation date for study time point (for HIV-positive subjects only)
Adverse Event • Assessment • Summary • Meds • Laboratory values • Tests	• Grade 3 unexpected AEs: within 3 business days of knowledge of the event • Grade 4-5 unexpected AEs: within 24 hours of knowledge of the event • Grade 5 expected AEs: within 24 hours of knowledge of the event
Unanticipated Problem (UPIRSO/UP)	Within 3 business days of the investigator becoming aware of the issue
Study Exit	Within 7 days of study exit event date

6.2 Adverse Event and Unanticipated Problem Reporting

6.2.1 Event Definitions

Adverse Event – Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of definite, probable, possible, unlikely, or unrelated).

Expected Adverse Event – Any AE listed in the informed consent, product inserts, or study materials.

Life-Threatening Adverse Event – Any AE that places the participant, in view of the investigator, at immediate risk of death from the reaction.

Serious Adverse Event (SAE) – Any AE that results in any of the following outcomes: death, a life threatening AE, in-patient hospitalization or prolongation of existing

hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.

Unexpected Adverse Event – Any AE, the specificity or severity of which is NOT listed in the study protocol, product inserts or informed consent document.

Unanticipated Problem – any incident, experience, or outcome that meets **all** of the following criteria:

1. unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
2. related or possibly related to participation in the research; and
3. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

See Appendix L for further details and examples.

Attribution – The determination of whether an AE is related to a medical treatment or procedure. Attribution categories:

- Definite – The AE is ***clearly related*** to the study procedure/treatment(s).
- Probable – The AE is ***likely related*** to the study procedure/treatment.
    - *The AE is not likely to be caused by the subject's underlying medical condition or other concomitant therapy, and the nature of the AE or the temporal relationship between the onset of the AE and study procedure/treatment administration lead the investigator to believe that there is a reasonable chance of causal relationship.*
- Possible – The AE ***may be related*** to the study procedure/treatment(s).
    - *The AE could be attributed to the subject's underlying medical condition or other concomitant therapy, but the nature of the AE or the temporal relationship between the onset of the AE and study procedure/treatment administration lead the investigator to believe that there could be a causal relationship.*
- Unlikely – The AE is ***doubtfully related*** to the study procedure/treatment(s).
- Unrelated – The AE is ***clearly NOT related*** to the study procedure/treatment(s).
    - *The AE is most plausibly explained by the subject's underlying medical condition or other concomitant therapy, or the AE has no plausible biological relationship to study procedure/treatment.*

Grade – Severity of the AE. All toxicities will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03. The CTCAE includes a grading (severity) scale for each AE term:

Grade 0 – No AE or within normal limits
1 – Mild AE
2 – Moderate AE
3– Severe AE
4– Life-threatening or disabling AE
5– Fatal AE

6.2.1.1 Reporting Requirements

Unexpected Adverse Events: Grades 3-5 unexpected AEs will be reported on the Adverse Event form in the Rave module. The CIBMTR Protocol Coordinator will review all submitted unexpected AEs and forward the information to the Medical Monitor for review.

- Grade 3 unexpected AEs, irrespective of the attribution of the event to the study procedure/treatment, must be reported within three business days of knowledge of the event.
- Grade 4-5 unexpected AEs, irrespective of the attribution of the event to the study procedure/treatment, must be reported within 24 hrs of knowledge of the event.

All unexpected AEs will be reviewed by the Medical Monitor within two business days of receiving the summary of the AE from the study site. If the Medical Monitor requires additional information to make his/her assessment, study sites will have 4 business days to respond to the request for additional information. The Medical Monitor will provide expedited notification with event assessment and any modification recommendation to the CIBMTR within seven days of the AE.

TABLE 6.2.1: REPORTING UNEXPECTED ADVERSE EVENTS

SEVERITY GRADE	ATTRIBUTION	STUDY SITE
5 – Fatal– 4 – Life-threatening or Disabling	All attributions	Submit the Adverse Event form to CIBMTR within 24 hours of learning of the event. • Grade 5 AEs: o AE form should include potential contributing causes of death o Study site must also complete all follow up forms through date of death, and study exit form
3 – Severe	All attributions	Submit the Adverse Event form to CIBMTR within 3 business days of the AE.

Note: For any AE that is ongoing at the time of death, the date of death should be reported as the resolution date.

Expected Adverse Events:
All Grade 5 expected AEs must be reported via the Adverse Event form within 24 hours of learning of the death. Expected AEs < Grade 5 need not be reported via the Adverse Event form, as they will be captured on other event-driven case report forms. The CIBMTR Protocol Coordinator will review Grade 5 expected AEs reported, and forward the information to the Medical Monitor for review.

The CIBMTR will prepare semi-annual summary reports of all Grade 5 expected AEs for the DSMB. See Table 6.2.2. for expected event reporting expectations.

The CIBMTR Protocol Coordinator and Medical Monitor will review the AEs monitored for stopping guidelines on a weekly basis.

Additionally, the CIBMTR Protocol Coordinator and Medical Monitor will review reported toxicity, GVHD, infection, and primary graft failure events on a weekly basis to assess whether there are safety concerns that should be referred to the DSMB.

Any concern regarding the type or frequency of an AE will be reported to the DSMB when warranted. The DSMB will determine if additional review is required and make recommendations to the study team concerning continuation of the study. If the DSMB recommends modification of protocol documents and/or determines that the event affects the conduct of the overall trial, the event will be reported to the NMDP IRB. The Principal Investigators and the Protocol Team will meet to determine protocol modifications based on DSMB recommendations.

The NMDP IRB will report events to regulatory agencies following NMDP IRB processes and procedures. CIBMTR will distribute reports of such events and any protocol modifications to study sites and study site Principal Investigators will be responsible for local IRB submission.

TABLE 6.2.2: REPORTING EXPECTED ADVERSE EVENTS

SEVERITY GRADE	ATTRIBUTION	STUDY SITE REPORTING REQUIREMENT
5 – Fatal	All attributions	Submit Adverse Event form within 24 hours of learning of the death. Submit death summaries and/or autopsy reports of the expected AE to CIBMTR as requested. The summaries should include potential contributing causes of death.

Adverse Event Updates: AEs should be followed until resolution of the event, death, or until the investigator concludes that the event is stable with no further improvement anticipated. Updates are reported by revising the Adverse Event form in the Rave module.

Unanticipated Problems: Unanticipated problems should be reported within three business days of the investigator becoming aware of the issue, via the Unanticipated Problem form in the Rave module.

All Unanticipated Problems will be reviewed by the Medical Monitor within three business days of receiving the summary of the Unanticipated Problem from the study site. The Medical Monitor will provide expedited notification with event assessment and any modification recommendation to the CIBMTR within seven days of the Unanticipated Problem.

