BLOOD AND MARROW
TRANSPLANT
CLINICAL TRIALS NETWORK

Allogeneic Hematopoietic Cell Transplant for Hematological Cancers and Myelodysplastic Syndromes in HIV-Infected Individuals

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BMT CTN PROTOCOL 0903 (AMC-080) VERSION 2.0

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PROTOCOL SYNOPSIS - BMT CTN PROTOCOL #0903

Allogeneic Hematopoietic Cell Transplant for Hematological Cancers and Myelodysplastic Syndromes in HIV-Infected Individuals

Study Chairpersons: Joseph Alvarnas, M.D. and Richard Ambinder, M.D.

Primary Objective: The primary objective is to assess the feasibility and safety of allogeneic hematopoietic cell transplantation (HCT) in HIV-infected patients. The primary endpoint is 100-Day Non-Relapse Mortality (NRM).

Secondary Objectives: Patients will be assessed for the following endpoints:

1. Disease status at Day 100 post-HCT
2. Time to hematopoietic recovery
3. Chimerism at 4 weeks, 100 days, and 6 months
4. Hematologic function at 100 days and 6 months
5. Infections
6. Six-month overall survival
7. Acute graft-vs-host disease
8. Chronic graft-vs-host disease
9. Immunologic reconstitution at 8 weeks, 6, 12 and 24 months
10. Impact of HCT on the HIV reservoir at Day 100, 6,12 and 24 months post-HCT

Study Design: This study is designed as a Phase II multi-center trial.

Accrual Objective: The trial will accrue 15 patients.

Accrual Period: The estimated accrual period is 2 years.

Eligibility Criteria: Patients ≥ 15 years old, HIV-infected and diagnosed with acute myeloid leukemia (AML) or acute lymphocytic leukemia (ALL) in first or second complete remission (CR); Int-2 or high-risk myelodysplastic syndrome (MDS) with < 10% marrow blasts and no circulating myeloblasts after their most recent therapy; or Hodgkin or non-Hodgkin lymphoma beyond first CR with at least a partial response to last treatment. Patients must have either an 8/8 matched related donor at HLA-A, -B, -C, (serologic typing or higher resolution) and –DRB1 (at high resolution using DNA based typing), or at least a 7/8 matched unrelated donor at HLA-A, -B, -C and DRB1 (at high resolution using DNA based typing). A 7/8 matched related donor match is permitted only if an 8/8 unrelated donor cannot be identified. A secondary matching

criterion is the presence of homozygosity for the CCR5delta32 mutation. Allogeneic transplantation using cord blood, T-cell depletion or prior allogeneic HCT are not allowed for this study.

Patients must have adequate organ function defined as 1) left ventricular ejection fraction at rest $\geq$ 40%; 2) DLCO, FEV_1, FVC $\geq$ 45% predicted; 3) total bilirubin $\leq$ 2.0 mg/dL, and ALT and AST $\leq$ 5 x upper limit of normal (ULN); 4) creatinine clearance > 40mL/min (measured or calculated). Karnofsky/Lansky performance status $\geq$ 70%.

Treatment Description: Patients with HIV infection and hematological malignancies or myelodysplastic syndromes (MDS) will be treated with either reduced-intensity or fully ablative allogeneic hematopoietic cell transplantation (HCT). Where feasible, an attempt will be made to identify hematopoietic cell donors who are homozygotes for the CCR5delta32 mutation. Patients will receive standard immunosuppressive therapy post-transplant that will be tapered as per the institutional standard of care. Graft-versus-host disease will be treated per the institutional standard of care.

Study Duration: Patients will be followed for two years following allogeneic HCT.

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1. BACKGROUND AND RATIONALE

1.1. Background

Survival in patients with Human Immunodeficiency Virus (HIV) has improved dramatically with the advent of Highly-Active Antiretroviral Therapy (HAART). Despite improvements in HIV-infection-related survival, the risk of malignancies remains significant in patients with HIV infection. While Non-Hodgkin lymphoma (NHL) is an AIDS-defining diagnosis, a comparison of a large cohort of HIV-infected patients to the Surveillance, Epidemiology and End Results (SEER) data demonstrates a significantly higher incidence of several cancers that are treated with allogeneic HCT, including Hodgkin lymphoma and leukemias in patients with HIV infection (Patel).

Prior to the advent of HAART, the treatment of patients with malignancy in the setting of HIV infection was hamstrung by limited chemotherapy tolerance and complications due to opportunistic infection. Since the widespread availability of HAART, there have been significant improvements in the capacity to treat this patient population in a fashion comparable to that of patients without HIV infection. This has, in turn, lead to marked improvements in the outcomes of patients with malignancies in the setting of HIV infection. The prognosis for patients with AIDS-related lymphoma is dramatically different in the era of HAART therapy. In a comparison of treatment outcomes for patients treated before and after the advent of HAART, there is a statistically significant improvement in the overall survival of patients treated with HAART ($p = 0.002$). While the International Prognostic Index (IPI) remains a useful tool for estimating the prognosis of patients with AIDS-related NHL, the CD4 count of less than 100 per microliter has independent prognostic value for this group of patients. The impact of HAART therapy upon the immunological and functional status of patients with HIV infection has permitted the extension of aggressive therapeutic regimens to this patient population. HAART allows a significant portion of patients to often reduce their viral load to undetectable levels and therefore tolerate far more aggressive therapeutic interventions than would have been possible previously.

1.2. Autologous Hematopoietic Cell Transplantation for Patients with HIV Infection

Based upon the opportunity afforded by this degree of viral suppression, high-dose therapy with autologous hematopoietic cell transplantation (HCT) has been extended to patients with AIDS-related NHL and Hodgkin lymphoma with results that appear to be comparable to those achieved in patients without HIV infection. Treatment-related complications appear to be comparable to those seen in the non-HIV-infected patient population. Across multiple trials, the probability of survival after high-dose conditioning followed by autologous HCT for patients with AIDS-related NHL ranges between 39% to 85% as seen in Table 1.2.

TABLE 1.2: CUMULATIVE OUTCOMES AFTER AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION IN PATIENTS WITH HIV-ASSOCIATED NHL AND HL*

Reference	Failed to Mobilize (n)	Patients Transplanted (n)	TRM	Median follow-up (months)	Patients Surviving (%)
Krishnan	0	20	5%	32	85
Spitzer	2	20	5%	5.8	50+??
Re	4	10	0%	18	39
Gabarre	?	14	?	1	71
Serrano	0	11	0%	32	73
EBMT	NA	68	7.5%	32	61

* Abstracts of references are provided in Appendix I

1.3. Allogeneic HCT for Patients with HIV Infection

The experience with allogeneic HCT transplantation for patients with HIV infection has been quite limited. Prior to the advent of HAART therapy, the key limiting factors in the use of allogeneic HCT in this patient population was control of the HIV infection and management of opportunistic infections. In a paper published in 1987, 3 patients who were diagnosed with HIV infection following transplantation were managed through the use of donor lymphocyte infusions and cytokine therapy (Vilmer). Two of the patients had undergone prior HCT for aplastic anemia and the third had been treated for acute leukemia. All of the patients demonstrated evidence of marrow failure. Patients were treated with 6 donor lymphocyte infusions, a 3-month course of alpha interferon and subsequently received gamma interferon. While all 3 patients had improvements in their blood counts following treatment, all had detectable HIV infection and suppressed CD4 levels.

In a case report published by the Johns Hopkins group, a 41-year-old man with AIDS-related lymphoma was treated with fully ablative allogeneic HCT using total body irradiation combined with cyclophosphamide (Holland). The patient received 2 weeks of pre-treatment with zidovudine prior to transplant and received the drug post-transplant. He achieved engraftment on Day + 17 but unfortunately died of complications related to progressive disease on Day + 47. Polymerase chain reaction (PCR) studies of peripheral blood were negative for HIV at Day + 32 and analysis of multiple autopsy tissues did not demonstrate detectable virus at the time of the patient's death.

Additional case reports from the pre-HAART era demonstrated the feasibility of performing allogeneic transplantation for patients with HIV infection who were treated with multiple anti-retroviral agents prior to and following allogeneic HCT, but in 2 of 3 cases, the patients remained PCR-positive for evidence of residual HIV infection (Torlontano, Giri). The 1 patient who did not demonstrate evidence of post-transplant HIV infection had poor count recovery following transplant and died of ARDS (Contu).

The availability of HAART therapy seems to have had an impact upon the feasibility of HCT in this patient population. In a review of the CIBMTR registry data, 23 patients with HIV infection were identified as having undergone allogeneic transplant (Gupta). Nine of the 23 (39%) underwent allogeneic HCT HIV after 1996, in the HAART era. At a median follow-up of 59 months, six of the 23 patients were alive. Four of the nine post-1996 patients were alive whereas only 2 of 14 pre-1996 patients were alive. The data suggest that allogeneic HCT is feasible for selected HIV-positive patients.

Despite the capacity of HAART therapy to suppress HIV viral loads to low or undetectable levels, the experience with allogeneic HCT remains quite limited. In a 2002 case report, a 33-year-old woman with acute myelogenous leukemia (AML) in first complete remission, HIV and hepatitis C infection underwent fully ablative CD34-selected allogeneic graft from a sibling donor (Sora). She was treated with HAART prior to transplant and achieved an undetectable viral load. The transplant course was complicated by grade II graft-versus-host disease (GVHD) that responded to treatment. At the time of the report, the patient remained in a complete remission with an undetectable HIV viral load at 39 months post-HCT.

One of the challenges in managing patients with HAART through the transplant course may be the risk of HIV-reactivation/graft infection during periods of anti-retroviral therapy interruption. In a 2001 report of 2 patients treated with reduced-intensity allogeneic transplant, 1 of the patients developed a febrile illness consistent with acute HIV reactivation (Kang). The viral load rose to 1,000,000 copies/mL during this time. This patient eventually died of relapsed Hodgkin lymphoma 12 months post-HCT. The other patient who underwent transplant for AML received a gene-modified graft that was engineered to inhibit wild type Rev. This patient demonstrated an undetectable HIV viral load post-transplant and remains free of disease. In another case report from 2007, a 34-year-old man with HIV infection and severe aplastic anemia (SAA) underwent allogeneic HCT (Wolf). During interruption of HAART therapy, the patient demonstrated a rapid rise in his HIV viral load. Despite this, the patient engrafted at Day + 18. He achieved viral suppression following retreatment with his anti-retroviral regimen.

Conversely there are some data that show that the donor graft may be capable of mounting and anti-HIV response mediated by CD8+ cells (Woolfrey). Two patients with AML underwent allogeneic HCT (1 matched sibling, 1 unrelated donor) following a non-myeloablative regimen (fludarabine 90 mg/m^2 and TBI 2 Gray) and were monitored with HIV RNA levels, proviral DNA levels and peripheral blood mononuclear cell quantitative HIV cultures post-transplant. Anti-retroviral therapy was chosen to avoid significant drug interactions and hepatotoxicity potentially related to nevirapine. GVHD prophylaxis consisted of cyclosporine and mycophenolate. HAART was administered throughout the transplant course. One patient suffered a relapse of his disease at Day + 44. Immunosuppression was withdrawn. This patient died as a result of complications related to GVHD and obliterative bronchiolitis. Both donors had wild type CCR5 coreceptor. Both patients had undetectable viral loads from prior to transplant through Day + 180. HIV proviral DNA was detected in 1 patient at Days + 56 and +180. Through an analysis of donor T-cell responses to multiple HIV epitopes, cells recovered from patient 2 demonstrated anti-HIV-specific T-cell responses to 8 of the 26 selected epitopes.

The experience with reduced-intensity allogeneic HCT for patients with HIV has expanded with the recent publication of the Ohio State University experience (Hamadani). Three patients with HIV underwent allogeneic HCT following conditioning with fludarabine and busulfan with or without antithymocyte globulin (1 patient without). GVHD prophylaxis consisted of tacrolimus and mini-dose methotrexate. The patients underwent transplant for AML in 2nd complete remission, Burkitt lymphoma and plasmablastic lymphoma, respectively. Patients continued HAART throughout the transplant course without interruption. One patient developed acute GVHD. At the time of the study publication, all patients were alive, free of disease and off of immunosuppressive therapy (range 368-802 days). One patient had a detectable viral load beyond 1 year following HCT.

The "Holy Grail" for allogeneic transplantation for patients with HIV-infection would be a cure of the underlying disorder for which transplant was performed and the concomitant eradication of the HIV infection. The recent case report of a patient who underwent allogeneic HCT for AML and received a graft from a donor with the CCR5delta32/delta32 mutation may herald this possibility (Hütter). One of the key mechanisms for HIV-1 entry into CD4 lymphocytes is chemokine receptor 5 (CCR5). The delta32 mutation represents a 32 base pair deletion in the allele that may confer significant resistance to HIV infection in homozygotes for the mutation. In this case report, a 40-year-old man with AML and concomitant HIV infection underwent allogeneic transplant from an unrelated donor. At the time of diagnosis with acute leukemia, the viral load was undetectable and the CD4 count was 415/mcl. The patient achieved a complete response following standard therapy, but relapsed 7 months following treatment. At the time of relapse, the patient underwent an unrelated donor transplant using a donor who was a homozygote for the delta32 allele. The patient received a CD34-selected graft. GVHD prophylaxis consisted of cyclosporine A and rabbit antithymocyte globulin. The patient engrafted on Day + 13. He experienced grade I GVHD of the skin that responded to therapy. The patient relapsed at Day +391 and was treated with a second transplant from the same donor following preparation with 2 Gray TBI. The patient's HIV viral load remains undetectable at 20 months following transplant despite discontinuation of HAART.

CHAPTER 2

2. STUDY DESIGN

2.1. Study Overview

The study is designed to evaluate the feasibility and safety of reduced-intensity and fully-ablative allogeneic hematopoietic cell transplantation (HCT) for patients with hematological malignancies or myelodysplastic syndromes (MDS) who have HIV infection. The goal of the study is to assess the 100 day Non-relapse Mortality as well as immunological reconstitution in this patient population. Where feasible, an attempt will be made to identify HLA-compatible hematopoietic stem cell donors who are homozygotes for the delta32 mutation of the chemokine receptor 5 (CCR5delta32). Patients will undergo a treatment plan review prior to registration on the trial. All patients will undergo allogeneic HCT from a matched sibling or unrelated donor.

2.2. Study Objectives and Rationale

The rationale for this trial is to demonstrate the feasibility and safety of allogeneic HCT for patients with chemotherapy-sensitive hematological malignancies and coincident HIV-infection. In particular the trial will focus upon the 100-day non-relapse mortality as an indicator of the safety of transplant in this patient population. Correlative assays will focus upon the incidence of infectious complications in this patient population, the evolution of HIV infection and immunological reconstitution. Where feasible (and when this can be accomplished without compromise of either the donor quality or the timeliness of transplantation), an attempt will be made to identify donors who are homozygotes for the delta32 mutation for CCR5.

2.2.1. Primary Objective

The primary objective of this multicenter study is to assess the feasibility and safety of allogeneic hematopoietic cell transplantation (HCT) in HIV-infected patients. The primary endpoint is 100-Day Non-Relapse Mortality (NRM).

2.2.2. Secondary Objectives

- Disease status at Day 100 post-HCT
- Time to hematopoietic recovery
- Chimerism at 4 weeks, 100 days and 6 months post-HCT
- Hematologic function at 100 days and 6 months post-HCT
- Infections
- Six-month overall survival
- Acute graft-vs.-host disease
- Chronic graft-vs.-host disease
- Immunologic reconstitution at 8 weeks and 6, 12, and 24 months post-HCT
- The impact of HCT on the HIV reservoir at Day 100, 6, 12 and 24 months post-HCT

2.3. Patient Eligibility

Patients must meet specified eligibility criteria for entry into the study.

2.3.1. Patient Inclusion Criteria

Patients fulfilling the following criteria will be eligible for entry into this study:

1. HIV-1 infection, as documented by a rapid HIV test or any FDA-Approved HIV-1 Enzyme or Chemiluminescence Immunoassay (E/CIA) test kit and confirmed by Western Blot at any time prior to study entry. HIV antigen, plasma HIV-1 RNA, or a secondary antibody test by a method other than rapid HIV and E/CIA is acceptable as an alternative test. Alternatively, if a rapid HIV test or any FDA-Approved HIV-1 Enzyme or Chemiluminescence Immunoassay (E/CIA) test is not available, two HIV-1 RNA values $\geq$ 2000 copies/mL at least 24 hours apart performed by any laboratory that has CLIA certification, or its equivalent, may be used to document infection.
2. Patients must be willing to comply with effective Antiretroviral Therapy.
3. Patients must be $\geq$ 15 years of age.
4. Hematological malignancy associated with a poor prognosis with medical therapy alone. Diagnoses to be included:
    a) Patients with the diagnosis of Acute Myeloid or Lymphocytic Leukemia (AML or ALL) in first or second complete remission.
    b) Patients with advanced myelodysplastic syndromes (MDS), including those with International Prognostic Scoring System (IPSS) Int-2 and high-risk disease with less than 10% marrow blasts and no circulating myeloblasts after most recent therapy. Patients with acute leukemia that develops from a pre-existing MDS must meet the inclusion criteria for patients with AML detailed above.
    c) Hodgkin Lymphoma beyond first remission achieving at least a partial response to most recent therapy with no evidence of progression prior to transplant.
    d) Non-Hodgkin Lymphoma beyond first remission achieving at least a partial response to most recent therapy with no evidence of progression prior to transplant.
5. Donor/Recipient HLA Matching:
    a) Related donor: must be an 8/8 match at HLA-A, -B, -C, (serologic typing or higher resolution) and –DRB1 (at high resolution using DNA based typing). A 7/8 related donor match is permitted only if an 8/8 unrelated donor cannot be identified.
    b) Unrelated donor: must be a 7/8 or 8/8 match at HLA-A, -B, -C, and -DRB1 (at high resolution using DNA based typing).

6. Patients with adequate organ function as measured by:
    a) Cardiac: Left ventricular ejection fraction at rest≥ 40% demonstrated by MUGA or echocardiogram. Patients with known heart disease must have a functional status no worse than American Heart Association Class I defined as patients with cardiac disease but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.
    b) Hepatic:
        i. Total Bilirubin $\leq$ 2.0 mg/dL (except for isolated hyperbilirubinemia attributed to Gilbert syndrome or antiretroviral therapy as specified in Appendix E) and ALT and AST $\leq$ 5x the upper limit of normal.
        ii. Concomitant Hepatitis: Patients with chronic hepatitis B or C may be enrolled on the trial providing the above bilirubin and transaminase criteria are met. In addition, there must be no clinical or pathologic evidence of irreversible chronic liver disease, and there must be no active viral replication as evidenced by an undetectable hepatitis viral load by a PCR-based assay.
    c) Renal: Creatinine clearance (calculated creatinine clearance is permitted) > 40 mL/min.
    d) Pulmonary: DLCO, FEV1, FVC $\geq$ 45% of predicted (corrected for hemoglobin).
7. Signed Informed Consent

2.3.2. Patient Exclusion Criteria

Patients with the following will be ineligible for registration onto this study:

1. Karnofsky/Lansky performance score < 70%.
2. Active CNS malignancy; however, patients with a history of positive CSF cytology that has become negative with intrathecal chemotherapy are eligible.
3. Uncontrolled bacterial, viral or fungal infection (currently taking medication and with progression or no clinical improvement).
4. Active CMV retinitis or other CMV-related organ dysfunction.
5. AIDS related syndromes or symptoms that pose a perceived excessive risk for transplantation-related morbidity as determined by the principal investigator.
6. Untreatable HIV infection due to multidrug antiretroviral resistance. Patients with a detectable 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 Antiretroviral Review (described in Appendix D). This Review Committee will make the final determination as to whether HIV viremia could potentially be suppressed with alternate antiretroviral therapy. .

7. Pregnant (positive β-HCG) or breastfeeding.
8. Fertile men or women unwilling to use contraceptive techniques from the time of initiation of mobilization until six-months post-transplant.
9. Prior allogeneic HCT.
10. Patients with psychosocial conditions that would prevent study compliance and follow-up, as determined by the principal investigator.
11. T-cell depletion (including ATG or alemtuzumab) is not allowed.
12. Use of cord blood as the source of hematopoietic cells is not allowed.

2.4. Donor Selection Criteria

Patients are eligible for transplantation using either compatible related donors or an appropriately compatible unrelated donor as identified through the National Marrow Donor Program (NMDP) that meets institutional criteria. Allogeneic transplantation using cord blood unit(s) as a hematopoietic stem cell source is prohibited under this protocol.

Donor Inclusion Criteria

1. Unrelated donors will be identified through the National Marrow Donor Program (NMDP).
2. No contraindications to donation of hematopoietic stem cells.
3. CCR5delta32 mutation: An additional matching criterion for this study will be the presence of homozygosity for the CCR5delta32 mutation. When feasible based on donor likelihood of CCR5delta32 homozygosity and timeliness, potential donors will be assessed for CCR5delta32 homozygosity. Among equivalently desirable donors as determined by transplant center donor selection criteria, a donor who is found to be a homozygote for CCR5delta32 will be favored as long as such a selection does not compromise the optimal timing of HCT. HLA matching as a selection criterion will always take precedence over CCR5delta32 homozygosity (see Appendix E for details).

2.4.1. Donor Exclusion Criteria

1. Monozygotic twin donors.
2. Females who are pregnant (positive serum β HCG) or breastfeeding.
3. HIV seropositive.

2.5. Study Treatments

In general reduced-intensity conditioning (RIC) is reserved for patients with co-morbidities or older patients in whom regimen-related mortality weighs against myeloablative conditioning (MAC). However, the selection of a RIC or MAC regimen in any particular patient is left to the discretion of investigators at the participating center in accordance with institutional standards.

Table 2.5A outlines the diseases, conditioning regimens and GVHD prophylaxis regimens allowed for this study.

TABLE 2.5A- DIAGNOSES, PREPARATIVE REGIMENS, AND GVHD PROPHYLAXIS REGIMENS

Diagnosis	RIC	MAC	GVHD
AML in morphologic CR1/2 **MDS**: IPSS int-2/high with <10% marrow blasts **HL** beyond CR1 in PR/CR **ALL** in morphologic CR1/2 **NHL** beyond CR1 in PR/CR	Flu/Bu		Tac/MTX or Tac/Sirolimus or Post-Tx Cy with Tac/MMF
		Bu/Flu	Tac/MTX or Post-Tx CY with Tac/MMF (Tac/MMF not required with post-tx CY for 8/8 matches)
	Flu/Mel	Cy/TBI	Tac/MTX or Tac/Sirolimus

2.5.1. Reduced Intensity Conditioning Regimens

Reduced intensity conditioning regimens permitted in this protocol are detailed below.

2.5.1.1. Fludarabine and busulfan (Flu/Bu)

Fludarabine will be given in a total dose of 120-180 mg/m^2 and busulfan will be given in a total dose of ≤ 8 mg/kg PO or 6.4 mg/kg IV with the recommended Flu/Bu regimen as follows:

- Days -6 to -2: Flu (30 mg/m^2/day, total dose of 150 mg/m^2)
- Days -5 to -4: Busulfan (4mg/kg/day PO or 3.2 mg/kg/day IV, 130 mg/m^2/day IV, total dose of 8 mg/kg PO or 6.4 mg/kg IV, or 260 mg/m^2 IV, respectively)

The sequence of fludarabine and busulfan administration in RIC regimens will be done according to institutional standards. Intravenous busulfan may be administered in divided doses or once daily. Oral busulfan must be administered in divided doses. Busulfan dose adjustments for obesity are listed in Section 2.5.2.4. Fludarabine dose adjustments for renal impairment are listed in Section 2.5.2.3.

See Section 2.5.4 and Table 2.6.1.4 for critical information about antiretroviral management in the setting of a busulfan-containing reduced intensity conditioning regimen when the antiretroviral regimen includes ritonavir.

2.5.1.2. Fludarabine and melphalan (Flu/Mel)

Fludarabine will be given in a total dose of 120-180 mg/m^2 and melphalan in a total dose of ≤ 150 mg/m^2 with the recommended Flu/Mel regimen as follows:

- Days -5 to -2: Flu (30mg/m^2/day, total dose of 120 mg/m^2)
- Day -1: Mel (140mg/m^2)

The sequence of fludarabine and melphalan administration in RIC regimens will be done according to institutional standards as long as the prescribed doses are the same as the regimen above. Dividing the dose of melphalan over two days is permitted. Fludarabine dose adjustments for renal impairment are listed in Section 2.5.2.3.

2.5.2. Myeloablative Conditioning Regimens

Myeloablative regimens permitted in this protocol are detailed below. See Section 2.5.4 and Table 2.6.1.4 for critical information about antiretroviral management in the setting of a myeloablative regimen when the antiretroviral regimen includes ritonavir.

2.5.2.1. Busulfan and fludarabine (Bu/Flu)

Fludarabine will be given in a total dose of 120-180mg/m^2 and busulfan will be given in a total dose of ≤16mg/kg PO or 12.8 mg/kg IV with the Bu/Flu regimen as follows:

- Days -5 to -2: Busulfan (4 mg/kg/day PO with Bu Css 900±100 ng/mL (or per institutional standard), 3.2 mg/kg/day IV or 130 mg/m^2/day IV; total dose of 16 mg/kg, 12.8 mg/kg or 520 mg/m^2, respectively)
- Days -5 to -2: Flu (30 mg/m^2/day, total dose of 120 mg/m^2)
- The sequence of busulfan and fludarabine administration in MAC regimens will be done according to institutional standards. Busulfan may be administered intravenously in divided doses or once daily. Appropriate busulfan dosing adjustments per institutional standard should be made when levels are available to achieve the target Css. Busulfan dose adjustments for obesity are listed in Section 2.5.2.4. Fludarabine dose adjustments for renal impairment are listed in Section 2.5.2.3.

2.5.2.2.Cyclophosphamide and total body irradiation (Cy/TBI)

Cyclophosphamide will be given in a total dose of 120 mg/kg and fractionated total body irradiation will be given in a total dose of 1200-1420 cGy with the recommended Cy/TBI regimen as follows:

- Days -7 to -4: TBI (total dose of 1200-1420 cGy)
- Days -3 to -2: Cy (60 mg/kg/day, total dose of 120 mg/kg)

- The sequence of cyclophosphamide, TBI and TBI administration practices in MAC regimens will be done accordingly to institutional standards. Cyclophosphamide dose adjustments for obesity are listed in Section 2.5.2.4.

(See Appendix F for detailed information on pharmacokinetic interactions.)

MESNA will be given to reduce the risk of cyclophosphamide-associated hemorrhagic cystitis. MESNA will be dosed based on cyclophosphamide dose and may be administered per institutional guidelines.

2.5.2.3.Conditioning agent dose adjustments for renal impairment

Fludarabine: Patients with a creatinine clearance of 40-70 ml/min (measured or calculated) should have a 20% dose reduction.

2.5.2.4.Conditioning agent dose adjustments for obesity

Busulfan: Busulfan will be dosed according to the recipient's ideal body weight (IBW), unless the patient weighs more than 125% of IBW, in which case the drug will be dosed according to the adjusted IBW (AIBW; see below for formulas).

Fludarabine: Fludarabine will not be dose adjusted for obesity.

Cyclophosphamide: Cyclophosphamide will be dosed according to the recipient's ideal body weight (IBW), unless the patient weighs less than IBW, in which case the drug will be dosed according to the actual body weight.

Ideal Body Weight (IBW) Formulas:

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

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

Adjusted Ideal Body Weight (AIBW) Formula:

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

2.5.3. GVHD Prophylaxis Regimen

Transplant centers must use the GVHD prophylaxis regimens that are listed below in Table 2.5B. Guidelines for continuing ARV therapy with various prophylaxis regimens are detailed below in Table 2.5C.

Table 2.5B GVHD PROPHYLAXIS REGIMENS

Conditioning Regimen / Match	GVHD Prophylaxis
Fludarabine/Busulfan (Flu/Bu) – RIC / 7/8 or 8/8 Match	**Tacrolimus** • Blood trough levels 5-15 ng/mL • Tacrolimus initiation tapering and discontinuation is per institutional guidelines **Methotrexate** • 15 mg/m^2 Day 1 • 5-10 mg/m^2 Days 3, 6 and 11
	Tacrolimus • Blood trough levels 5-10 ng/mL • Tacrolimus initiation tapering and discontinuation is per institutional guidelines **Sirolimus** • 12 mg loading dose on Day -3 • 4 mg daily starting on Day -2 adjusted to maintain a trough level between 3-12 ng/ml • Sirolimus tapering and discontinuation is per institutional guidelines
	Post-transplant Cyclophosphamide • 50 mg/kg Days 3 and 4 (with MESNA) **Tacrolimus** • Beginning on Day 5, administer tacrolimus to achieve trough levels 5-15 ng/mL for 6 months • 1 mg Day 5 and dose adjusted weekly after Day 7 and continued for 6 months post-transplantation • Tacrolimus tapering and discontinuation is per institutional guidelines **MMF** • 15 mg/kg po TID (max dose 1 gm po TID) Day 5 to Day 35
Busulfan/Fludarabine (Bu/Flu) – MAC / 7/8 or 8/8 Match	**Tacrolimus** • Blood trough levels 5-15 ng/mL • Tacrolimus initiation tapering and discontinuation is per institutional guidelines **Methotrexate** • 15 mg/m^2 Day 1 • 5-10 mg/m^2 Days 3, 6 and 11
Busulfan/Fludarabine (Bu/Flu) – MAC 8/8 Match	**Post-transplant Cyclophosphamide (8/8 match)** • 50 mg/kg Days 3 and 4 (with MESNA) • Note that tacrolimus/MMF is not required in this setting

Conditioning Regimen / Match	GVHD Prophylaxis
Busulfan/Fludarabine (Bu/Flu) – MAC **7/8 Match**	**Post-transplant Cyclophosphamide** • 50 mg/kg Days 3 and 4 (with MESNA) **Tacrolimus** • Beginning on Day 5, administer tacrolimus to achieve trough levels 5-15 ng/mL for 6 months • 1 mg Day 5 and dose adjusted weekly after Day 7 and continued for 6 months post-transplantation • Tacrolimus tapering, and discontinuation is per institutional guidelines **MMF** 15 mg/kg po TID (max dose 1 gm po TID) Day 5 to Day 35
Fludarabine/Melphalan (Flu/Mel) - RIC **Cyclophosphamide/Total Body Irradiation (Cy/TBI) – MAC/** **7/8 or 8/8 Match**	**Tacrolimus** • Blood trough levels 5-15 ng/mL • Tacrolimus initiation tapering and discontinuation is per institutional guidelines **Methotrexate** • 15 mg/m^2 Day 1 • 5-10 mg/m^2 Days 3, 6 and 11
	Tacrolimus • Blood trough levels 5-10 ng/mL • Tacrolimus initiation tapering and discontinuation is per institutional guidelines **Sirolimus** • 12 mg loading dose on Day -3 • 4 mg daily starting on Day -2 adjusted to maintain a trough level between 3-12 ng/ml • Sirolimus tapering and discontinuation is per institutional guidelines

TABLE 2.5C CONTINUATION OF ANTIRETROVIRAL THERAPY BASED ON GVHD PROPHYLAXIS REGIMEN[1]

GVHD Prophylaxis Agent	Raltegravir Based Regimen	NNRTI[2] Based Regimen	Boosted PI[3, 4] Based Regimen	Other
Tacrolimus	Continue antiretroviral therapy as tolerated	Continue antiretroviral therapy. Tacrolimus dose adjustments will be required (Appendix F)	Continue antiretroviral therapy. Tacrolimus dose adjustments will be required (Appendix F)	Consultation recommended, reviewed on a case by case basis
Sirolimus	Continue antiretroviral therapy as tolerated	Continue antiretroviral therapy. Sirolimus dose adjustments will be required (Appendix F)	Continue antiretroviral therapy. Sirolimus dose adjustments will be required (Appendix F)	Consultation recommended, reviewed on a case by case basis
Cyclophosphamide (Cy)	Continue antiretroviral therapy as tolerated	Continue antiretroviral therapy as tolerated with caution and frequent monitoring (Appendix F)	Hold antiretroviral therapy during Cy prophylaxis. Restart antiretrovirals at Day +5 as tolerated.[4]	Consultation recommended, reviewed on a case by case basis[5]

[1]Patients should be switched to raltegravir-based regimens whenever possible.
[2]NNRTI (Non-nucleoside Reverse Transcriptase Inhibitors).
[3]PI (Protease Inhibitors).
[4]Ritonavir – containing antiretroviral therapy.
[5]Restart antiretroviral therapy after cyclophosphamide prophylaxis (Day +5) as soon as patient can tolerate oral medications.