7 PROTOCOL DEVIATIONS

7.1 Investigator Reporting Responsibilities

Circumstances may arise when it is necessary for subject safety to deviate from the study protocol. When feasible, Investigators should contact the protocol chairs and CIBMTR Protocol Coordinator beforehand to discuss the intended deviation(s). All deviations, whether approved beforehand or not, are required to be documented and explained by the Investigator, and reported to the study sponsor within 7 business days.

All protocol deviations will be documented in the Rave module by the study sponsor.

Study sites are also required to note deviations in patient medical records and report them to their local IRB in accordance with local policies. All protocol deviations will be compiled centrally by the CIBMTR Protocol Coordinator and reported to the NMDP DSMB.

Non-emergency minor deviations from the protocol will be permitted with approval from the CIBMTR Protocol Coordinator.

7.2 Study Monitoring

7.2.1 Data Safety Monitoring

The NMDP DSMB will review a summary of AEs at least twice yearly.
The report will be made available to study sites. If the DSMB recommends protocol or informed consent changes, they will be distributed to the participating Principal Investigators. It is the responsibility of each Principal Investigators to forward the distributed communication to their local IRB.

7.2.2 Study Site Monitoring Plan

The Principal Investigator [or his/her deputy] will permit study-related onsite and/or centralized monitoring visits by representatives of the CIBMTR or designees, and regulatory inspection(s) (e.g., FDA) to ensure proper conduct of the study and compliance with all FDA safety reporting requirements. Access must be provided to source documents, data collection forms, informed consent and assent forms, and any other study documents.

The Principal Investigator [or his/her deputy] agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

Details about monitoring can be found in the Study Monitoring Plan.

8 REGULATORY CONSIDERATIONS

8.1 Institutional Review Board/Ethics Committee approval

The protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki and the Belmont Report. The review of this protocol by the IRB and the performance of all aspects of the study, including the methods used for obtaining informed consent, must also be in accordance with principles enunciated

in the Belmont Report, as well as International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guidelines, Title 21 of the Code of Federal Regulations (CFR), Part 50 Protection of Human Subjects and Part 56 Institutional Review Boards.

The NMDP IRB serves as the Coordinating Center IRB for the protocol. The study site Principal Investigators will be responsible for preparing documents for submission to the institutional IRB of their study sites and obtaining written approval for this study. The approval will be obtained prior to the initiation of the study at the study site.

The approval for both the protocol and informed consent must specify the date of approval, protocol number and version, or amendment number.

Any approved amendments to the protocol must be submitted by the study site Principal Investigator to the local IRB for approval. The study site Principal Investigator is also responsible for notifying the institutional IRB of any serious deviations from the protocol, or anything else that may involve added risk to subjects.

8.2 Informed Consent

All subjects in this study must provide informed consent prior to any study related procedures as per Good Clinical Practices as set forth in the CFR and ICH guidelines. Legal guardian permission must be obtained for subjects < 18 years of age. Pediatric subjects will be included in age appropriate discussion in order to obtain assent.

Documentation that informed consent (or minor assent with parental permission) occurred prior to the subject's entry into the study and the informed consent process must be recorded in the subject's source documents. The original informed consent form signed and dated by the subject and by the person obtaining the subject's informed consent prior to the subject's entry into the study, must be maintained in the transplant center's study files.

LIST OF APPENDICES:

APPENDIX A

PERFORMANCE STATUS

KARNOFSKY SCALE, ≥ 16 YEARS

100% – normal, no complaints, no signs of disease
90% – capable of normal activity, few symptoms or signs of disease
80% – normal activity with some difficulty, some symptoms or signs
70% – caring for self, not capable of normal activity or work
60% – requiring some help, can take care of most personal requirements
50% – requires help often, requires frequent medical care
40% – disabled, requires special care and help
30% – severely disabled, hospital admission indicated but no risk of death
20% – very ill, urgently requiring admission, requires supportive measures or treatment
10% – moribund, rapidly progressive fatal disease processes

LANSKY PLAY-PERFORMANCE SCALE, < 16 YEARS

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

APPENDIX B

LIST OF ABBREVIATIONS

µL	microliter
µM	micrometer
ABL	acute biphenotypic leukemia
ADV	adenovirus
AE	adverse event
aGVHD	acute graft versus host disease
AIDS	acquired immunodeficiency syndrome
ALL	acute lymphoblastic leukemia
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AML	acute myelogenous leukemia
ANC	absolute neutrophil count
ARV	antiretroviral therapy
AST	aspartate aminotransferase
ATG	anti-thymocyte globulin
ATP	adenosine triphosphate
AUC	area under the curve
AUL	acute undifferentiated leukemia
AZT	zidovudine
BID	two times a day (*bis in die*)
BMT CTN	Blood and Marrow Transplant Clinical Trials Network
BOOP	bronchiolitis obliterans organizing pneumonia
BSA	body surface area
CBC	complete blood count
CFR	Code of Federal Regulations
cGVHD	chronic graft versus host disease
cGy	centigray
CI	confidence interval
CIBMTR	Center for International Blood and Marrow Transplant Research
CLL	chronic lymphocytic leukemia
cm	centimeter
CML	Chronic myelogenous leukemia
CMV	Cytomegalovirus
CNI	calcineurin inhibitors
CNS	central nervous system

CR	complete remission/complete response
CrCl	creatinine clearance
CRID	CIBMTR Research Identification Number
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
Cy	cyclophosphamide
dL	deciliter
DLCO	diffusing capacity of the lungs for carbon monoxide
DCI	donor cellular infusion
DNA	deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
EBV	Epstein–Barr virus
EDTA	ethylenediaminetetraacetic acid
FCBP	females of childbearing potential
EMD	extramedullary disease
FDA	U.S. Food and Drug Administration
FEV_1	forced expiratory volume
FIC	full intensity conditioning
FISH	fluorescence in situ hybridization
FVC	forced vital capacity
g	gram
G-CSF	granulocyte-colony stimulating factor
GFR	glomerular filtration rate
GM-CSF	granulocyte-macrophage colony-stimulating factor
GVHD	graft versus host disease
H&P	history and physical
HAART	highly active antiretroviral therapy
HCT	hematopoietic cell transplantation
HCT-CI	hematopoietic cell transplantation comorbidity index
HHS	U.S. Department of Health & Human Services
HHV-6	human herpesvirus 6
HI	Hematologic Improvement
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HPLC	high performance liquid chromatography
hr	hour
HSV	herpes simplex virus