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

Recommendations on GVHD prophylaxis are included in Sections 2.5.3.1 to 2.5.3.4.

2.5.3.1. Tacrolimus

Tacrolimus doses are adjusted to target whole blood levels between 5 and 15 ng/mL, except when the patient is receiving both tacrolimus and sirolimus, in which case it is recommended that serum trough levels of tacrolimus do not exceed 10 ng/mL. When a patient is switched from intravenous to oral tacrolimus, the dose is increased by 3-4 fold to adjust for the lower bioavailability of oral compared to intravenous tacrolimus.

Drugs that may affect tacrolimus levels are:

1. Caspofungin, phenobarbital, phenytoin, rifampin, carbamazepine, rifabutin, St. John's Wort (**lowers levels**);
2. Glucocorticoids, fluconazole, voriconazole, posaconazole, ketoconazole, itraconazole, grapefruit juice, amprenavir, bromocriptine, chloramphenicol, cimetidine, cisapride, clarithromycin, clotrimazole, danazol, diltiazem, erythromycin, ethinyl estradiol, metoclopramide, metronidazole, mibefradil, nefazodone, nelfinavir, nifedipine, omeprazole, quinupristin/dalfopristin, ritonavir, saquinavir, theophylline, troleandomycin, verapamil (**increases levels**).

Per the tacrolimus package insert, when initiating therapy with posaconazole or voriconazole in patients already receiving tacrolimus, it is recommended that the tacrolimus dose be reduced to one-third of the original dose and followed with frequent monitoring of the tacrolimus blood levels. Increased tacrolimus levels have been associated with nephrotoxicity. When posaconazole or voriconazole are discontinued, tacrolimus levels should be carefully monitored and the dose increased as necessary (see Appendix F). Cyclosporine may be substituted for tacrolimus if the patient is intolerant of tacrolimus.

2.5.3.2. Methotrexate

The regimen of methotrexate for GVHD prophylaxis will employ intravenous doses of 15 mg per m^2 on Day 1 post-transplant, and 10 or 5 mg/m^2 on Days 3, 6, and 11 post-transplant according to institutional standards. Third space syndromes with large accumulation of ascites or pleural effusions are a contraindication to the use of methotrexate. Dose reductions per institutional guidelines should be made for renal, hepatic and mucosal toxicity. Determinations of blood levels are indicated 24-72 hours after administration in patients with impaired renal function. Leucovorin rescue should be considered in patients with decreased clearance, severe toxicity or fluid accumulation/effusions.

Drugs that may increase methotrexate levels are:

1. Non-steroidal anti inflammatory drugs
2. Penicillins
3. Diuretics

2.5.3.3. Sirolimus

Sirolimus is recommended to be given to adult patients in a loading dose of 12 mg on Day –3 followed by a daily oral dose of 4 mg per day. Doses may be repeated if the subject vomits within 15 minutes of an oral dose.

Levels of sirolimus need to be monitored frequently during initiation of treatment until target level is reached. Monitoring should be done weekly or monthly once the level reaches steady

state. Frequent monitoring is required if a new medication is initiated or if the patient develops renal insufficiency. The target serum level for sirolimus is 3-12 ng/mL.

Dose adjustments are based on clinical judgment of the treating physician after considering clinical toxicity, serum levels, GVHD, concomitant drug use and the rate of rise or decline of the serum level. For levels < 3 ng/mL, it is suggested, but not required, that the dose of sirolimus be increased by approximately 25% increments no more frequently than every 2 days, rounded to the nearest full milligram until the target range is achieved. Conversely, for levels > 12 ng/mL, it is suggested, but not required, that the dose be decreased by approximately 25% no more frequently than every 2 days until the target level is achieved. Alternatively, sirolimus can be held entirely as long as serum levels are monitored and the drug is restarted when the level returns to the therapeutic range and the treating physician feels it is appropriate to restart the agent.

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

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

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

- Anticonvulsants: carbamazepine, phenobarbital, phenytoin.
- Antibiotics: rifabutin, rifapentine.
- Herbal preparations: St. John's Wort (*Hypericum perforatum*) could result in reduced sirolimus concentrations.

Due to extreme interactions with sirolimus, voriconazole is contraindicated during sirolimus therapy. In the event of suspected or documented fungal infection, alternative antifungal therapy should be used wherever possible (see Appendix F for further details). The uses of sirolimus and myeloablative doses of busulfan are associated with hepatic veno-occlusive disease (sinusoidal obstructive syndrome) and thus should be avoided.

2.5.3.4.Post-transplantation Cyclophosphamide with MESNA

Post-transplantation cyclophosphamide with MESNA may only be used with fludarabine/busulfan for non-myeloablative and busulfan/fludarabine for myeloablative conditioning regimens.

MESNA will be given to reduce the risk of cyclophosphamide-associated hemorrhagic cystitis. MESNA will be dosed based on cyclophosphamide dose and may be administered per institutional guidelines.

It is crucial that no immunosuppressive agents are given until 24 hours after the completion of the post-transplant Cy. This includes corticosteroids for any reason.

Tacrolimus and MMF should not be used with post-transplant cyclophosphamide if the patient received a myeloablative conditioning regimen and the donor/recipient match is 8/8.

Additional immunosuppression for patients receiving post-transplant cyclophosphamide:

- Full myeloablative regimen and donor/recipient match is 8/8: no additional prophylaxis is given
- Full myeloablative regimen with a 7/8 match and for Flu/Bu non-myeloablative regimen:
  - On Day 5, patients will begin prophylaxis with Tacrolimus and Mycophenolic Acid Mofetil (MMF). Tacrolimus will be given at a dose of 1 mg IV daily then will be changed to a PO dosing schedule once a therapeutic level is achieved or as per institutional standards.
  - MMF will be given at a dose of 15 mg/kg PO TID (based upon actual body weight) with the maximum total daily dose not to exceed 3 grams (1 g PO TID).
  - MMF prophylaxis will be discontinued after the last dose on Day 35 and Tacrolimus prophylaxis will be discontinued per institutional standards typically around Day 180.

2.5.4. Ritonavir-Based Antiretroviral Therapy Dosing Considerations

Serious and prolonged toxicity is possible for patients concurrently receiving ritonavir-based (boosted-PI) therapy and the immunosuppressive agents tacrolimus or sirolimus. The introduction of immunosuppressive therapy must be systematic, carefully monitored and consistent throughout the peri-transplantation period. At the inception of immunosuppression, patients may or may not be receiving antiretroviral therapy based on the conditioning regimen selected. Patients receiving ritonavir throughout conditioning are to be managed differently than those who are not. This is due to the potent inhibition of CYP3A4 and P-glycoprotein by ritonavir, resulting in the delayed metabolism and consequently higher serum levels of tacrolimus, and sirolimus.

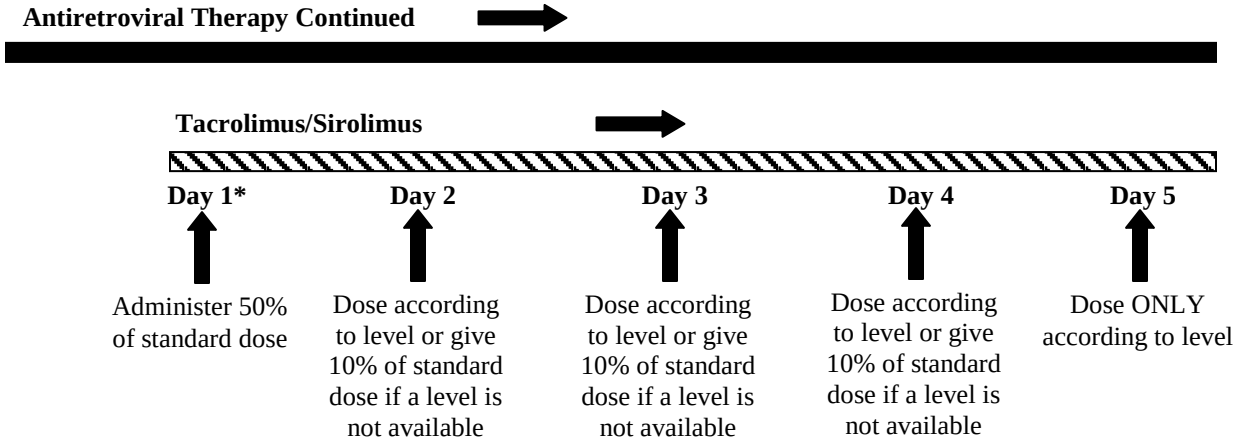
Daily monitoring of serum trough levels of cyclosporine, tacrolimus and sirolimus is the most accurate way to provide effective immunosuppression while minimizing toxicity. In the setting of less frequent monitoring, dose adjustments can be predicted based on the metabolic pathways for each agent; however, the drug-drug interactions with ritonavir have not been studied extensively and must be supported by routine therapeutic drug monitoring as soon as possible. Only the doses of tacrolimus and sirolimus should be adjusted; antiretroviral therapy must be continued at the dose prescribed.

For all patients, the following is recommended:

1) Intravenous tacrolimus is preferred to avoid gastrointestinal cytochrome P450 co-enzyme inhibition during the peri-transplantation period, however oral therapy with tacrolimus, or sirolimus is permitted
2) Serum trough levels should be obtained no later than Day 5 of concurrent immunosuppressive and antiretroviral therapy
3) If clinical signs or symptoms of severe toxicity (neurotoxicity) are present OR if serum trough levels of tacrolimus/sirolimus exceed the upper limits of acceptable (*tacrolimus >25 ng/mL, sirolimus >25 ng/mL*), then BOTH antiretroviral therapy and tacrolimus/sirolimus should be held, a level repeated in 24 hours and therapy resumed once the level is within the target range
4) No dose adjustments are required for mycophenolate mofetil or methotrexate
5) Dosing recommendations based on serum levels are provided below and in Appendix F
6) Recommended standard dosing **(or per institutional standard)**:
   a. Tacrolimus
      i. IV: 0.03 mg/kg every 24 hours (based on Ideal Body Weight)
      ii. PO: 0.12 mg/kg/day divided into 2 doses
   b. Sirolimus
      i. PO: 12 mg once, then 4 mg daily as a single dose

Dosing recommendations are provided for each of the following:

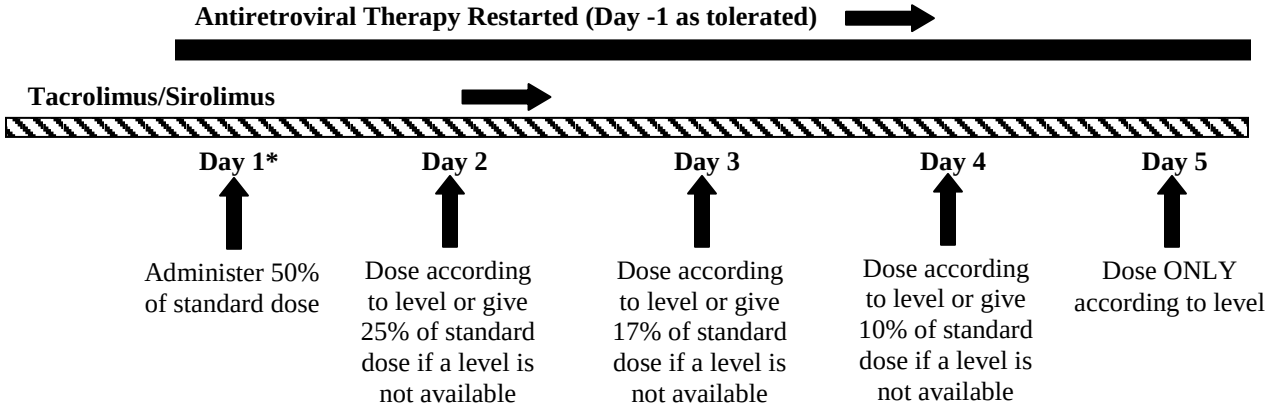
1) Patients continuing ritonavir-based therapy throughout conditioning



*Day 1 represents the first day of concurrent therapy with cyclosporine, tacrolimus, or sirolimus and ritonavir-based antiretroviral therapy.

2) Patients discontinuing ritonavir-based therapy prior to conditioning

a. Antiretroviral therapy should be discontinued 4-7 days prior to any myeloablative conditioning regimen and busulfan-containing reduced-intensity conditioning
b. Antiretroviral therapy should be restarted no sooner than Day -1 as tolerated
c. For patients receiving post-transplant cyclophosphamide for GVHD prophylaxis, antiretroviral therapy will be restarted on Day +5 as tolerated



*Day 1 represents the first day of concurrent therapy with tacrolimus, or sirolimus and ritonavir-based antiretroviral therapy. For patients restarting antiretroviral therapy, **this should be Day -1**, unless they are unable to tolerate oral medications or receive cyclophosphamide for GVHD prophylaxis.

3) Dosing based on a serum trough level of tacrolimus, or sirolimus while receiving ritonavir-based therapy

Immunosuppressive Agent	Target Trough	Recommendation[1]
Tacrolimus	5 – 15 ng/mL	**< 5 ng/mL:** Administer 1 mg (PO) or 0.25 mg (IV)[2] **>5 - 15 ng/mL:** Administer 0.5 mg (PO) or 0.125 mg (IV) [2] **>15 - 25 ng/mL:** HOLD tacrolimus & repeat level in 24h **> 25 ng/mL:** HOLD both tacrolimus and ARV
Sirolimus	3 – 12 ng/mL	**< 3 ng/mL:** Administer 0.4 mg (PO) [2] **>3 - 12 ng/mL:** Administer 0.3 mg (PO) [2] **>12-25ng/mL:** HOLD sirolimus & repeat level in 24h **>25ng/mL:** HOLD both sirolimus and ARV

ARV= Antiretroviral Therapy
[1]See Appendix F (Table F-3) for detailed information
[2]Administer a one-time dose in response to the observed level

2.5.5. Initiation of Antifungal Prophylaxis in Ritonavir-Based Antiretroviral Therapy

Detailed information is available in Appendix F Table F-6 for patients receiving ritonavir-based antiretroviral therapy.

Initiating antifungal prophylaxis in patients concomitantly receiving ritonavir may result in serious toxicity. Moreover, many antifungal agents affect the metabolism of the immunosuppressive agents, tacrolimus, sirolimus and cyclophosphamide, also resulting in the increased risk of serious adverse events. ***Careful selection of the most appropriate antifungal agent and routine therapeutic drug monitoring are strategies to minimize the risk of severe toxicity.***

Fluconazole is effective against yeasts other than *Candida krusei* and some *C. glabrata*, but is not effective against moulds. For patients at high risk for mould infections (such as those with cell-mediated immunodeficiency from GVHD) or those with a prior history of invasive aspergillosis, prophylaxis with an anti-mould agent is recommended. Given the three-way interaction between selected immunosuppressive drugs, HIV medications (especially protease inhibitors) and triazole agents (especially voriconazole, itraconazole, and to a lesser extent posaconazole), the echinocandins (anidulafungin, caspofungin, micafungin) and amphotericin B (either the aerosolized or intravenous formulations) are preferred for prophylaxis. The echinochandins are effective against *Aspergillus* mould only, whereas amphotericin B has a

broader spectrum of mould activity. Aerosolized liposomal amphotericin B has been shown to be effective in reducing invasive pulmonary aspergillosis in leukemic and HCT patients.

2.5.5.1.Voriconazole

Voriconazole is metabolized by, and inhibits the activities of, human cytochrome P450 enzymes CYP2C19, CYP3A4 and CYP2C9. The affinity of voriconazole is the highest for CYP2C19, followed by CYP2C9 and CYP3A4; however there is a large degree of inter-individual variability in voriconazole pharmacokinetics. The interactions between voriconazole and the antiretroviral agents, specifically ritonavir, are complex. ***Concurrent use of high-dose ritonavir (400-600 mg twice daily) is contraindicated*** due to significant reduction in plasma concentration of voriconazole. Low-dose ritonavir (100mg twice daily) has been shown to decrease the voriconazole AUC by 39% in healthy subjects after 9 days; however, upon initiation of voriconazole, the AUC may be increased by 4.5 fold. This is due to a time- and dose-dependent interaction where ritonavir initially inhibits voriconazole metabolism, followed by delayed induction.

Moreover, voriconazole may further inhibit the metabolism of immunosuppressive agents, such as tacrolimus, sirolimus and cyclophosphamide. The following is recommended for initiating voriconazole prophylaxis in patients receiving protease inhibitor-based antiretroviral therapy:

1) No high-dose ritonavir (contra-indicated due to significant reduction of plasma voriconazole concentrations)
2) Avoid loading doses (due to significant voriconazole effect on calcineurin inhibitors and sirolimus levels); initiate prophylaxis with voriconazole 4 mg/kg IV or 200mg PO every 12 hours
3) Therapeutic drug monitoring of voriconazole is required within 7 days of concomitant therapy (due to significant effect of protease inhibitors on voriconazole)
    a. Serum trough levels ≥ 1 mcg/ml are desired for optimal efficacy
    b. Serum trough levels > 6 mcg/ml are associated with greater toxicity
4) Close monitoring (daily if possible) of serum levels for cyclosporine, tacrolimus or sirolimus is strongly recommended (due to significant effect of voriconazole on these agents)
    a. In the absence of an appropriate level, administer 10% of the standard dose of cyclosporine, tacrolimus or sirolimus (see Section 2.5.4)
5) Monitor closely for signs or symptoms of voriconazole toxicity (hepatotoxicity, CNS toxicity)

2.5.5.2.Posaconazole

Posaconazole also inhibits the activity of human cytochrome P450 enzymes especially the isoform CYP3A4, which can increase the plasma concentration and exposure of drugs metabolized by this enzyme. However, posaconazole does not inhibit the CYP450 isoforms CYP1A2, 2C8/9, 2D6, or 2E1, and thus potentially has a lower drug interaction potential than

voriconazole. Unlike voriconazole, posaconazole metabolism is mediated primarily by uridine diphosphate glucuronosyltransferase. In general, the metabolic pathways of posaconazole and ritonavir suggest that posaconazole may increase the plasma concentrations of ritonavir, and ritonavir may decrease the plasma concentration of posaconazole. Posaconazole may further inhibit the metabolism of immunosuppressive agents, such as tacrolimus, sirolimus and cyclophosphamide. The following is recommended for initiating posaconazole prophylaxis in patients receiving protease inhibitor-based antiretroviral therapy:

1) No high-dose ritonavir (contra-indicated due to significant reduction of plasma posaconazole concentrations)
2) Therapeutic drug monitoring of posaconazole is required within 7 days of concomitant therapy (due to significant effect of protease inhibitors on posaconazole). Therapeutic levels of posaconazole have not been established, but low levels have been encountered in the setting of impaired absorption with poor food intake, acute GVHD, or diarrhea.
3) Close monitoring (daily if possible) of serum levels for cyclosporine, tacrolimus or sirolimus is strongly recommended (due to significant effect of posaconazole on these agents)
    a. In the absence of an appropriate level, administer 10% of the standard dose of tacrolimus or sirolimus (see Section 2.5.4)

2.6. Supportive Care

2.6.1. Post-HCT

All supportive care will be given in keeping with BMT CTN MOP and local institutional guidelines.

2.6.1.1. Prophylaxis against infections

All patients will receive prophylaxis against bacterial, fungal and viral infections during the post-HCT period according to the BMT CTN MOP. Additional guidelines for HIV patients in this study are summarized in Appendix E.

2.6.1.2. Cryopreservation

Donor's stem cells may be cryopreserved prior to transplantation at the discretion of treating institution and in keeping with NMDP policy if an unrelated donor.

2.6.1.3. Post-HCT growth factors

Use of Post-HCT growth factors will be according to institutional standards.

2.6.1.4. Antiretroviral therapy

Antiretroviral (ARV) therapy 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 therapy should be administered only when it is anticipated that the ARV regimen can be taken on a consistent basis (i.e., preparative 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 therapy should be continued at least through Day 100.

Patients on Reduced Intensity Conditioning Regimens: Patients will continue on ARV therapy.

Patients on Myeloablative Conditioning Regimens: Ritonavir-containing ARV therapy will restart not sooner than 12 hours after the last chemotherapy infusion including post-transplant cyclophosphamide as tolerated.

Table 2.6.1.4 outlines continuation guidelines for ARV regimens based on the conditioning regimen used.

TABLE 2.6.1.4: CONTINUATION OF ANTIRETROVIRAL THERAPY (ARV) BASED ON THE CONDITIONING REGIMEN

Reduced Intensity Conditioning	Raltegravir Based Regimen	NNRTI Based Regimen	Boosted PI Based Regimen**	Other
Fludarabine Busulfan	Continue throughout conditioning as tolerated	Continue throughout conditioning as tolerated†	Discontinue ARV 96 hours prior to conditioning and resume after conditioning as tolerated	Consultation recommended, reviewed on a case by case basis
Fludarabine Melphalan	Continue throughout conditioning as tolerated	Continue throughout conditioning as tolerated	Continue throughout conditioning as tolerated	Consultation recommended, reviewed on a case by case basis
Myeloablative Conditioning				
Fludarabine Busulfan *	Continue throughout conditioning as tolerated	Continue throughout conditioning as tolerated†	Discontinue ARV 96 hours prior to conditioning and resume after conditioning as tolerated	Consultation recommended, reviewed on a case by case basis
Cyclophosphamide Total Body Irradiation (1200 – 1420 cGy)	Continue throughout conditioning as tolerated	Continue throughout conditioning as tolerated†	Discontinue ARV 96 hours prior to conditioning and resume after conditioning as tolerated	Consultation recommended, reviewed on a case by case basis

*****PO doses adjusted to maintain BU Css at 900 ± 100 ng/mL or per institutional standard
** Patients receiving cyclophosphamide for GVHD prophylaxis should not resume boosted PI based regimens until Day +5 as tolerated
†Busulfan levels may be decreased when co-administered with NNRTIs

See Appendix F [Table F-1] for detailed information on each interaction.

2.7. Participant Risks

HCT recipients incur risks from high-dose conditioning and post-HCT therapy, which must be weighed against the risk of the disease for which the HCT is prescribed. Major risks following transplantation include: 1) Infection which can be bacterial, viral, parasitic, or fungal. Often, these infections are life-threatening, particularly when caused by viral or fungal agents, and are associated with high mortality in the transplant population. The published experience shows increased risk of CMV viremia, but no increase in mortality related to that virus; 2) Damage of all or any of the major organs may occur as a result of reactions to drugs (e.g., chemotherapy, antibiotics, anti-fungal medications), and as a result of destructive processes (e.g., infection), and may have a fatal outcome; brain damage can result in severe loss of cognitive or neurologic function; 3) Relapse or progression may occur, especially in patients with advanced disease status at time of treatment; 4) Unknown toxicities may occur in any individual patient due to

multiple events and cumulative effects which may involve any and all organs, including the brain; and, 5) Death.

2.8. Therapy Toxicities

All toxicities will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. All of the following listed agents are commercially available. Refer to www.fda.gov for full adverse event information regarding the agents listed below. All of the following agents should be administered per institutional standards, and stored per package insert instructions.

2.8.1. Busulfan

Busulfan is a bi-functional alkylating agent. Toxicities include the following: Severe bone marrow hypoplasia, which would be fatal without administration of bone marrow, stem cells; nausea and vomiting, which can be decreased by the use of sedation and anti-emetics; stomatitis and diarrhea, which can be treated symptomatically with fluid replacement and atropine or diphenoxylate HCl; pulmonary fibrosis characterized by delayed onset of cough, shortness of breath and low-grade fever; hepatic damage, which can occur in combination with cyclophosphamide or as a single agent and can result in significant hepatic toxicity, which can be fatal; temporary hyperpigmentation of the skin and nail bed changes; and grand mal seizures, which can be prevented by the prophylactic administration of phenytoin.

2.8.2. Melphalan

Melphalan, an alkylating agent, is a phenylalanine derivative of nitrogen mustard. At high doses, the likely toxicities include myelosuppression, gastrointestinal toxicity and alopecia. The duration of profound myelosuppression decreases with the use of stem cell transplantation and colony stimulating factors. Gastrointestinal toxicity, which includes potentially severe stomatitis, esophagitis and diarrhea, may require intravenous narcotics for mucositis related pain, intravenous hydration and alimentation, and antibiotics. Less likely is hepatotoxicity. Rare but serious toxicities reported include pulmonary fibrosis and interstitial pneumonitis, veno-occlusive disease of the liver, skin hypersensitivity, vasculitis, hemolytic anemia, allergic reactions, and a small risk of developing second cancers.

2.8.3. Fludarabine

a. Neurotoxicity: Agitation or confusion, blurred vision, loss of hearing, peripheral neuropathy or weakness have been reported. Severe neurologic effects, including blindness, coma, and death are seen in 36% of patients treated with doses approximately four times greater than recommended; severe CNS toxicity is rarely seen with doses in the recommended range for non-transplant therapy of hematologic malignancies. Effect of chronic use on the CNS is unknown, although patients have received recommended doses for up to 15 courses. The dose used in this study is approximately 1.5 times the usual one-course dose given in non-transplant settings. Doses and schedules such as

those used in this study have been used in adult and pediatric patients and increased neurotoxicity has not been observed.

b. Anemia: Life-threatening and sometimes fatal autoimmune hemolytic anemia has been reported after one or more cycles of therapy in patients with or without a previous history of autoimmune hemolytic anemia or a positive Coombs' test and who may or may not be in remission; no mechanisms for development of this complication have been identified. Corticosteroids may or may not be effective in controlling these episodes. The majority of patients re-challenged developed a recurrence of the hemolytic process.

c. Cardiovascular: Deep venous thrombosis, phlebitis, transient ischemic attack, and aneurysm (1%) are reported.

d. Fever: 60% of patients develop fever.

e. Skin Rash: 15% of patients develop a skin rash, which may be pruritic.

f. Digestive: Gastrointestinal side effects include: nausea/vomiting (36%), diarrhea (15%), stomatitis (9%), anorexia (7%), GI bleeding and esophagitis (3%), mucositis (2%), liver failure, abnormal liver function test, constipation, dysphagia (1%) and mouth sores.

g. Some other effects are: Chills (11%), peripheral edema (8%), myalgia (4%), osteoporosis (2%), pancytopenia, arthralgia (1%), dysuria (4%), urinary tract infection and hematuria (2%); renal failure, abnormal renal function test, and proteinuria (1%); and, very rarely, hemorrhagic cystitis and pulmonary toxicity.

2.8.4. Cyclophosphamide

Cyclophosphamide side effects include: nausea/vomiting, cardiomyopathy, skin rash, mucositis, stomatitis, sterility, diarrhea, hemorrhagic cystitis, fluid weight gain/edema, alopecia and hemolytic anemia.

2.8.5. Total Body Irradiation

TBI can cause: nausea and vomiting, diarrhea, parotitis (rapid onset within 24-48 hours, usually self-limited), generalized mild erythema, hyperpigmentation, fever, mucositis, and alopecia.

Late effects include: possible growth retardation, vertebral deformities, cataracts, probable increased risk of secondary malignant neoplasms, sterility, nephropathy, interstitial pneumonitis and veno-occlusive disease.

2.8.6. Tacrolimus

Side effects include: reversible renal insufficiency, hypertension, hypomagnesemia, hypokalemia, and neurologic toxicity. A syndrome of thrombotic microangiopathy, comprised of microangiopathic hemolytic anemia, thrombocytopenia and renal dysfunction has been described in association with sirolimus and tacrolimus use. In common with all other immunosuppressants, it may increase the risk of opportunistic infections and post-transplant lymphoproliferative disorders.

2.8.7. Sirolimus

Despite the similarity between sirolimus and tacrolimus, sirolimus is not associated with neurotoxicity or nephrotoxicity because of its inability to inhibit calcineurin. Phase III clinical trials have indicated that the primary toxicities are hypertriglyceridemia, hypercholesterolemia, mild thrombocytopenia, anemia, leukopenia, hypokalemia, elevated LDH, arthralgia, epistaxis, edema, and infections. Clinically significant elevations in hepatic transaminases without sequelae were noted in the prior Phase II study with this drug.

2.8.8. Methotrexate

The most frequently reported adverse reactions associated with methotrexate use as GVHD prophylaxis include ulcerative stomatitis, leucopenia and suppressed hematopoiesis, nausea, and abdominal distress. Other frequently reported adverse effects are malaise, undue fatigue, chills and fever, dizziness and decreased resistance to infection. Methotrexate may be associated with increased rates of pulmonary complications after transplantation. The risk of infections is due to the suppression of hematopoiesis after transplantation.

2.8.9. Mycophenolate Mofetil (MMF)

The most frequent reported adverse reactions associated with MMF include infection, upset stomach and nausea. Less common reported adverse effects are low blood counts, vomiting and diarrhea. Some rare but serious reported adverse reactions include serious injury to the gut including bloody stools and vomit, secondary cancers such as lymphoproliferative disease or lymphoma, serious infections of the brain, risks to a baby in pregnancy and progressive multifocal leukoencephalopathy (PML).

CHAPTER 3

3. STUDY ENDPOINTS

3.1. Primary Endpoints

Patients will be assessed for 100-Day Non-Relapse Mortality. The events for non-relapse mortality are death due to any cause other than relapse of the underlying malignancy.

3.2. Secondary Endpoints

3.2.1. 100-day Disease Status Following Transplant

Patients will be assessed for disease status at Day 100 post-HCT: complete remission, partial remission (HL, NHL), stable disease (HL, NHL), relapse.

3.2.2. Time to Hematopoietic Recovery

Time to neutrophil recovery will be the first of three consecutive days of $\geq$ 500 neutrophils/μL following the expected nadir. Patients who never nadir will be censored for time to neutrophil recovery. Time to platelet engraftment will be the date platelet count is $\geq$ 20,000/μL for the first of three consecutive labs with no platelet transfusions 7 days prior.

3.2.3. Chimerism

Blood samples will be evaluated for T cell and myeloid chimerism at 4 weeks, Day 100, and 6 months post-transplant.

3.2.4. Hematologic Function

Hematologic function will be defined by ANC >1500, Hemoglobin >10g/dL without transfusion support, and platelets >100,000 and measured at Day 100 and6 months. Use of growth factors will be noted.

3.2.5. Occurrence of Infections

Microbiologically documented infections will be reported by site of disease, date of onset, severity, and resolution, if any.

3.2.6. Six Month Overall Survival

Overall survival is defined as time from transplant to death or last follow-up.

3.2.7. Acute Graft-versus-Host Disease (GVHD)

Acute GVHD will be graded according to the BMT CTN MOP. The time to onset of acute grades II-IV GVHD and grades III-IV GVHD will be recorded, as well as the maximum grade experienced.

3.2.8. Chronic Graft-versus-Host Disease (GVHD)

Chronic GVHD will be scored according to the BMT CTN MOP. The time to onset of limited and extensive chronic GVHD will be recorded.

3.2.9. Immunologic Reconstitution

This will be measured in all patients at 8 weeks, 6, 12 and 24 months. Tests to be performed on peripheral blood at those time points include CD2, CD3, CD4, CD8, CD19, CD3+/CD25+, CD45 RA/RO, CD56+/CD3-, and quantitative immunoglobulins (IgM, IgG and IgA).