HUS	hemolytic uremic syndrome
IBW	ideal body weight
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
INSTI	integrase strand transfer inhibitor
INR	international normalized ratio
IPSS	International Prognostic Scoring System
IRB	Institutional Review Board
IV	intravenous
kg	kilogram
L	liter
LVEF	left ventricular ejection fraction
LVFS	left ventricular shortening fraction
m^2	square meter
mm^3	cubic millimeter
MAC	M. Avium Complex
mcg	microgram
MDS	myelodysplastic syndrome
MFI	mean fluorescence intensity
mg	milligram
min	minute
mL	milliliter
MMF	mycophenolate mofetil
MMUD	mismatched unrelated donor
MOP	manual of procedures
MPA	mycophenolic acid
MRI	magnetic resonance imaging
MUGA	multiple-gated acquisition scan
ng	nanogram
NIH	National Institutes of Health
NMDP	National Marrow Donor Program
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitors
NRM	non-relapse mortality
NRTI	Nucleoside Reverse Transcriptase Inhibitors
OHRP	Office for Human Research Protections
OS	overall survival
PCR	polymerase chain reaction

PET	positron emission tomography
PFS	progression-free survival
PFTs	pulmonary function tests
PK	pharmacokinetic
PML	progressive multifocal leukoencephalopathy
PO	by mouth (*per os*)
PR	partial response
PTCy	post-transplantation cyclophosphamide
PTLD	post-transplant lymphoproliferative disorder
QD	one day (*quaque die)*
RCI BMT	Resource for Clinical Investigation in Blood and Marrow Transplantation
RIC	reduced intensity conditioning
RNA	ribonucleic acid
SAE	serious adverse event
SC	subcutaneous
SCr	serum creatinine
SOS	sinusoidal obstruction syndrome
TBI	total body irradiation
TID	three times a day (*ter in die*)
T-LBL	T lymphoblastic lymphoma
TMA	thrombotic microangiopathy
TMP/SMX	trimethoprim/sulfamethoxazole
TRM	transplant-related mortality
TTP	thrombotic thrombocytopenic purpura
ULN	upper limit of normal
URI	upper respiratory infection
UTI	urinary tract infection
VOD	hepatic veno-occlusive disease
VZV	varicella–zoster virus

APPENDIX C

NMDP PROCEDURE FOR IDENTIFICATION OF CCR5DELTA32 MUTATION HOMOZYGOUS DONORS RELATED TO THIS PROTOCOL

Background

A recent case report by Hütter et al. (NEJM, 2009) documented the outcome of an allogeneic HCT performed for the treatment of AML in which the well-matched donor was selected for homozygosity for the CCR5delta32 mutation. The recipient had been known to be HIV-infected prior to developing AML and the German transplant team hypothesized that hematopoietic cells from such a donor could confer natural resistance to HIV infection in the transplanted hematopoietic and immune cells. A single HLA-matched donor homozygous for the CCR5delta32 mutation was identified among approximately 80 well-matched donors and an allogeneic HCT for the treatment of the patient's AML was performed using that donor. Following HCT, the recipient's ARV medications were discontinued and viral titers were monitored. No subsequent recrudescence of HIV replication was detected. The recipient relapsed with AML approximately one year later but a second HCT using the same donor was successfully performed with continued primary disease remission and HIV control with undetectable HIV viral loads two years following the second HCT (Hütter, personal communication).

The 15-MMUD Protocol Team would like to replicate these findings in the context of allogeneic HCT as proposed under this protocol. It is known that the incidence of CCR5delta32 homozygosity is essentially limited to European populations, with highest incidence in Northern Europeans (Galvani and Novembre, 2005); from these data, it is apparent that non-European populations manifest homozygosity too rarely to warrant evaluation. Additionally, it is also known that donor ethnicity strongly correlates with recipient ethnicity.

In view of that fact that this protocol allows the use of HLA MMUDs there is a statistically higher chance of finding a CCR5delta32 mutation homozygous donor for the patient.

Assessment of donor CCR5delta32 homozygosity should be considered for any HIV-positive patient who meets the criteria for inclusion in the 15-MMUD protocol.

CCR5delta32 homozygosity status will be considered for donors who have been previously typed for this polymorphism and have the information available at the time of the search. No additional sample collection or informed consent is required for these donors.

References

Hütter G, Nowak D, Mossner M, Ganepola S, ßig A, Allers K, Schneider T, Hofmann J, Kücherer K, Blau O, Blau IW, Hofmann WK, Thiel E. Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation. *New Engl J Med.* 2009; 360:692-8.

Galvani A, Novembre J. The evolutionary history of the CCR5-D32 HIV-resistance mutation. *Microbes Infect*. 2005; 7: 302–309.

APPENDIX D

TREATMENT REVIEW COMMITTEE FOR HIV-POSITIVE SUBJECTS

Due to the complex nature of the patient population and the potential for numerous drug-drug interactions between the ARV regimen and the allogeneic HCT conditioning regimen, each subject's conditioning regimen must be reviewed with the Treatment Review Committee. This review should typically occur at least 4 weeks prior to the planned conditioning regimen start date in order to allow sufficient time for any necessary adjustments to the subject's medication regimen.

The treating institution will review subjects with the Treatment Review Committee prior to the initiation of HCT therapy by a review panel composed of experts in HIV drug resistance, HIV care, pharmacology, HCT infectious diseases and oncology. Recommendations for HCT are based on the following supporting documentation:

− Current diagnosis
− Past medical history
− Prior ARV history
− Documented ARV drug toxicities
− Drug allergies
− History of HLA B57 antigen testing, if available
− Current creatinine and liver function tests
− Past and recent HIV drug resistance test results
− Current ARV drug regimen
− Current concomitant medications

The Committee will review available past and current HIV-1 genotype results for subjects with detectable viremia so as to exclude subjects with multi-drug resistant HIV-1 who are unlikely to achieve viral suppression on alternate ARV regimens. The Committee will also advise with regard to various drug interactions anticipated during the course of HCT. The Treatment Review Committee will include well-qualified investigators from the Johns Hopkins University. Review materials will be sent to the panel 4 weeks prior to initiation of HCT therapy to allow sufficient time for implementation of recommendations for the ARV regimen. The Treatment Review form and de-identified copies of the supporting documentation (noted above) must be submitted to the CIBMTR Protocol Coordinator. The CIBMTR Protocol Coordinator will then provide these materials to the Treatment Review Committee to conduct their review.

APPENDIX E

SUGGESTED PROPHYLAXIS FOR HIV-POSITIVE SUBJECTS

Infectious prophylaxis for HIV-positive subjects undergoing HCT will include prophylaxis for:

1. Bacteria: In keeping with the BMT CTN MOP and local institutional standards.
2. *Pneumocystis Jiroveci* Pneumonia: Prophylaxis will be administered until CD4 counts are >200 and for a minimum of 6 months. Several effective regimens are available. Subject tolerance (nausea, allergic reaction, G6PD or other considerations) may contraindicate a particular regimen. Choices in order of preference are: 1) TMP/SMX 1 double-strength daily; 2) TMP/SMX 1 single strength daily; 3) dapsone 100 mg daily (may be decreased to 50 mg daily if given in combination with pyrimethamine as described below for toxoplasmosis prophylaxis; 4) atovaquone 1500 mg daily; and, 5) aerosolized or IV pentamidine monthly, or per institutional standard.
3. Toxoplasmosis: Subjects on TMP/SMX do not require additional prophylaxis. If toxoplasma IgG is positive and TMP/SMX cannot be used subjects should be prophylaxed for at least 3 months after HCT and until CD4>100. This prophylaxis may be either: 1) dapsone 50 mg PO daily and pyrimethamine 50 mg/week and leucovorin 25 mg/week or 2) atovaquone 1500 mg daily plus pyrimethamine 25 mg/day plus leucovorin 10 mg/day. Since all medications for toxoplasmosis prophylaxis are oral, and some subjects may be unable to take PO medications in the peri-HCT period, polymerase chain reaction (PCR) for toxoplasmosis should be checked at least weekly whenever prophylaxis must be held in toxoplasma IgG positive subjects for more than a week and the CD4 is < 100.
4. Fungi: Anti-fungal prophylaxis will be per local institutional practice. It is noted that in histoplasma endemic areas (Midwest and Puerto Rico) antifungal prophylaxis is standard for CD4 <150 and would be appropriate for at least 3 months after HCT and until CD4>150 (see Appendix F for detailed interactions with ARVs).
5. Herpes simplex virus (HSV)/varicella–zoster virus (VZV): One of the following regimens should be used for 1-year after HCT, or per institutional standard, unless the subject remains on immunosuppressive acyclovir 400 - 800 mg PO BID, valaciclovir 500 mg PO BID, or famciclovir 500 mg PO BID.
6. M. Avium Complex (MAC): If CD4 < 50, azithromycin suggested at 1200 mg q week or 600 mg twice weekly.
7. Hepatitis:
    a. Subjects with positive hepatitis B surface antigen should be evaluated for viral DNA replication (viral load) by a quantitative PCR method before enrolling the subject on the study.
    b. Lamivudine or newer generation of anti-hepatitis B agents, like tenofovir, should be started in those with detectable hepatitis B viral load according to institutional preferences. The goal of the treatment should be achieving undetectable (<500 copies/ml) viral load status.
    c. Subjects should be maintained on anti-hepatitis B treatment throughout chemotherapy, throughout the HCT, and at least 1-year post-HCT.
    d. Subjects with hepatitis C must have achieved a sustained virologic response for 12 weeks after cessation of antiviral treatment to be eligible for the study.

APPENDIX F

PK DRUG-DRUG INTERACTIONS WITH ARVs

Preferred ARV Regimens for Subjects Undergoing Allogeneic HCT
Tier 1: Raltegravir (INSTI) based therapy (no interruption of therapy is required)
Tier 2: NNRTI based therapy (interruption of therapy may be required, see Table F-1)

Summary of ARV Agents and Drug-Drug Interactions

1. **NNRTIs** (efavirenz, nevirapine, etravirine)
    a. Dosing: Efavirenz is the preferred agent in this class, nevirapine should be used with caution due to toxicity
    b. Toxicity: Class adverse effects include, rash, hepatotoxicity and gastrointestinal intolerance
    c. Metabolism: NNRTIs are metabolized hepatically via cytochrome P450 co-enzymes
    d. Drug Interactions
        i. All NNRTIs induce CYP3A4 (etravirine > efavirenz > nevirapine)
        ii. Efavirenz
            1. Substrate for CYP3A4 (major), 2B6 (major)
            2. Inhibits CYP2C9 (moderate), 2C19 (moderate), 3A4 (moderate)
            3. Induces CYP2B6 (weak), 3A4 (strong)

2. **Nucleoside Reverse Transcriptase Inhibitors (NRTI)** (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir)
    a. Dosing: Zidovudine (AZT) must not be used in subjects receiving HCT
    b. Toxicity: Adverse effects of the class include lactic acidosis, peripheral neuropathy and hepatic steatosis
    c. Metabolism: Not extensively metabolized, eliminated renally (except abacavir)
    d. Drug Interactions
        i. None of the NRTIs are metabolized via CYP450 co-enzymes
        ii. Avoid additive toxicities

3. **Other Agents**
    a. Raltegravir (INSTI): Preferred agent due to excellent tolerability and lack of drug-drug interactions
    b. Maraviroc: Substrate of CYP3A4, but does not induce or inhibit the metabolism of other agents
    c. Enfuviritide: No clinically relevant drug interactions, reserved for salvage therapy only
    d. Ritonavir: must not be used in subjects receiving HCT
    e. Cobicistat: must not be used in subjects receiving HCT

TABLE F-1: SUMMARY OF ARV DRUG-DRUG INTERACTIONS

Conditioning Regimen	Metabolism (Primary Site)	Interaction Potential* (Predicted Effect)	Recommendation/Evidence
Regimen A: **Flu/Cy/TBI**	**Fludarabine:** • Rapid dephosphorylation to 2-fluoro-ara-A, then phosphorylated to 2-fluoro-ara-ATP intracellularly **Cy (Liver):** • Prodrug is converted by hepatic microsomal enzymes to the active form. The activation pathway involves CYP2B6, CYP2C9 and CYP3A4 isoenzymes. • Substrate for CYP2A6 (minor), 2B6 (major), 2C8/9 (minor), 2C19 (minor), 3A4 (major) • Inhibits CYP3A4 (weak) • Induces CYP2B6 (weak), 2C8/9 (weak) • Active metabolites include acrolein	**Low** • Efavirenz has been given in combination with fludarabine. **High** • NNRTIs may increase levels of acrolein through CYP induction. Other CYP inducers like phenytoin have been shown to increase the AUC of active metabolites by 50%.	**Raltegravir-Based:** May continue highly active antiretroviral therapy (HAART) as tolerated **NNRTI-Based:** May continue HAART as tolerated ARV should not be resumed until, at least, 72 hours after the last dose of Cy and once the subject is able to consistently tolerate oral medications.

*Combinations have not been studied and the interaction potential has been predicted based on the metabolic pathways of each agent.