3.2.10. Impact of Therapy on the HIV Reservoir

HIV-1 RNA in plasma will be measured by standard real-time RT-PCR (detection limit 40 copies/ml) and by the investigational single copy assay (SCA, detection limit 0.38 copy/ml). HIV-1 DNA in peripheral blood mononuclear cells (PBMCs) and other cells will be quantified using the same primers and probes used for SCA but without a reverse transcription step. HIV-1 DNA levels will be normalized by cell number and amplifiable cellular DNA (GAPDH). Assays will be performed in triplicate on each sample. HIV-1 RNA levels will be measured in plasma prior to the initiation of ablative chemotherapy, and at Day 100, 6, 12 and 24 months post-transplant. The latent HIV reservoir measurement assay at approximately 13 months will only occur in those patients who have no measurable viral load by single copy HIV assay at 12 months (Appendix C).

3.3. Disease Status Assessment

3.3.1. Lymphoma

Patients at each data collection period are classified into one of the disease statuses specified below (CR, PR, SD, PD). After HCT and until relapse/progression, all disease status classifications are relative to the patient's pre-HCT disease status. Tests used for evaluation of disease status would be physical examination, laboratory testing, bone marrow biopsy and aspirate, PET scans, and CT scans of neck, chest, abdomen and pelvis as indicated.

Segments of this section are excerpts from the Bruce Cheson, et al, article "Revised Response Criteria for Malignant Lymphoma."

TABLE 3.1: RESPONSE DEFINITIONS – NON-HODGKINS'S LYMPHOMA*

Response	Definition	Nodal Masses	Spleen, Liver	Bone Marrow
CR	Disappearance of all evidence of disease	(a) FDG-avid or PET positive prior to therapy; mass of any size permitted if PET negative (b) Variably FDG-avid or PET negative; regression to normal size on CT	Not palpable, nodules disappeared	Infiltrate cleared on repeat biopsy; if indeterminate by morphology, immunohistochemistry should be negative
PR	Regression of measurable disease and no new sites	≥ 50% decrease in SPD of up to 6 largest dominant masses; no increase in size of other nodes (a) FDG-avid or PET positive prior to therapy; one or more PET positive at previously involved site (b) Variably FDG-avid or PET negative; regression on CT	≥ 50% decrease in SPD of nodules (for single nodule in greatest transverse diameter); no increase in size of liver or spleen	Irrelevant if positive prior to therapy; cell type should be specified
SD	Failure to attain CR/PR or PD	(a) FDG-avid or PET positive prior to therapy; PET positive at prior sites of disease and no new sites on CT or PET (b) Variably FDG-avid or PET negative; no change in size of previous lesions on CT		
Relapsed disease or PD	Any new lesion or increase by ≥ 50% of previously involved sites from nadir	Appearance of a new lesion(s) > 1.5 cm in any axis, ≥ 50% increase in SPD of more than one node, or ≥ 50% increase in longest diameter of a previously identified node > 1 cm in short axis Lesions PET positive if FDG-avid lymphoma or PET positive prior to therapy	> 50% increase from nadir in the SPD of any previous lesions	New or recurrent involvement

Abbreviations: CR, complete remission; FDG, [18F]fluorodeoxyglucose; PET, positron emission tomography; CT, computed tomography; PR, partial remission; SPD, sum of the product of the diameters; SD, stable disease; PD, progressive disease.

* Cheson B, Pfistner B, Juweid M, et al. Revised Response Criteria for Malignant Lymphoma. J Clin Oncol 25: 579-586, 1997.
** In relation to Response Definitions, be aware of HIV adenopathy, this can be avid in patients with equivocal scans.
*** Additional follow-up PET needed if 90-day results are equivocal.

Complete Remission (CR):

The designation of CR requires the following (Table 3.1):

- Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy.
- Typically FDG-avid lymphoma: in patients with no pretreatment PET scan or when the PET scan was positive before therapy, a post-treatment residual mass of any size is permitted as long as it is PET negative.
- Variably FDG-avid lymphomas/FDG avidity unknown: in patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, all lymph nodes and nodal masses must have regressed on CT to normal size ($\leq$ 1.5 cm in their greatest transverse diameter for nodes > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their long axis and more than 1.0 cm in their short axis before treatment must have decreased to $\leq$ 1.0cm in their short axis after treatment.
- The spleen and/or liver, if considered to be enlarged before therapy on the basis of a physical examination or CT scan, should not be palpable on physical exam and should be considered normal size by imaging studies, and nodules related to lymphoma should disappear. However, determination of splenic involvement is not always reliable because a spleen considered normal in size may still contain lymphoma, whereas an enlarged spleen may reflect variations in anatomy, blood volume, the use of hematopoietic growth factors, or causes other than lymphoma.
- If bone marrow was involved by lymphoma before treatment, the infiltrate must have cleared on repeat bone marrow biopsy. The biopsy sample on which this determination is made must be adequate (with a goal of > 20 mm unilateral core). If the sample is indeterminate by morphology, it should be negative by immunohistochemistry. A sample that is negative by immunohistochemistry but that demonstrates a small population of clonal lymphocytes by flow cytometry will be considered a CR until data become available demonstrating a clear difference in patient outcome.

Complete Remission Undetermined (CRu):

- The use of the above definition for CR and that below for PR eliminates the category of CRu.

Partial Remission (PR):

The designation of PR requires all of the following:

- At least a 50% decrease in sum of the product of the diameters (SPD) of up to six of the largest dominant nodes or nodal masses. These nodes or masses should be selected according to all of the following: they should be clearly measurable in at least 2 perpendicular dimensions; if possible they should be from disparate regions of the body; and they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.
- No increase should be observed in the size of other nodes, liver or spleen.

- Splenic and hepatic nodules must regress by ≥ 50% in their SPD or, for single nodules in the greatest transverse diameter.
- With the exception of splenic and hepatic nodules, involvement of other organs is usually assessable and no measurable disease should be present.
- Bone marrow assessment is irrelevant for determination of a PR if the sample was positive before treatment. However, if positive, the cell type should be specified (eg, large-cell lymphoma or small neoplastic B cells). Patients who achieve a CR by the above criteria, but who have persistent morphologic bone marrow involvement will be considered partial responders. When the bone marrow was involved before therapy and a clinical CR was achieved, but with no bone marrow assessment after treatment, patients should be considered partial responders.
- No new sites of disease should be observed.
- Typically FDG-avid lymphoma: for patients with no pretreatment PET scan or if the PET scan was positive before therapy, the post-treatment PET should be positive in at least one previously involved site.
- Variably FDG-avid lymphomas/FDG-avidity unknown: for patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, CT criteria should be used. In patients with follicular lymphoma or mantle-cell lymphoma, a PET scan is only indicated with one, or at most two, residual masses that have regressed by more than 50% on CT; those with more than two residual lesions are unlikely to be PET negative and should be considered partial responders.

Stable Disease (SD):

Stable disease (SD) is defined as the following:

- A patient is considered to have SD when he or she fails to attain the criteria needed for a CR or PR, but does not fulfill those for progressive disease (see Relapsed Disease [after CR]/Progressive Disease [after PR, SD]).
- Typically FDG-avid lymphomas: the PET should be positive at prior sites of disease with no new areas of involvement on the post-treatment CT or PET.
- Variably FDG-avid lymphomas/FDG-avidity unknown: for patients without a pretreatment PET scan or if the pretreatment PET was negative, there must be no change in the size of the previous lesions on the post-treatment CT scan.

Relapsed Disease (RD, after CR)/ Progressive Disease (PD after PR, SD):

Lymph nodes should be considered abnormal if the long axis is more than 1.5 cm regardless of the short axis. If a lymph node has a long axis of 1.1 to 1.5 cm, it should only be considered abnormal if its short axis is more than 1.0. Lymph nodes$\leq 1.0 \times \leq 1.0$cm will not be considered as abnormal for relapse or progressive disease.

- Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased FDG uptake in a

previously unaffected site should only be considered relapsed or progressive disease after confirmation with other modalities. In patients with no prior history of pulmonary lymphoma, new lung nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the PET without histologic confirmation.

- At least a 50% increase from nadir in the SPD of any previously involved nodes or in a single involved node, or the size of other lesions (e.g, splenic or hepatic nodules). To be considered progressive disease, a lymph node with a diameter of the short axis of less than 1.0 cm must increase by ≥ 50% and to a size of 1.5 x 1.5 cm or more than 1.5 cm in the long axis.
- At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis.
- Lesions should be PET positive if observed in a typical FDG-avid lymphoma or the lesion was PET positive before therapy unless the lesion is too small to be detected with current PET systems (< 1.5 cm in its long axis by CT). Measurable extranodal disease should be assessed in a manner similar to that for nodal disease. For these recommendations, the spleen is considered nodal disease. Disease that is only assessable (e.g. pleural effusions, bone lesions) will be recorded as present or absent only, unless, while an abnormality is still noted by imaging studies or physical examination, it is found to be histologically negative. In clinical trials where PET is unavailable to the vast majority of participants, or where PET is not deemed necessary or appropriate for use (e.g. a trial in patients with MALT lymphoma), response should be assessed as above, but only using CT scans. However, residual masses should not be assigned CRu status, but should be considered partial responses.

3.3.2. Acute Leukemia and Myelodysplastic Syndromes

Assessment after transplantation response will be assessment as follows:

Complete Remission:

- Bone marrow myeloblasts < 5% by morphologic assessment;
- No circulating blasts;
- Neutrophil count ≥ 1,000/μL;
- Absence of previous cytogenetic or molecular abnormality identified prior to transplantation in the bone marrow aspirate.

Disease Relapse for Patients with AML:

- Increase in bone marrow blast to≥ 5% by morphologic assessment not attributed to other causes (e.g., bone marrow regeneration); or if < 5%, reappearance of blasts with the same leukemia phenotype as present at diagnosis.
- Reappearance of blasts with aberrant phenotype by flow cytometry.

- Reappearance of leukemic blasts in the peripheral blood.
- Reappearance of previous cytogenetic or molecular marker of disease present prior to transplantation.
- The development of extramedullary leukemia or leukemic cells in the cerebral spinal fluid.

Disease Relapse for Patients with MDS:

- Satisfying above criteria for evolution into acute leukemia; or,
- Reappearance of pre-transplant morphologic abnormalities, detected in two consecutive bone marrow specimens taken at least one month apart; 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.

CHAPTER 4

4. PATIENT ENROLLMENT AND EVALUATION

4.1. Enrollment Procedures

4.1.1. Screening and Eligibility Procedures

Patients will be registered using the BMT CTN Electronic Data Capture System (AdvantageEDC^{SM}). The following procedures should be followed:

1. An authorized user at the transplant center completes the Segment 0 Screening Form (patient demographic data and the date that informed consent was signed). A study number will be generated for the patient with the submission of the Segment 0 Screening Form.

   - Potential Unrelated Donor Transplant: Patients requiring an unrelated donor should be enrolled in the Segment 0 at least 3 months prior to the proposed start of conditioning. In some cases, patients will not be able to be enrolled at least 3 months prior to the proposed start of conditioning. If this is the case, the patient should be enrolled as early as possible but no less than 2 weeks prior to the proposed start of conditioning.
   - Potential Related Donor Transplant: Patients with an available related donor should be enrolled in Segment 0 approximately 8 to 10 weeks (but no less than 2 weeks) prior to the proposed start of conditioning.

   Note: There are two reasons for completing the screening enrollment phase early. First, blood specimens are collected at baseline to address important research questions in this study. Secondly, for patients requiring an unrelated donor, sufficient time is needed for the unrelated donor search.

2. The transplant coordinator at the transplant center should proceed with their institution's standard procedure to identify a sibling donor, if applicable. If an unrelated donor search is to be pursued, then the coordinator should inform the transplant center's assigned NMDP coordinator through the standard procedure for any unrelated donor.

3. Due to the complex nature of the patient population and the potential for numerous drug-drug interactions between the antiretroviral regimen and the allogeneic HCT treatment regimen, each patient's treatment regimen must be reviewed with the Treatment Review Committee. Appendix D details the process for getting Treatment Review Committee approval. This review should typically occur at least 4 weeks prior to the planned transplant date in order to allow sufficient time for any necessary adjustments to the patient's medication regimen. In some patients' circumstances, a 4-week lead time may not be possible; this alone will not make the patient ineligible for the study.

4. Prior to initiation of conditioning therapy (typically 1-2 weeks), an authorized user at the transplant center completes Segment A of the Enrollment Form in AdvantageEDC.

Segment A includes questions that verify eligibility and captures the proposed start date of conditioning.

4.2. Study Monitoring

4.2.1. Follow-up Schedule

The follow-up schedule for scheduled study visits is outlined in Table 4.2.1. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User's Guide. The Data Management Handbook, including the Forms Submission Schedule, is available on the homepage of the Internet data entry system.

Follow-up Visits: Follow-up visits will begin as soon as patients are enrolled onto the study. The follow-up period is 24months.

TABLE 4.2.1: FOLLOW-UP SCHEDULE

Study Visit	Target Day (± 2 Days to Day 100 Post-HCT) (± 28 Days After Day 100 Post-HCT)
1 week	7 days
2 week	14 days
3 week	21 days
4 week	28 days
5 week	35 days
6 week	42 days
8 week	56 days
100 day	100 days
6 month	180 days
12 month	365 days
13 month	395 days
24 month	730 days

Criteria for Forms Submission: Criteria for timeliness of submission for all study forms are detailed in the Data Management Handbook and User's Guide. Forms that are not entered into AdvantageEDC within the specified time will be considered delinquent. A missing form will continue to be requested either until the form is entered into the AdvantageEDC and integrated into the Data and Coordinating Center's (DCC) master database or until an exception is granted

and entered into the Missing Form Exception File, as detailed in the Data Management Handbook.

Reporting Patient Deaths: Recipient Death Information must be entered into AdvantageEDC within 24 hours of knowledge of the patient's death on the Death Form. If the cause of death is unknown at that time, the cause of death field may be left blank. However, once the cause of death is determined, the form must be updated in AdvantageEDC. In addition, all deaths must be reported via the Unexpected Grade 3-5 Adverse Event Forms in AdvantageEDC for this study.

CIBMTR Data Reporting: Centers participating in BMT CTN trials must register pre- and post-transplant outcomes on all consecutive hematopoietic stem transplants done at their institution during their time of participation to the Center for International Blood and Marrow Transplant Research (CIBMTR). Registration is done using procedures and forms of the Stem Cell Transplant Outcomes Database (SCTOD). (Note: Federal legislation requires submission of these forms for all US allotransplant recipients.) Enrollment of BMT CTN #0903 must be indicated on the SCTOD pre-transplant registration form. Additionally, CIBMTR pre- and post-transplant Report Forms must also be submitted for all patients enrolled on this trial. CIBMTR forms will be submitted directly to the CIBMTR at the times specified on the Form Submission Schedule.

Weekly GVHD Monitoring: GVHD should be monitored in accordance with BMT CTN guidelines as specified in the Manual of Procedures. Patients should be assessed weekly until Day 56 post-transplant for GVHD. After Day 56, patients will be assessed at each follow-up visit (Day 100, and 6, 12, and 24 months) for the presence of GVHD. For scheduling, a target day range has been provided in Table 4.2.1.

4.2.2. Adverse Event Reporting

Unexpected, grade 3-5 adverse events (AE) and all deaths will be reported through an expedited AE reporting system via AdvantageEDC. Unexpected, grade 4-5 AEs must be reported within 24 hours of knowledge of the event. Unexpected, grade 3 AEs must be reported within three business days of knowledge of the event. Expected AEs will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 at regular intervals as defined on the Form Submission Schedule.

Tables 4.2.2.A and 4.2.2.B summarize patient clinical assessments over the course of the study.

4.2.3. Patient Evaluations prior to the HCT conditioning therapy

The following observations need to be performed within 3 months of initiation of conditioning:

1. CMV IgG, hepatitis panel (HAV Ab, HBsAb, HBsAg, HBcAb, HCVAb), HSV 1 and 2 IgG, RPR or VDRL, toxoplasma IgG, VZV IgG, and HTLV1 antibody.
2. EKG
3. Creatinine clearance (calculated creatinine clearance is permitted).

4. CMV PCR or antigenemia assay.
5. D_LCO, FEV1 and FVC.
6. HIV-1 RNA level (HIV viral load by standard assay).
7. CD4 count.
8. If hepatitis B core and/or hepatitis C antibodies are positive, hepatitis B DNA PCR and/or hepatitis C RNA PCR must be checked.
9. Cardiac ejection fraction by MUGA or echocardiography.
10. Signed informed consent form.