TABLE F-2: PK INTERACTIONS FOR GVHD PROPHYLAXIS AND ARVs

Concomitant Medication	**Effect on sirolimus**	**Mechanism**	Recommendation
Integrase Inhibitor • Raltegravir (RLV)	None	N/A	No adjustments necessary. Monitor sirolimus trough levels 2-3 times/week. Raltegravir based regimens may be preferred over NNRTI- based regimens as it is primarily metabolized via glucuronidation and not by CYP3A4.
Fusion Inhibitor • Enfuviritide (ENF, T20)	None	N/A	No adjustments necessary. Monitor sirolimus trough levels 2-3 times/week.
CCR5 Antagonist • Maraviroc (MVC)	None	N/A	No adjustments necessary. Monitor sirolimus trough levels 2-3 times/week.

NNRTIs • Efavirenz (EFV) • Nevirapine (NVP)	↓ Sirolimus Levels	Strong induction of CYP3A4	Continue sirolimus at the same dose and monitor trough levels daily. Based on level: • **< 3 ng/mL:** Increase sirolimus daily dose by 25% • **3 - 12 ng/mL:** No adjustment • **>12 ng/mL:** Decrease sirolimus daily dose by 25% **Full induction effects may take up to two weeks. Daily sirolimus doses are substantially increased for most patients with co-administration of NNRTIs. Efavirenz is a more potent inducer of CYP3A4 than is nevirapine.**
NNRTIs • Etravirine (ETV)	↓↓ Sirolimus Levels (Thoeretical)	Potent induction of CYP3A4	Concomitant use of etravirine and sirolimus should be discouraged.
NRTIs • Abacavir (ABC) • Didanosine (ddI) • Emtricitabine (FTC) • Lamivudine (3TC) • Stavudine (d4T) • Tenofovir (TDF)	None	N/A	No adjustments necessary. Monitor sirolimus trough levels 2-3 times/week. Frequent monitoring of renal function is required for those patients on tenofovir due to the increased risk of nephrotoxicity. For patients with renal impairment, adjust NRTI doses according to local guidelines.

APPENDIX G

ADDITIONAL EVALUATIONS FOR HIV-POSITIVE SUBJECTS

PRE-CONDITIONING

In addition to the Pre-Conditioning evaluations stated in section 2.6.3, the following Pre-Conditioning evaluations must be completed for subjects who are HIV-positive:

Within **6 months** prior to start of conditioning:

1. Obtain hepatitis B core and hepatitis C antibody test results. If hepatitis B core and/or hepatitis C antibodies are positive, hepatitis B DNA PCR and/or hepatitis C RNA PCR must be tested and results provided.
    a. HIV-positive subjects with hepatitis C must have achieved a sustained virologic response for 12 weeks after cessation of antiviral treatment to be eligible for the study.

Within **8 weeks** prior to start of conditioning:

1. CMV PCR or antigenemia assay results
2. HIV-1 RNA level (HIV viral load by standard assay) results
3. CD4 count results
4. Duration of HIV/AIDS diagnosis, history of prior opportunistic illnesses
5. Medication list to include all current antiviral, antibiotics and opportunistic infection prophylaxis
6. Peripheral blood collected for Latent HIV Cellular Reservoir Analysis (see Appendix I)
7. Tropism assay results

Within **4 weeks** prior to start of conditioning:

1. Treatment Review Committee approval (see Appendix D)

POST-HCT

In addition to the Post-HCT evaluations stated in section 2.6.3, the following Post-HCT evaluations must be completed for subjects who are HIV-positive:

1. Day+100 (+/- 7 days)
    - CD4 count results
2. Day+180 (+/- 28 days)
    - CD4 count results
    - Peripheral blood collected for Latent HIV Cellular Reservoir Analysis (see Appendix I)
3. Day+270 (+/- 28 days)
    - CD4 count results
4. 1-year (Day+365 (+/- 28 days))
    - CD4 count results
    - Peripheral blood collected for Latent HIV Cellular Reservoir Analysis (see Appendix I)

APPENDIX H
DIAGNOSIS AND SEVERITY SCORING FOR ACUTE AND CHRONIC GVHD

1. Acute GVHD organ staging

Stage	Skin	GI	Liver
1	< 25% rash	Diarrhea > 500ml/d or persistent nausea	Bilirubin 2-3mg/dl
2	25-50%	> 1000 ml/d	Bilirubin 3-6 mg/dl
3	> 50%	> 1500 ml/d	Bilirubin 6-15 mg/dl
4	Generalized erythroderma with bullae	Large volume diarrhea and severe abdominal pain ± ileus	Bilirubin > 15 mg/dl

2. Consensus Acute GVHD Grading (PRZEPIORKA, ET. AL., 1995)

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

3. Grading of Chronic GVHD (NIH Criteria)

	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, capabl of self-care, >50% of waking hours ou 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):*_____

	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**

	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):*				

4. 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 5** (below)

5. Signs and Symptoms of Chronic GVHD

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
Skin	Poikiloderma	Depigmentation	Sweat impairment	Erythema
	Lichen planus-like features		Ichthyosis	Maculopapular rash
	Sclerotic features		Keratosis pilaris	Pruritus
	Morphea-like features		Hypopigmentation	
	Lichen sclerosis-like features		Hyperpigmentation	
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) Scaling, papulosquamous lesions	Thinning scalp hair, typically patchy, coarse, or dull (not explained by endocrine or other causes)	
			Premature gray hair	
Mouth	Lichen-type features	Xerostomia		Gingivitis
	Hyperkeratotic plaques	Mucocele		Mucositis
	Restriction of mouth opening from sclerosis	Mucosal atrophy Pseudomembranes† Ulcers†		Erythema Pain
Eyes		New onset dry, gritty, or painful eyes‡	Photophobia	
		Cicatricial conjunctivitis Keratoconjunctivitis sicca‡ Confluent areas of punctate keratopathy	Periorbital hyperpigmentation	

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
			Blepharitis (erythema of the eyelids with edema)	
Genitalia	Lichen planus-like features	Erosions†		
	Vaginal scarring or stenosis	Fissures†		
		Ulcers†		
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, ALP >2 × ULN
				ALT or AST >2 × ULN†
Lung	Bronchiolitis obliterans diagnosed with lung biopsy	Bronchiolitis obliterans diagnosed with PFTs and radiology‡		BOOP
Muscles, fascia, joints	Fasciitis Joint stiffness or contractures secondary to sclerosis	Myositis or polymyositis‡	Edema Muscle cramps Arthralgia or arthritis	
Hematopoietic and immune			Thrombocytopenia	
			Eosinophilia	
			Lymphopenia	
			Hypo- or hypergammaglobulinemia	
			Autoantibodies (AIHA and ITP)	
Other			Pericardial or pleural effusions	
			Ascites	

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
			Peripheral neuropathy	
			Nephrotic syndrome	
			Myasthenia gravis	
			Cardiac conduction abnormality or cardiomyopathy	

***** Can be acknowledged as part of the cGVHD symptomatology if the diagnosis is confirmed.

† In all cases, infection, drug effects, malignancy, or other causes must be excluded.

‡ Diagnosis of cGVHD requires biopsy or radiology confirmation (or Schirmer test for eyes).

APPENDIX I

CORRELATIVE STUDY PROCEDURES

The research samples supporting the correlative laboratory studies described below in sections A, B, and C will be required of all subjects enrolled on this trial. The laboratory study described in section D below applies only to HIV-positive subjects. All correlative studies were designed to address very specific questions relevant to this HCT trial. Detailed instructions regarding sample collection, packaging and shipping can be found in the 15-MMUD Clinical Trial Laboratory Manual provided to study sites.

A. Gene Expression Profile Based Model to Predict Donor Cell Immune Tolerance

In a previous investigation, Pidala and colleagues examined peripheral blood transcriptional markers in tolerant and non-tolerant HCT recipients with the goals of developing an accurate phenotypic classifier and dissecting biologic mechanisms of immune tolerance following HCT with promising results. We will use this approach in this correlative study to prospectively investigate tolerance development following PTCy/sirolimus/MMF therapy among MMUD HCT.

Aim 1: Identify and verify transcriptional markers predictive of immune tolerance development

(a) Day+100 and/or Day+180 samples will be used for prediction of immune tolerance, defined by complete discontinuation of immunosuppression and absence of GVHD.

Aim 2: Investigate longitudinal change in transcriptional markers for prediction of immune tolerance development

(a) Change in gene expression from Day+21 to Day+180 will be examined for prediction of immune tolerance development.

Research blood samples will be collected from recipients on Day+21, Day+100, and Day+180. Two PAXgene 2.5 mL-fill Blood RNA (IVD) tubes will be drawn at each time point. This whole blood collection system stabilizes RNA, and thus is ideal for collection and shipment from multiple study sites to H. Lee Moffitt Cancer Center and Research Institute for analysis. Based on expected yield of 0.5-1ug RNA/mL whole blood, total RNA isolated (~ 2.5-5ug) will be more than sufficient for planned mRNA profiling platforms (Affymetrix gene array, Nanostring nCounter technology). RNA will be isolated from whole blood (total RNA, inclusive of small RNA to permit microRNA analysis), and RNA integrity confirmed.

The collection of these samples should be scheduled for Monday-Thursday of any given week. Samples must be stored at room temperature and shipped on the day of collection in an ambient sample shipping kit for next morning receipt at the H. Lee Moffitt Cancer Center and Research Institute.
In rare cases where samples must be collected on Friday, Saturday, or Sunday, samples must be stored in the refrigerator at 2 – 8°C until shipped in a validated insulated cooled

sample shipping kit to the H. Lee Moffitt Cancer Center and Research Institute on Monday or the next business day in the case of a holiday.

B. **Clonal Hematopoiesis Assessments**

Age-related clonal hematopoiesis has recently been found to affect significant proportion of otherwise asymptomatic older individuals. In order to examine the incidence of HCT recipients developing donor clonal hematopoiesis and potential functional and clinical consequences we will study post-HCT DNA obtained from HCT recipients in the current study. Post-HCT DNA will be obtained from recipient bone marrow research samples collected at the time of standard of care bone marrow aspirates performed on Day+100 and Day+365. A custom panel (TBD) designed as a clinical leukemia panel of genes important in oncogenesis will be evaluated by targeted next-generation sequencing. To investigate the propensity of donor clones for expansion and malignant transformation we will analyze the clonal architecture of donor-derived hematopoiesis in the recipients over time. We will apply targeted next-generation sequencing (as described above) to better understand the clonal dynamics in the recipients. We will analyze the changes in variant allele frequency of previously identified premalignant clones as well as the occurrence of additional cooperating mutations.

Aim 1: To define the population of bone marrow recipients who develop donor clonal hematopoiesis

Aim 2: To determine the clinical and functional consequences of the hematopoietic clones marked by somatic mutations in donor-derived hematopoiesis post-HCT

A 4 mL research bone marrow aspirate aliquot will be collected at the time of standard of care bone marrow aspirates performed at Day+100 and Day+365. The 4 mL aliquot of bone marrow will be added to two 2 mL-fill PAXgene Blood DNA tubes (IVD) and shipped priority overnight to the CIBMTR Biorepository. Samples will be frozen for later use for this correlative study, which will be conducted by a qualified investigator (TBD).

The collection of these samples should be scheduled for Monday-Friday of a given week. Samples must be stored at room temperature and shipped on the day of collection in an ambient sample shipping kit for next morning receipt at the CIBMTR Biorepository. If shipped on a Friday for Saturday delivery, the shipment must be designated for Saturday delivery.
In rare cases where samples must be collected on Saturday or Sunday, samples must be stored upright in the refrigerator at 2 – 8°C until ambient temperature shipment to the CIBMTR Biorepository on Monday or the next business day in the case of a holiday.

C. **Immune Reconstitution Studies**

Immune system reconstitution dynamics will be assessed by measuring absolute number of leukocytes and the percentage of T, B, NK, monocyte, and dendritic cell subsets as defined by multiparameter flow cytometry. Post-HCT peripheral blood samples will be

collected to explore the relationships of immune cell populations with the development of acute and/or chronic GVHD, and with outcomes including PFS, TRM and OS.

A peripheral blood research sample (10 mL) will be collected from recipients on Day+100 and Day+365. The 10 mL blood sample will be added to one 10 mL (or two 6mL) sodium heparin blood tube and shipped priority overnight to Roswell Park Cancer Institute.

The collection of these samples must be scheduled for Monday-Friday of a given week. Samples must be stored at room temperature and shipped on the day of collection in an insulated ambient sample shipping kit for next morning receipt at Roswell Park Cancer Institute. If shipped on a Friday for Saturday delivery, the shipment must be designated for Saturday delivery.

D. **Latent HIV Cellular Reservoir Analysis (HIV-positive patients only)**

In patients with undetectable viral load as measured in conventional assays, there is persistent viremia that can be measured at single copy/mL by specialized PCR analysis. This viremia may reflect virus being released by the decay of latently infected cells or possibly ongoing viral replication. Therapies that impact on the latently infected reservoir might be expected to change the viremia if it mainly reflected the decay of latently infected cells. Pre-HCT conditioning therapies may kill cells that constitute the latency reservoir and thus might thus be expected to impact on the reservoir and the very low level viremia that can be assessed by this assay.