The following observations need to be performed within 8 weeks prior to initiation of conditioning:

1. History, physical examination, height and weight, body surface area, neurologic examination, measurement of all palpable peripheral lymph nodes and measurement of other sites of disease present on physical.
   a. Lumbar puncture(s) for determination of presence of CNS disease for patients with either non-Hodgkin's lymphoma or acute lymphocytic leukemia is required.
   b. Duration of HIV/AIDS diagnosis, history of prior opportunistic illnesses.
   c. Presence or absence of "B"-symptoms (unexplained fevers, night sweats, involuntary weight loss greater than 10% normal body weight).
   d. Medication list to include all antiviral, antibiotics and opportunistic infection prophylaxis.
2. β-HCG serum pregnancy test for females of childbearing potential.
3. Blood samples for evaluation of immune reconstitution by flow cytometry (CD2, CD3, CD4, CD8, CD19, CD3+/CD25+, CD45RA/RO, and CD56+/CD3-) and quantitative immunoglobulins (IgM, IgG, and IgA).
4. Blood collected for latent virus recovery assay; will be collected for only those patients that have documented undetectable plasma HIV-1 by standard assay at the time of enrollment (see Appendix C).

The following tests and observations will be performed within 4 weeks prior to initiation of conditioning:

1. For lymphoma patients: CT scans of neck, chest, abdomen and pelvis. Neck CT only required if previous site of disease. PET scan should be performed.
2. Bone marrow aspiration and biopsy, including cytogenetics

The following tests and observations will be performed within 1-3 weeks prior to initiation of conditioning:

1. Blood for HIV single copy measurements (see Appendix C).

2. Optional future research sample collection for consenting patients (see Appendix C).

The following tests and observations will be performed within 1 week prior to initiation of conditioning:

1. Karnofsky/Lansky performance status.
2. CBC with differential, platelet count, creatinine, bilirubin, LDH, alkaline phosphatase.
3. ALT, AST, sodium, magnesium, potassium, chloride, and CO_2.
4. Blood for Microbial Translocation Markers collected within 1 week prior to the initiation of conditioning and on Day -3 of conditioning (see Appendix C).
5. Blood for HIV single copy measurement (see Appendix C).

4.2.4. Patient Post-HCT Evaluations

1. Physical examination and weight weekly until 4 weeks then at 8 weeks, 100 days, 6, 12 and 24 months post-HCT.
2. CBC at least twice a week from Day 0 until ANC > 500/mm^3 for 2 days after nadir reached. Thereafter CBC twice per week until Day 28 (or 4 weeks), then at 8 weeks, Day 100, 6, 12 and 24 months post-HCT.
3. Toxicity assessments at 4 weeks, 8 weeks, Day 100, 6, 12, and 24 months post-HCT.
4. Evaluation of immune reconstitution by flow cytometry (CD2, CD3, CD4, CD8, CD19, CD3+/CD25+, CD45 RA/RO, and CD56+/CD3-) and quantitative immunoglobulins (IgM, IgG and IgA) at 8 weeks, 6, 12, and 24 months post-HCT.
5. CMV PCR or antigenemia assay measured weekly Day 0 through 8 weeks; follow-up at Day 100.
6. HIV-1 RNA level at 100 days, 12 and 24 months (viral load) by standard assay.
7. Blood for HIV single copy measurement at Day 100, and 6, 12, and 24 months post-HCT (see Appendix C).
8. Blood collected for latent virus recovery assay to be scheduled for only those patients where HIV-1 RNA by standard assay and the single copy research assay are negative at 12 months post-transplant to be done at approximately 13 months post-HCT (see Appendix C).
9. Blood collected for Microbial Translocation Monitoring at Days 7, 14 and 100 (see Appendix C).
10. Blood collected for Immune Reconstitution Studies (Research) at 8 weeks and 6 and 12 months (see Appendix C).
11. Acute Graft versus Host Disease assessments weekly Day 0 through 8 weeks; follow-up at Day 100.
12. Chronic Graft versus Host Disease assessments at 100 days, 6, 12, and 24 months.

13. Chimerism (T cell and myeloid) will be assessed at 4 weeks, 100 days and 6 months.
14. Disease restaging:
    a) Lymphoma
        - CT scan at 100 days, 6 and 12 months post-transplant
        - PET or PET CT at 100 days post-transplant, with a 6 month PET or PET-CT performed if the Day 100 scan results are equivocal
        - Bone marrow biopsy/aspirate for patients with previously documented marrow involvement at Day 100 and 12 months post-transplant
    b) Acute Leukemia or Myelodysplastic Syndromes
        - Bone marrow biopsy/aspirate for pathology and cytogenetics at 100 days and 12 months post-transplant.

TABLE 4.2.2A: PRE-HCT EVALUATIONS

Required Studies/Testing	Prior to Conditioning					
	–3 months	**–8 weeks**	**–4 weeks**	**–1-3 weeks**	**–1 week**	**Day –3 of Condition-ing**
CMV IgG, Hepatitis Panel (HAV Ab, HBsAg, HBcAb, HBsAb, HCV Ab), HSV 1/2 IgG, Toxoplasma IgG, VZV IgG, Syphilis (RPR or VDRL), HTLV1 antibody	X					
EKG	X					
DLCO, FEV1, FVC	X					
Creatinine Clearance	X [1]					
CMV PCR or Antigenemia Assay	X					
HIV-1 RNA Viral Load, CD4 Count	X					
HepB DNA PCR and HepC RNA PCR if HepB and/or HepC Ab are positive, respectively	X					
Ejection Fraction (MUGA or ECHO)	X					
History, Physical Examination, Height and Weight, Body Surface Area, Neurologic Examination, careful measurement of all palpable peripheral lymph nodes and measurement of other sites of disease present on physical.		X [2]				
For lymphoma patients: CT scans of neck, chest, abdomen and pelvis. Neck CT only required if previous site of disease. PET scan should be performed			X [2]			
Lumbar puncture(s) for determination of presence of CNS disease for patients with either non-Hodgkin's lymphoma or acute lymphocytic leukemia		X [2]				
Bone Marrow Aspiration and Biopsy, including cytogenetics			X			
β –HCG Serum Pregnancy Test for Females of Childbearing Potential		X				
Informed Consent	X					
Immune Reconstitution Assays (Transplant Center)		X [3]				
Karnofsky/Lansky Performance Score					X	
CBC with Differential, Platelet Count, Creatinine, Bilirubin, Alkaline Phosphatase, AST, ALT, LDH, Sodium, Magnesium, Potassium, Chloride and CO_2					X	
Blood for Microbial Translocation Monitoring (See Appendix C)					X	X
Blood for HIV Single Copy PCR Assessment (See Appendix C)				X	X	

Required Studies/Testing	Prior to Conditioning					
	–3 months	**–8 weeks**	**–4 weeks**	**–1-3 weeks**	**–1 week**	**Day –3 of Condition-ing**
Blood for Latent HIV Reservoir Measurements (See Appendix C)		X[4]				
Optional Future Research Blood Sample				X		

[1] Calculated creatinine clearance is permitted.

[2] To include:

a. Lumbar puncture(s) for determination of presence of CNS disease for non-Hodgkin's lymphoma and Acute Lymphoblastic Leukemia patients only.

b. Duration of AIDS diagnosis, history of prior opportunistic illnesses.

c. Presence or absence of "B"-symptoms (unexplained fevers, night sweats, involuntary weight loss greater than 10% normal body weight) for lymphoma patients.

d. Medication list to include all antiviral, antibiotics and opportunistic prophylaxis.

[3] To include: CD2, CD3, CD4, CD8, CD19, CD3+/CD25+, CD45RA/RO, and CD56+/CD3- and quantitative immunoglobulins (IgM, IgG, and IgA).

[4] Collection of blood for Latent HIV Reservoir testing. Once the patient is consented and enrolled on the trial, a peripheral blood sample will be collected at approximately 8 weeks prior to initiation of conditioning for only those patients that have documented undetectable plasma HIV-1 by standard assay upon enrollment (see Appendix C).

TABLE 4.2.2B: POST-HCT EVALUATIONS

Study Assessments/ Testing	Weeks Post-HCT								Months Post-HCT			
	1	2	3	4	5	6	8	Day 100	6	12	13	24
CBC[1], Physical Exam, and Weight[1]	X	X	X	X			X	X	X	X		X
Immune Reconstitution Assays[2] (Transplant Center)							X		X	X		X
HIV-1 RNA Viral Load[3]								X		X		X
PET or PET-CT (lymphoma patients)								X[4]	X			
CT Scan (lymphoma patients)								X	X	X		
Bone marrow biopsy/aspirate for pathology (acute leukemia, MDS patients and lymphoma patients with previously documented marrow involvement; include cytogenetics for acute leukemia and MDS patients)								X		X		
Toxicity Assessment				X			X	X	X	X		X
Chimerism (T cell and myeloid)				X				X	X			
CMV PCR or antigenemia	X	X	X	X	X	X	X	X				
Acute GVHD Assessment	X	X	X	X	X	X	X	X				
Chronic GVHD Assessment								X	X	X		X
Blood for Microbial Translocation Monitoring (See Appendix C)	X	X						X				
Blood for HIV Single Copy PCR Assessment (See Appendix C)								X	X	X		X
Blood for Latent HIV Reservoir Measurements[6] (See Appendix C)											X	
Blood for Immune Reconstitution Assays (Research)							X		X	X		

Notes for Table 4.2.2B:

[1] To be performed at least twice weekly from Day 0 until ANC > 500/mm^3 for 2 days after nadir reached. Thereafter, twice weekly until Day 28 (or 4 weeks), then at 8 weeks, Day 100, 6, 12 and 24 months post-HCT.

[2] Immune reconstitution assays by flow cytometry (CD2, CD3, CD4, CD8, CD19, CD3+/CD25+, CD45 RA/RO, and CD56+/CD3) and quantitative immunoglobulins (IgM, IgG and IgA).

[3] HIV-1 viral RNA plasma level (viral load) at 100 days, 12 and 24 months by standard assay.

[4] PET or PET-CT performed if the Day 100 Scan results are equivocal at 6 months.

[5] For lymphoma patients, CT scans of neck, chest, abdomen and pelvis. Neck CT only required if previous site of disease.

[6] Collection of blood sample to be scheduled for only those patients where HIV-1 RNA by standard assay is undetectable at 12 months post-transplant (see Appendix C).

CHAPTER 5

5. STATISTICAL CONSIDERATIONS

5.1. Study Overview

This study is designed as a Phase II multi-center trial to assess the feasibility and safety of allogeneic hematopoietic cell transplantation (HCT) in HIV-infected patients. The target enrollment is 15 patients.

5.1.1. Accrual

Accrual will be across multiple sites and remain open until 15 patients are transplanted. It is estimated that two years of accrual will be necessary to enroll the targeted sample size.

5.1.2. Primary Endpoint

The primary endpoint for the study is 100-day Non-Relapse Mortality (NRM).

5.2. Sample Size Calculations and Stopping Guidelines

The sample size is 15 patients for this trial. Allogeneic transplant for patients with HIV is considered unacceptable if 100-day non-relapse mortality probability is 45% or higher. The objective of the study is to show that 100-day NRM is lower than 45%. In patients without HIV, non relapse mortality probability 100 days after allogeneic transplant is expected to be lower than 15%. Therefore, we framed this objective as a hypothesis test of the null hypothesis H0:p>=0.45, where p is the 100 day NRM probability, and this study is adequately powered against the alternative hypothesis H1:p=0.15. The stopping rule and study design described in detail below has a 8% chance of concluding that allogeneic transplant for patients with HIV has less than 45% NRM when in fact the 100 day NRM rate is 45% (type I error), and an 83% chance of concluding that the NRM rate is lower than 45% when the 100 day NRM rate is 15% (power).

To guard against excessive mortality, non-relapse mortality will be monitored up to 100 days post transplant and if rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures. The stopping guidelines serve as a trigger for consultation with DSMB for additional review, and are not formal "stopping rules" that would mandate automatic closure of study enrollment.

A truncated Sequential Probability Ratio Test (SPRT) based on a binomial test of proportions for non-relapse mortality will be used as described below. This sequential testing procedure conserves type I error across all of the monitoring looks for NRM. The SPRT can be represented graphically. At each interim analysis, the number of patients enrolled is plotted against the total

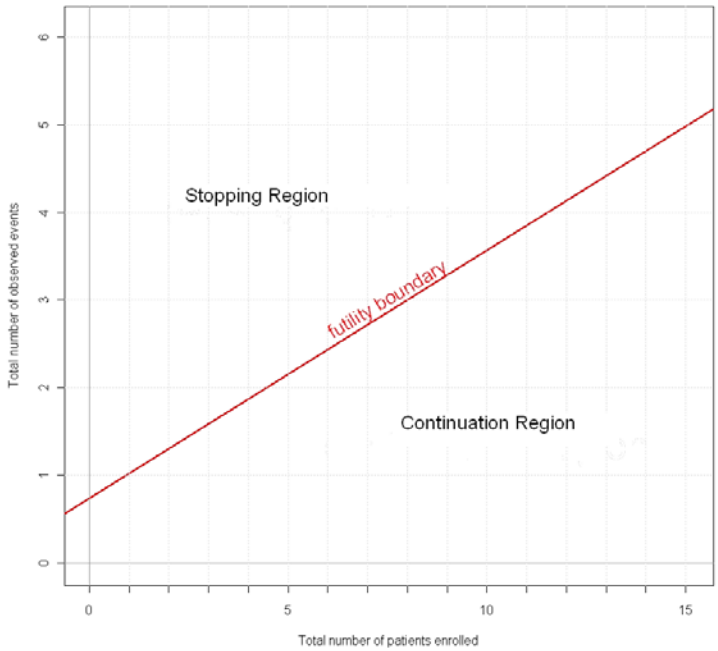
number of patients who have experienced NRM. The continuation region of the SPRT is defined by two parallel lines. For a hypothesis test H_0: $\theta = \theta_0$ versus H_1: $\theta = \theta_1$ where $\theta_0 > \theta_1$, if the graph falls above the upper boundary, accept H_0 and if the graph falls below the lower boundary reject H_0 and conclude H_1. Only the upper boundary will be used to protect against excessive NRM which would make it unlikely that we could conclude that the NRM is less than 45% by the end of the study.

The usual measures of performance of an SPRT are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$ and of accepting H_0 when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta_i)$. Note that since the test uses only the upper boundary, and is truncated by a finite sample size, the size of the test will be slightly lower than the nominal level. The test to be used in this protocol was developed from the following SPRT:

- An SPRT testing H_0: p = 45% versus H_1: p = 15% NRM, with nominal type I and II errors of 7% and 30%, respectively.
- The slope of the parallel lines for monitoring NRM is 0.283 and the intercepts are –1.53 and 0.74.
- ***Note that the stopping rule shown in Table 5.1 was constructed using the futility boundary of this SPRT test. The futility boundary was used to ensure that the DSMB will be notified if it is no longer likely that we will be able to demonstrate that 100-day NRM is lower than 45%, thus protecting against excessive NRM.***

The futility stopping boundary is shown in Figure 5.1 for the SPRT test.

FIGURE 5.1 SPRT STOPPING BOUNDARY FOR 100-DAY NRM



The futility stopping rules are summarized in Table 5.1 for the SPRT test.

TABLE 5.1 STOPPING GUIDELINES FOR 100-DAY NRM AMONG PATIENTS ENROLLED

Number of patients enrolled (n)	SPRT stopping boundary (x)
2-4	2
5-7	3
8-11	4
12-15	5

* Stopping guideline is triggered if x patients out of n patients enrolled experience NRM.

The actual operating characteristics of the truncated test (shown in Table 5.2) were determined in a simulation study. The simulation assumed uniform accrual of 15 patients over a two-year time period.

TABLE 5.2 OPERATING CHARACTERISTICS OF SEQUENTIAL TESTING PROCEDURE FOR 100-DAY NRM FROM A SIMULATION STUDY WITH 10,000 REPLICATIONS

Non-relapse Mortality				
True 100-day rate	45%	35%	25%	15%
Probability stop and accept the null hypothesis	.92	.75	.47	.17
Probability continue to full recruitment and reject the null hypothesis	0.08	0.25	0.53	0.83
Mean month stopped	11.8	15.6	20.2	24.5
Mean # endpoints	2.7	2.9	2.8	2.0
Mean patients with 100 days follow up	6.0	8.2	10.8	13.4

The SPRT stops and accepts the null hypothesis 92% of the time when the true 100-day NRM is 45% and 17% of the time when the true 100-day NRM is 15%. This corresponds to a type I error of 8% and type II error of 17%. When the true 100-day NRM rate is 45%, on average the DSMB will be consulted 11.8 months after opening, when 2.7 events have been observed in 6 patients.

5.3. Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, race/ethnicity, performance status, disease stage, genotype, donor type, donor gender, donor-recipient CMV, HLA matching, graft type, HIV load, CD4 counts, and number of prior chemotherapy regimens as treatment of primary malignancy and number of prior HIV regimens.

5.4. Analysis Plan

5.4.1. Analysis of the Primary Endpoint

The primary analysis will consist of estimating the 100-day NRM probability along with a 95% confidence interval using the cumulative incidence function.

In a secondary analysis, a Cochran-Mantel-Haenszel test will be used to compare 100-day NRM probabilities between patients enrolled in this study to matched non-HIV-infected patients from the CIBMTR database. Closely matched controls will be identified at ratio 1:4 using the following criteria: age, gender, year of transplant, performance status, interval from diagnosis to transplant, diagnosis, disease status, preparative regimen/intensity, GVHD prophylaxis regimen, cytogenetics if available for acute leukemias.

5.4.2. Analysis of Secondary Endpoints

Disease Status
The proportion of patients in complete remission, partial remission, stable disease, and relapse at 100 days post-transplant will be described.

Time to Hematopoietic Recovery
Time to neutrophil recovery and platelet engraftment will be described separately in the myeloablative and reduced intensity groups, using cumulative incidence function with death prior to engraftment as the competing risk.

Chimerism
Donor T-cell and myeloid chimerism at4 weeks, 100 days, and 6 months will be described separately in the myeloablative and reduced intensity groups, according to proportions with mixed (5-95% donor cells), full (>95%), or graft rejection (<5%).

Hematologic Function
The proportions of patients with hematologic function at 100 days and 6 months will be described in patients surviving to these time points.

Occurrence of Infections
Microbiologically documented infections will be reported by site of disease, date of onset, severity, and resolution, if any. This data will be captured via an event-driven case report form and will be collected from Day 0 until two years post-transplant or relapse. The incidence of viral, fungal and bacterial infections will be tabulated for each patient according to Chapter 5 of the BMT CTN Technical Manual of Procedures.

Overall Survival
The 6-month overall survival probability with 95% confidence interval will be estimated using the Kaplan-Meier product limit estimator. In an additional analysis, a Cochran-Mantel-Haenszel test will be used to compare 6-month overall survival probabilities between patients in the study to matched controls selected for the secondary analysis of 100-day NRM of Section 5.5.1.

Acute Graft versus Host Disease:
Cumulative incidence of grade II-IV and III-IV acute GVHD will be estimated at 100 days, treating death as a competing risk.

Chronic Graft versus Host Disease:
Cumulative incidence of chronic GVHD will be estimated at 100 days, 6 and 24 months, treating death as a competing risk.

Immunologic Reconstitution
Immune reconstitution assays on peripheral blood which include CD2, CD3, CD4, CD8, CD19, CD3+/CD25+, CD45 RA/RO, CD56+/CD3-, and quantitative immunoglobulins (IgM, IgG and IgA) will be measured in all patients prior to the conditioning, at 8 weeks, and 6 and 12 months post-transplant. These will be summarized at each time point using descriptive statistics.

Impact of Therapy on HIV-1 Reservoir
HIV RNA levels will be measured prior to initiation of ablative chemotherapy, and at Day 100, 12 months, 13 months (for some patients) and 24 months post-transplant. Standard assay will measure HIV RNA levels (viral load) and for patients with no detectable viral RNA using the standard assay, a single copy assay will be performed to assess persistent viremia. The proportion of patients with undetectable viral loads at Day 100, and 12 and 24 months will be examined and an exact binomial confidence interval will be calculated. Additionally, change in viral loads status between consecutive time points will be described by the proportion of patients whose viral loads changed from detectable to undetectable between assessment times.

APPENDIX A

HUMAN SUBJECTS

APPENDIX A

HUMAN SUBJECTS

1. Subject Consent

A conference will be held with the patient and family to discuss this study and alternative treatments available for the treatment of the underlying disease. The Principal Investigator or another designated physician will conduct the conference. Potential risks associated HCT should be discussed as objectively as possible.

The consent document should be reviewed with the patient and family prior to proceeding to ablative therapy.

Informed consent from the patient will be obtained using a form approved by the Institutional Review Board of the institution enrolling the patient.

2. Confidentiality

Confidentiality will be maintained by masking of individual names and assignment of a patient identifier code. The identifier code representing the patient's identity will be kept separately from the research file at the center. The ID code will be transmitted to the BMT CTN Data and Coordinating Center upon enrollment.

3. Participation of Women and Minorities and Other Populations

Women, ethnic minorities, and other populations will be included in this study. Accrual of women and minorities at each center will be monitored to determine whether their rates of enrollment are reflective of the distribution of potentially eligible women and minorities expected from data reported to the CIBMTR and from published data on the incidence of AML, ALL, MDS, and Hodgkin and Non-Hodgkin Lymphoma. Centers will be notified if their rates differ significantly from those expected and asked to develop appropriate recruitment strategies.

APPENDIX B

INFORMED CONSENT AND ASSENT

Informed Consent to Participate in Research

Your Name: _____

Study Title: Allogeneic Hematopoietic Cell Transplant for Hematological Cancers and Myelodysplastic Syndromes in HIV-Infected Individuals

Protocol: BMT CTN #0903 (AMC 080)

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Transplant Center Investigator: _____
(Insert contact information for PI at your site)

Sponsor: The National Institutes of Health (NIH) is sponsoring this study by providing financial support through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN).

1. Introduction

We invite you to join this clinical trial, also known as a research study. We are doing this study because we want to find out how well allogeneic marrow transplant (a marrow transplant using cells from someone other than the patient) treats specific blood cancers in people with HIV (human immunodeficiency virus).

This study will take at least two (2) years and will include 15 participants.

This Consent Form will tell you about the purpose of the study, its possible risks and benefits, other options available to you, and your rights as a participant in the study.

Everyone who takes part in research at [insert facility name] should know that:

- Being in any research study is voluntary.
- You may or may not benefit from being in the study. Knowledge we gain from this study may benefit others.
- If you join the study, you can quit the study at any time.
- If you decide to quit the study, it will not affect your care at [insert name of facility or institution].
- Please ask the study staff questions about anything that you do not understand, or if you would like to have more information.
- You can ask questions now or any time during the study.
- Please take the time you need to talk about the study with your doctor, study staff, and your family and friends. It is your decision to be in the study. If you decide to join, please sign and date the end of the Consent Form.

You and your doctor will discuss other treatment choices if you do not want to participate in this study.

2. Background

The National Institutes of Health (NIH), through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), are providing staff support and money for this research study.

This study will look at the risks and benefits of allogeneic transplant for people with specific kinds of blood cancers and HIV infection.

Blood Cancer and Transplant

For people with a blood cancer, the main treatment choices include chemotherapy, radiation therapy, immunotherapy, and a transplant using cells that can make blood. The blood-making cells are most often collected from bone marrow and the bloodstream. These cells are called **hematopoietic cells** and transplant using these kinds of cells is called **hematopoietic cell transplant**, or **HCT**. These treatments may be used alone or used in combination. For some people with a blood cancer, a HCT transplant may offer the best chance to be free of signs of disease for a long time (long-term remission).

This clinical trial will study how well allogeneic transplant works as a treatment for people who have a blood cancer and HIV. An allogeneic transplant uses blood-making cells from a family member or an unrelated donor to remove and replace the abnormal blood cells in the patient.

An allogeneic transplant is a common treatment for patients with a blood cancer that has either returned (relapsed) or does not respond well to other treatments (refractory), or is known to be likely to return (high risk). The blood cancers we will include in this study are: acute myeloid or lymphocytic leukemia (AML or ALL), myelodysplastic syndrome (MDS), Hodgkin lymphoma and non-Hodgkin lymphoma.

Conditioning Regimen

We will use a combination of chemotherapy and in some cases radiation as a treatment to destroy cancer cells and help donor cells start to grow in your bone marrow. Depending on the combination used, each treatment (or "conditioning regimen") can have a different intensity or strength.

- High intensity treatment uses a combination of chemotherapy that uses strong or higher amounts of drugs and sometimes radiation. This is also called "myeloablative conditioning".
- Reduced intensity treatment uses a combination of chemotherapy, using less strong or lower amounts of drugs and sometimes radiation. This is also called "reduced intensity conditioning".

Both kinds of treatments are used by blood and marrow transplant doctors around the world and are not experimental. You and your doctor will decide which conditioning regimen intensity is the best choice for you. This study will help us to better understand how to use these treatments in people with HIV who have an allogeneic transplant to treat their blood cancer.

If you volunteer to join this study, the treating physician at the center will assign you to have either a high intensity or a reduced intensity treatment before you receive the blood stem cells or bone marrow from your donor.

3. Purpose

The main purpose of this study is to learn how well allogeneic transplant treats blood cancers in people with HIV. We invite you to participate in this study because you have HIV and a blood cancer.

4. Right to Ask Questions

You have the right to ask questions about **t**he study at any time. If you have questions about your rights as a participant or you want to leave the study, please contact:

[insert contact info]

5. Procedures

Before you join the study, we will evaluate your general health, medical history, and your current medications.

Study Participation

Your study participation will last for **2 years** after your transplant.

Follow-up tests for your transplant (described below) will last as long as you need care.

We would like to keep track of your medical condition for the rest of your life. We would contact you and the doctor who gives your regular medical care by phone or mail once a year. Following up with you every year helps us learn about the long-term effects of the study and transplant. Many transplant centers include this type of long-term follow-up as part of their regular medical care.

Before You Start Your Treatment

You will need to have health evaluations before you start treatment, while you receive your treatment, and for several years after you finish your treatment. These tests are standard care for patients with blood cancer and would happen even if you were not part of this study. It will be up to your study doctor to decide what tests you need and when.

If you decide to join, we will ask you to sign this Consent Form, and you will get a copy of the signed form to keep.

You will have several tests to find out if you can be in the study. The tests include:

- Medical history
- Physical examination, including height and weight
- Blood and urine tests
- Heart function tests, including a MUGA or an electrocardiogram (ECG)

- Lung (pulmonary) function tests
- Tests to evaluate your blood cancer including a bone marrow biopsy/aspirate
- If you have lymphoma, you will also have PET (positron-emission tomography) and/or CT (computed tomography) scans
- A blood pregnancy test if you are a woman able to have children. If you are pregnant, you will not be able to take part in this study.

During The Study

- **Study Evaluations**
  We will evaluate your health at specific points during your participation. These tests and how often they are scheduled are standard care for patients receiving an allogeneic transplant and would be done even if you were not part of this study.
- **Research Study Evaluations**
  Researchers are trying to learn more about HIV and the effect of HCT on HIV. Much of this research is done using blood samples. As a result, blood samples will be collected from you during the study to help researchers learn more about the effect of HCT on your HIV.
  - In patients that do not have any detectable HIV using standard tests, 30 mL (about 6 teaspoons) of blood will be drawn at two times before your HCT and at 100 days, 6 months, 12 months and 24 months after your HCT. Another 180 mL (about 36 teaspoons) of blood will also be drawn before your HCT and 13 months after your HCT if necessary. This blood will be used to look closer at any HIV that may still be present in your body.
  - In all patients, 10 mL (about 2 teaspoons) of blood will be drawn at two times before your HCT and at 1 week, 2 weeks and 100 days after your HCT. Another 37 mL (about 7 teaspoons) will be drawn at 8 weeks, 6 months and 12 months after your HCT. This blood will be used to look at how your immune system is responding to the treatment.
- **Antiretroviral Therapy**
  Your antiretroviral therapy (your HIV therapy) will be reviewed. An outside review committee will look at your therapy and discuss with your doctor the best way to adjust your therapy throughout your HCT. Your doctor may or may not adjust your therapy depending on what is best for you.
- **Conditioning Regimen (Chemotherapy)**
  The conditioning regimen is a combination of chemotherapy and/or radiation given to patients before the donor cells are infused. This treatment allows donor cells to start growing (or "engraft") in your bone marrow. The conditioning regimen also helps to destroy remaining cancer cells that might not be found in tests.

Several chemotherapy drugs can be used as part of the conditioning treatment. The choice and amount of drugs or radiation determines the treatment intensity (strength). Common drug combinations used in allogeneic transplant include:

1) Busulfan and fludarabine
2) Fludarabine and melphalan
3) Radiation and cyclophosphamide (**Cytoxan)**

Allogeneic transplantation kills cancer through the conditioning regimen and through the immune cells from the donor that might recognize cancer cells and destroy them. Conditioning regimens with high intensity are also known as myeloablative conditioning regimens. High intensity treatments work very well to destroy remaining cancer cells because they use very high amounts chemotherapy or radiation. High intensity treatments can also have more side effects during and after transplant.

Using a lower or "reduced" intensity treatment before transplant can have fewer serious problems from the chemotherapy drugs. While the cancer killing effects may also be lower, studies show that immune cells given during the transplant can help destroy remaining cancer cells. Allogeneic transplants with reduced intensity conditioning (RIC) regimens are often used for people who cannot have high doses of chemotherapy drugs or radiation because of their age or other medical problems.

Your doctor will decide which type of conditioning treatment is the best choice for you, depending on the kind of blood cancer you have and your overall health. The table below lists the 4 conditioning regimens that will be used in this study.

TABLE B-1: CONDITIONING REGIMENS

Reduced Intensity Treatments	
A	Fludarabine + Busulfan (Flu/Bu)
B	Fludarabine + Melphalan (Flu/Mel)

High Intensity Treatments	
C	Busulfan + Fludarabine (Bu/Flu)
D	Cyclophosphamide + Total Body Irradiation (Cy/TBI)

- **Reinfusion of Stem Cells (Transplant)**
  We will use your intravenous catheter or central line to give you the blood or bone marrow stem cells that were collected before your transplant. The cells will travel to your bone marrow where they will start to make healthy, new blood cells after several weeks.

6. Alternative Treatments

Participation in this study is optional. If you choose not to participate you may still receive an allogeneic transplant for treatment of your disease. It is possible that the treatment and the evaluations you would receive could very similar to what would be if you were enrolled in this clinical trial.

Your study doctor will talk with you about your options. If you decide not to participate in this research study, your medical care will not be affected in any way.

Your other choices may include:

- Treatment with other drugs, radiation, or a combination of drugs and radiation without a transplant.
- An allogeneic hematopoietic cell transplant that is not part of the study, or another type of transplant.
- Participation in another clinical trial (if available, check with your doctor).
- No treatment for your blood cancer at this time.

Every treatment option has benefits and risks. Talk with your doctor about your treatment choices before you decide if you will take part in this study.

7. Risks and Discomforts

You will have side effects while on the study. Side effects can range from mild to very serious.

The risks and discomforts in participating in this study will be similar to what you may have with a blood or bone marrow cell transplant if you do not participate in this trial. There are differences in side effects from medications based on the strength of your conditioning regimen. Myeloablative regimens often cause more side effects early after transplant compared to reduced intensity regimens. Other complications from transplants, such as graft-versus-host disease (GVHD) and infections happen equally in patients who have either type of regimen.

Your health care team will give you medicines to help lower side effects such as feeling sick to your stomach (nausea). In some cases, side effects can be long lasting or may never go away.

A. <u>Side Effects of Allogeneic Transplant</u>

Short term (days to weeks): During the transplant, you will have a higher risk of infection and bleeding from low blood counts. There is also the risk that your blood counts will not recover even though we gave you donated blood stem cells. This risk is rare, but it can happen. After your transplant, you have a higher risk of infections. This is because the new immune system needs time to learn again how to best fight some infections and because you will need to take medicines that lower immunity to reduce the risk of graft-versus-host disease (GVHD). See below for more information about GVHD.