Required Latent HIV Research Sample Collections on HIV-positive Patients

Once an HIV-positive patient with one or more available MMUDs that are homozygous for the CCR5delta32 mutation is consented and enrolled on the trial, a required peripheral blood research sample will be collected at approximately 8 weeks prior to initiation of ablative therapy. The peripheral blood research sample (180 mL) will be collected and placed into eighteen (18) 10 mL EDTA containing, lavender-top Vacutainer tubes. Additional scheduled required Latent HIV research samples will be collected at Day+180 and Day+365.

HIV-positive patients may be contacted by researchers at the Sidney Kimmel Comprehensive Cancer Centre at Johns Hopkins regarding additional research requests after study completion. A separate informed consent would be required to participate in that research.

The collection of these samples should be scheduled on only a Monday, Tuesday or Wednesday of a given week. Samples must be stored at room temperature and shipped on the day of collection in an insulated ambient shipping kit for next morning receipt at the Johns Hopkins Laboratory. In all cases, this large volume peripheral blood sample collection should be scheduled with consideration of other standard of care and required research sample collections so that the collection of the entire sample volume might be possible. **Study sites must notify the CIBMTR Protocol Coordinator prior to sample collection at each time point to advise of the planned collection date.**

Required Research Samples Associated with Protocol Associated Correlative Studies					
Purpose	**Sample Type**	**Sample Collection Summary**	**Collection Time Points**	**Shipping Specifications**	**Shipping Location**
Gene Expression Profile Testing	5 mL Peripheral Blood	• Collect 5 mL peripheral blood sample and place 2.5 mL into each of two PAXgene Blood RNA (IVD) tubes. Gently mix sample by inversion 8-10 times to mix sample well with RNA stabilization solution. • Store at room temperature while preparing to ship to project laboratory. • Samples are to be collected Monday - Thursday only	Day+21; Day+100; Day+180	PAXgene tubes will be shipped at ambient temperature on the day of collection by priority overnight FedEx delivery to H. Lee Moffitt Cancer Laboratory.	H. Lee Moffitt Cancer Laboratory
Clonal Hematopoiesis Assessment	4 mL Bone Marrow Aspirate	• Collect 4 mL bone marrow aspirated sample and place a 2 mL volume into each of two PAXgene Blood DNA (IVD) tubes. • Store at room temperature while preparing to ship to project laboratory. • Samples may be collected Monday -Friday	Day+100 and Day+365	PAXgene tubes will be shipped at ambient temperature on the day of collection by priority overnight FedEx delivery to the CIBMTR Biorepository for storage for future clonal hematopoiesis assessment.	CIBMTR Biorepository
Immune Reconstitution Studies	10 mL Peripheral Blood	• Collect 10 mL peripheral blood sample and place into one 10 mL (or two 6mL) green top plastic BD Vacutainer® tubes, containing Sodium-Heparin anticoagulant. Gently mix sample by inversion 8-10 times to mix sample well with heparin anticoagulant. • Store at room temperature while preparing to ship to project laboratory. • Samples may be collected Monday -Friday	Day+100 and Day+365	Blood sample tubes will be shipped on the day of collection at ambient temperature by priority overnight FedEx delivery to Roswell Park Cancer Institute Laboratory.	Roswell Park Cancer Institute Laboratory

Additional Required Research Samples for <u>HIV-positive subjects only</u>					
Purpose	**Sample Type**	**Sample Collection Summary**	**Collection Time Points**	**Shipping Specifications**	**Shipping Location**
Latent HIV Cellular Reservoir Analysis	180 mL Peripheral Blood	• Collect 180 mL peripheral blood sample and place into 18, 10 mL-fill lavender top plastic BD Vacutainer® tubes, containing EDTA anticoagulant. Gently mix sample by inversion 8-10 times to mix sample well with heparin anticoagulant. • Store at room temperature while preparing to ship to project laboratory. • Samples are to be collected Monday - Wednesday only	Pre-Conditioning; Day+180 and Day+365	Blood sample tubes will be shipped on the day of collection at ambient temperature by priority overnight FedEx delivery to Johns Hopkins Laboratory.	Johns Hopkins Laboratory

APPENDIX J

MMUD SELECTION ALGORITHM

Given no available suitable 8/8 matched related or unrelated donor, study sites may utilize the proposed MMUD search strategy process noted below. This selection algorithm prioritizes donor age, CMV serostatus, and ABO match over HLA.

1. Among donors who are at least 4/8 allele matched (HLA-A, -B, -C, -DRB1), exclude any donor with HLA alleles against which the patient has specific antibodies.
2. Attempt to find a donor that meets all following criteria:
    a. Young donor <30 years over 31-40 years over 41-50 years over 51-60 years
    b. ABO matched to patient over minor ABO mismatch over major ABO mismatch
    c. CMV serostatus matched to patient CMV (i.e. CMV positive donor for CMV positive patient) over CMV mismatch
3. If donor meeting all criteria in #2 above is not available, the transplant team may choose among donors using institutional practices regarding prioritizing among these characteristics.
4. Once donor age, ABO match and CMV status have been optimized, the following characteristics should be pursued (if possible):
    a. Male over nulliparous female over parous female
    b. Donor having body weight sufficient for adequate cell collection per patient size, if data available
    c. Donor availability information (last donor contact date with NMDP)
5. After optimizing non-HLA criteria, the transplant team may select the donor based on HLA factors:
    a. Best allele match out of 8 alleles (HLA-A, -B, -C, -DRB1; 7>6>5>4)
    b. Consideration of -DPB1 allele matching (allele matching or T-cell-epitope permissive)
    c. Consideration of -DQB1 matching
    d. Consideration of other HLA-loci or genetic markers (e.g. -DRB3/4/5, Killer Immunoglobulin-like Receptor)

Selection for any HIV-positive patient will prioritize donors homozygous for CCR5delta32 mutation as the primary selection criteria. Additional donor selection criteria with consideration of age, gender, donor weight, ABO matching, CMV matching and donor availability will be made by the transplant team.

Multiple NMDP resources are available to assist study sites with donor selection assistance:

- **Traxis application**: may be used by study sites to search for donors. Please note the following limitations:
    - Traxis results do not include donors <5/6 HLA-A, -B, -DRB1 antigen level match to the recipient.
    - This protocol allows for lower overall matching; many potential donors will not be available in the default NMDP HapLogic listing in Traxis.

- **NMDP Immunogenetic Operations group**: may use additional methods to identify donors.
- **NMDP Case Management group:** may use the MMUD selection algorithm with specific search factor(s) at the request of the study site. NMDP Case Management then provides a Search Strategy Advice (SSA) report to the study site within 5 business days of the request. The results included in the SSA balance the MMUD algorithm with the study site's specifications.

During donor selection, study sites may consider other factors that may impact the product and/or the infusion:

- Plans for cell processing/manipulation may increase the potential for a reduction in TNC yield.
- Product travel time may delay the planned infusion date (and conditioning regimen schedule in relation to the infusion date).
    - Late product pick-up time: some collection centers do not allow product pick-up until later in the day, which may result in product delivery delay.
- In instances where the product requires processing prior to infusion, it may be beneficial to delay Day 0 by one day (especially if the product requires transcontinental travel).

APPENDIX K

RECOMMENDED FORMULAS FOR CALCULATION OF ADJUSTED AND IDEAL BODY WEIGHT

Adjusted IBW = IBW + 0.25 (Actual weight – IBW)

The following formulas for pediatric and adult IBW calculations are recommended, but IBW may be calculated according to institutional SOPs.

- Recommended IBW Calculation for Age 15 - 17 years (Traub-Johnson Equation for Children):

$$IBW = [(\text{Height in cm})^2 \times 1.65]/1000$$

- Recommended IBW Calculation for Age > 18 years:

$$IBW(\text{men}) = 50\text{kg} + 2.3\text{kg} * (\text{heightover60inches})$$

$$IBW(\text{women}) = 45.5\text{kg} + 2.3\text{kg} * (\text{heightover60inches})$$

APPENDIX L

GUIDANCE ON REVIEWING AND REPORTING UNANTICIPATED PROBLEMS

For more information, see http://www.hhs.gov/ohrp/policy/advevntguid.html#Q1.

Below are pertinent excerpts from the Office for Human Research Protections (OHRP) Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects.

I. What are *unanticipated problems*?
The phrase "unanticipated problems involving risks to subjects or others" is found but not defined in the U.S. Department of Health & Human Services (HHS) regulations at 45 CFR part 46. OHRP considers *unanticipated problems*, in general, to include any incident, experience, or outcome that meets **all** of the following criteria:

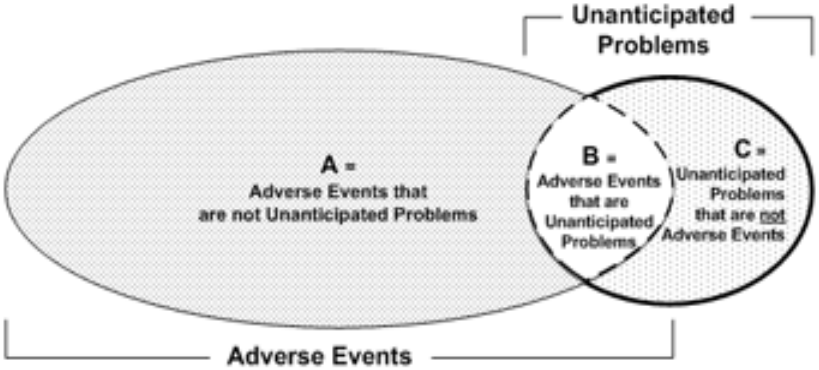
1. unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;

2. related or possibly related to participation in the research (in this guidance document, *possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

3. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

OHRP recognizes that it may be difficult to determine whether a particular incident, experience, or outcome is unexpected and whether it is related or possibly related to participation in the research.

II. How to determine which *AEs* are *unanticipated problems*?
In OHRP's experience, most IRB members, investigators, and institutional officials understand the scope and meaning of the term *AE* in the research context, but lack a clear understanding of OHRP's expectations for what, when, and to whom AEs need to be reported as unanticipated problems, given the requirements of the HHS regulations at 45 CFR part 46.
The following Venn diagram summarizes the general relationship between AEs and unanticipated problems:

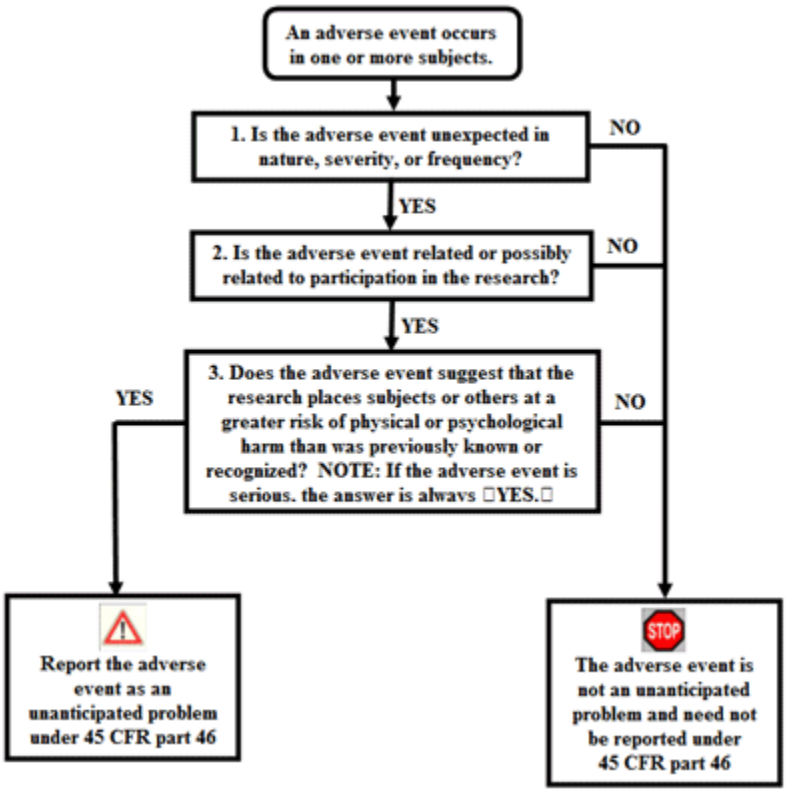


Under 45 CFR part 46: Do not report A, Do report (B+C)

To determine whether an AE is an unanticipated problem, the following questions should be asked:

- Is the AE unexpected?
- Is the AE related or possibly related to participation in the research?
- Does the AE suggest that the research places subjects or others at a greater risk of harm than was previously known or recognized?

If the answer to **all three questions** is *yes,* then the AE is an unanticipated problem.



III. Some examples of unanticipated problems:

- A series of related AEs that individually may not be unexpected but indicate a trend that places research participants or others at a greater risk of harm than was previously known or recognized.
- A breach of confidentiality.
- Incarceration of a participant in a protocol not approved to enroll prisoners.
- Complaint of a participant when the complaint indicates unexpected risks.
- A paper published from another study that shows that the risks or potential benefits of the research may be different than initially presented to the IRB.

APPENDIX M
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