Long term (years): If you have an allogeneic transplant, you will have a higher risk of developing cancers that are caused by the chemotherapy given as part of the transplant or to treat the blood cancer before your transplant. These secondary cancers can be of any type, including blood cancers such as leukemia. Your HIV infection also gives you a higher risk for some cancers, so the true risk for allogeneic transplant for HIV-associated cancers is not yet known.

B. Risks Related to Medications or Radiation Used in Conditioning Regimens

All chemotherapy and radiation treatments used as conditioning regimens in this study are commonly used in allogeneic hematopoietic cell transplantation. The side effects may vary, based on the dose that is given. This applies to busulfan, which is used in different amounts for myeloablative and reduced intensity regimens.

TABLE B-2 – ADVERSE EVENTS

	Likely Side Effects (May happen in 20% of patients or more)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
Busulfan (Bu)	Abdominal discomfort Constipation Diarrhea Dizziness Fluid retention Headache Heartburn Insomnia Lack of appetite Mouth sores Nausea and vomiting Running nose Skin rashes Irregular or no menstrual cycles Tachycardia	Cough Hepatic Veno-occlusive disease High blood pressure High magnesium and phosphorus levels in the blood High sugar levels in the blood Infertility Low blood pressure Seizures	Cataracts Lung fibrosis

	Likely Side Effects (May happen in 20% of patients or more)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
Cyclophosphamide (Cy)	Damage to male (testes) and female (ovaries) sex glands Diarrhea Fluid retention Hair loss Infertility Irregular or no menstrual cycles Loss of appetite Nausea, Vomiting Suppression of the immune system	Bleeding in the bladder Inflammation of the heart muscle (heart failure) Shortness of breath	Allergic reaction Lung fibrosis Serious skin rashes
Fludarabine (Flu)	Diarrhea Mouth sores Nausea and vomiting Suppression of the immune system	Fever Numbness in the extremities Sleepiness Visual changes Weakness	Coma Cough Inflammation of the lung Interstitial Pneumonia Skin rash
Melphalan (Mel)	Constipation Diarrhea Hair loss Mucositis Nausea and vomiting	Heart rhythm abnormalities Hepatitis Kidney failure	Allergic reaction Interstitial Pneumonia Seizure Lung fibrosis
Total Body Irradiation (TBI)	Fatigue Hair loss Infertility Loss of appetite Mouth sores Nausea and vomiting	Cataracts Inflammation of the parotid glands Skin pigmentation (reversible) Stunned Growth	Lung fibrosis Second cancers

C. Risks Related to the Medication Used to Help Prevent Graft-versus-Host Disease (GVHD)

Graft-versus-Host Disease (GVHD) is a medical condition that can become very serious and may cause death. GVHD is a common development after allogeneic stem cell transplant. It happens when the donor cells attack and damage your organ tissues after transplant. GVHD can cause:

- **Skin problems** including rashes, sores or blisters

- **Feeling sick** to your stomach (nausea),
- **Throwing up** (vomiting),
- **Abdominal pain**
- **Diarrhea**
- **Liver damage** or jaundice (yellowing of the skin or eyes)
- **There are other side effects that may be seen**
- **GVHD may be bad enough to cause death**

The most common forms of GVHD are relatively mild. Other forms of GVHD can be chronic and last well past your transplant date. GVHD can be very hard to predict. You will be monitored closely for this condition.

We will give you medications to try and prevent GVHD. These medications are usually started around the time you receive the donor cells and can continue many months after the transplant. These medications do not completely prevent GVHD and more medications might be needed if you do develop GVHD.

Your doctor will decide which GVHD prevention treatment you will receive. This choice is not part of this research study and your doctor will decide on the medications based on what is routinely used in this institution as part of allogeneic transplants. Below is a list of commonly used medications to prevent GVHD. Your doctor may choose to use other medications than what is listed here.

- **Tacrolimus:** This medication is used to try to prevent GVHD. The immediate side effects you may experience include nausea (feeling sick to your stomach) or vomiting (throwing up) when the medications are given orally. Other side effects you may experience include high blood pressure (hypertension), shaking of the hands (tremor), increased hair growth and possibly an effect on mental function.

  If you have these effects, they generally go away if your doctor lowers the amount of medication you take. A few patients have had a seizure while on this medication.

  Your liver or kidneys might not work as well as they did before. If this happens, your doctor may lower the amount of drug you take or stop giving the drug completely. The effect on kidneys seems to increase when other medications, which might cause kidney problems, are given at the same time, especially antibiotics. Occasionally, the kidney damage caused may require the use of an artificial kidney machine (hemodialysis).

  Some patients given tacrolimus develop diabetes and must take insulin while taking tacrolimus.

Rarely, patients receiving tacrolimus may develop low platelets and kidney problems that require stopping the tacrolimus, which can increase the risk of GVHD. This condition, called TTP/HUS Syndrome, can also be life-threatening.

It is very important that you do not eat grapefruit or drink grapefruit juice. Grapefruit has an ingredient called bergamottin, which can affect some of the treatment drugs, including tacrolimus, used in this study. Common soft drinks that have bergamottin are Fresca, Squirt and Sunny Delight.

- **Sirolimus:** This medication is used to try to prevent GVHD. The immediate side effects you may experience include fast heart rate, pain during breathing, shortness of breath, chest pain, nausea or vomiting. Other side effects you may experience include pale skin, easy bruising, fever, chills, body aches, night sweats, swelling in your face, stomach, hands or feet, pain or burning when you urinate, headache or skin rash. If you experience these effects, they generally go away when the dose of the medication is decreased.

  Sirolimus may increase your risk of developing lymphoma or other forms of cancer. Talk with your doctor about your specific cancer risk.

  Rarely, patients receiving sirolimus may develop low platelets and kidney problems that require stopping the sirolimus, which can increase the risk of GVHD. This condition, called TTP/HUS Syndrome, can also be life-threatening.

  It is very important that you do not eat grapefruit or drink grapefruit juice. Grapefruit has an ingredient called bergamottin, which can affect some of the treatment drugs, including tacrolimus, used in this study. Common soft drinks that have bergamottin are Fresca, Squirt and Sunny Delight.

- **Mycophenolate Mofetil:** MMF is a potent immunosuppressive drug that blocks the growth of the immune cells that can cause GVHD. Side-effects you might experience include nausea and vomiting, diarrhea, infection, low blood counts, serious injury to your gut (digestive tract) including bloody stools and vomit, secondary cancers, such as lymphoproliferative disease or lymphoma, serious infections of the brain, risk to an unborn child, or Progressive Multifocal Leukoencephalopathy (PML).

- **Cyclophosphamide:** This is a chemotherapy drug that works by slowing or stopping cell growth. Side-effects you might experience include nausea and vomiting, bone marrow suppression, stomach ache, diarrhea, darkening of the skin or nails, hair loss (alopecia) or thinning of hair, changes in color and texture of the hair, and feeling tired. Blood in your urine (hemorrhagic cystitis) is a common complication. Although it is used to treat cancer, it may increase your risk of developing another kind of cancer, sometimes months to years after treatment.

- **Methotrexate:** This is also a medication used to try to prevent GVHD. Methotrexate causes damage to cells, and therefore can affect many different tissues of your body. It may cause or can worsen the mouth sores or inflammation of the mouth which you may have already developed from the procedures and medications used to prepare you for the transplant. Methotrexate may slow down the recovery of blood cells after transplantation. Methotrexate can cause kidney damage. If your kidneys are already damaged for other reasons, it can worsen kidney function. If kidney damage does occur, the methotrexate dose may be reduced or the medication may not be given at all.

- **Tacrolimus and Methotrexate:** These medications interfere with the body's defense system (the immune system). This may cause you to have more infections (especially viral infections and pneumonia) for several months after transplant.

D. Risks Related to the Transplant Procedure

The following risks are part of the transplant process and not connected to any one medication or the transplanted donor cells.

- **Bleeding:** Platelets help your blood to clot. Your platelets will be low until the new bone marrow grows and, as a result, bleeding may occur. This can be minor bleeding, such as nosebleeds or bruising, but more serious, life-threatening bleeding in the lungs and brain can occur if the platelet count remains low. Usually, there is success in preventing major bleeding problems with transfusions of platelets. However, some patients may not respond well to transfused platelets and may be at serious risk for bleeding.

- **Veno-Occlusive Disease (VOD):** This can occur as a result of high dose chemotherapy, radiation therapy and/or medications used to prevent GVHD. VOD causes severe damage to the liver. Symptoms include jaundice (yellowing of the skin and eyes), weight gain, and extra fluid build-up in the abdominal cavity and other parts of the body. It can often be managed successfully, and completely resolve. However, complications can arise that may be fatal.

- **Mouth Sores and Diarrhea:** The large doses of chemotherapy and radiation cause irritation in the lining of the mouth and intestines. This can result in painful mouth sores and diarrhea. If you have severe mouth sores you will be given medicine to help control the pain. If your mouth sores are severe you may not be able to eat normally until the sores are healed. Mouth sores get better when the white blood count starts to rise, and engraftment occurs.

- **Capillary Leak Syndrome:** This may occur as a result of chemotherapy and radiation therapy. The blood vessels may become 'leaky' and fluid enters the abdominal cavity, lungs, and other tissues. You may gain water weight and not go to the bathroom as often as you normally do. Capillary leak syndrome can be difficult to manage if extra fluid enters the lungs and causes difficulty breathing, and can be life-threatening. You may die if there is continued fluid collection in the lungs.

- **Unexpected Organ Damage and Other Side Effects:** It is possible you may experience unexpected, life-threatening heart, lung, kidney, or liver damage as a result of the transplant. Occasionally, the high doses of chemotherapy and radiation cause severe lung damage that cannot always be treated. If this happens, you may need to use oxygen or even a respirator. The lung damage may get worse and be life-threatening. Rarely, multi-organ failure (such as lung and kidney failure) may occur, which is often fatal.
- **Late Effects:** You may experience side effects that occur several months to many years after your transplant. You may experience poor function of the thyroid gland, requiring you to take thyroid medication. As a result of radiation, cataracts may occur earlier in life compared to a person who had not had a transplant. If you develop cataracts (cloudiness in the eyes) they may require treatment. It is rare, but your kidneys could be affected, causing anemia (low red blood cell count) or high blood pressure. There is also a risk you may develop a second cancer as a result of the chemotherapy, radiation and/or underlying disease. If secondary cancers occur they generally do not occur until 10 to 15 years after the transplant. The long-term effects upon heart, lung, and brain are unknown.
- **Fluid Build-up:** You will receive intravenous fluids during the transplant process and your body may have trouble eliminating this fluid.

E Additional Side Effects for People with HIV

Most of the risks in this Consent Form can happen to all patients undergoing transplant, but some, such as risk of infection or organ damage, may be different in patients with HIV. There is a risk that a temporary stop to antiretroviral treatment could lead to HIV resistance to the medications you are taking.

Your HIV viral load will be monitored by your doctor and if HIV resistance were to develop, alternate antiretroviral therapy may be needed.

Many of the medications used for HIV will react with important medications to reduce the complications of the transplant (HCT). These reactions may require frequent blood checks to make sure the amount of medications in the blood are correct. It is possible those medications interactions will result in complications.

F Infections

You will need to take several antibiotics to prevent infection. You will also be watched carefully for any infections while you are being treated for HIV related Leukemia or Lymphoma. Tell your doctors right away if you get a fever, chills, cough or any other symptoms that might be a sign of an infection. Infections may be bad enough to cause death.

G Reproductive Risks

Risk to the unborn: The treatments in this study have NOT been proven to be safe at any stage of pregnancy. Therefore, if you are pregnant or nursing, you are not eligible for

this study. Women who have the potential of becoming pregnant must use some form of effective birth control while receiving chemotherapy and GVHD prophylaxis. Effective birth control is defined as the following:

1) Refraining from all acts of vaginal intercourse (ABSTINENCE)
2) Consistent use of birth control pills
3) Injected birth control methods (Depro-Provera, Norplant)
4) Tubal sterilization or male partner who has undergone a vasectomy
5) Placement of an IUD (intrauterine device)
6) Use, with every act of intercourse, of a diaphragm with contraceptive jelly and/or condoms with contraceptive foam.

Sterility and future childbearing potential for men and women: Chemotherapy and/or irradiation may affect your ability to have children. Male patients may become sterile (unable to produce sperm) and should discuss with their doctor regarding sperm banking prior to transplantation. Female patients who have attained puberty may find that their menstrual cycle becomes irregular or stops permanently. However, this DOES NOT MEAN THAT YOU CANNOT BECOME PREGNANT, and you must use some effective method of birth control during transplant and afterwards until you are off GVHD prophylaxis. Damage to reproductive tissue may result in infertility (inability to have children). It is not known if the damage could result in birth defects.
You should discuss risks and options in detail with your doctor before entering this study.

H Unforeseen Risks

New risks might appear at any time during the study that are different from the risks listed in this Consent Form. We will promptly tell you of any new information that may affect your decision to participate.

I Other Treatments or Medications

Some medicines react with each other, and it is important that you tell the study doctor or staff about any other drugs, treatments, or medicines you are taking. This includes non-prescription medications, vitamins and herbal treatments.

It is also important that you tell the study staff about any changes to these medications during your participation in the study.

For more information about risks and side effects, ask your study doctor.

8. Possible Benefits

Taking part in this study may or may not make your health better. The information from this study will help doctors learn more about transplant as a treatment for people with a blood cancer and HIV. This information could help future people with HIV who may need a transplant.

9. New Information Available During the Study

During this research study, the study doctors may learn about new information about the study drug or the risks and benefits of the study. If this happens, they will tell you about the new information. The new information may mean that you can no longer participate in the study, or that you may not want to continue in the study.

If this happens, the study doctor will stop your participation in the study and will offer you all available care to suit your needs and medical conditions.

10. Privacy, Confidentiality and Use of Information

Your confidentiality is one of our main concerns. We will do our best to make sure that the personal information in your medical record is kept private. However, we cannot guarantee total privacy.

We may give out your personal information if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

Information about your transplant from your original medical records may be seen or sent to national and international transplant registries, including:

- The Center for International Blood and Marrow Transplant Research (CIBMTR)
- The National Marrow Donor Program (NMDP)
- The Food and Drug Administration (FDA)
- The National Institutes of Health (NIH), which include the National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI)
- Data and Coordinating Center of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN)
- Other authorized study organizations.

We will not identify you by name in any publications or reports that come from these organizations or groups.

Information that does not include personally identifiable information about this clinical trial has been or will be submitted, at the appropriate and required time, to the government-operated

clinical trial registry data bank, which contains registration, results, and other information about registered clinical trials.

This data bank can be accessed by you and the general public at www.ClinicalTrials.gov. Federal law requires clinical trial information for certain clinical trials to be submitted to the data bank.

11. Ending Your Participation

Being in this study is voluntary. You can choose to not be in this study, or leave this study at any time. If you choose not to take part or leave this study, your regular medical care will not be affected in any way. This includes standard care for your blood cancer and HIV. Tell your doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop safely.

The study doctor or the study sponsor may stop the study at any time, and we may ask you to leave the study. We may ask you to leave the study if you do not follow directions or if you suffer from side effects of the treatment. If we ask you to leave the study, the reasons will be discussed with you. Possible reasons to end your participation in this study include:

- You do not meet the study requirements.
- You need a medical treatment not allowed in this study.
- The study doctor decides that it would be harmful to you to stay in the study.
- You are having serious side effects.
- You become pregnant.
- You cannot keep appointments or take study drugs as directed.
- The study is stopped for any reason.

If you decide to leave this study after taking the study treatment, or are asked to leave by your doctor for medical reason, you will need to come back to the doctor's office for tests for your safety. Even if you leave the study, the information collected from your participation will be included in the study evaluation, unless you specifically ask that it not be included.

12. Physical Injury as a Result of Participation

It is important that you tell your doctor, _____ *[investigator's name(s)]* or study staff if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him/her at _____ *[telephone number]*.

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment.

In case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

13. Compensation or Payment

You will not be paid for your participation in the research study. You will not get compensation or reimbursement for any extra expenses (travel, meals, etc.) you may have through your participation on this trial.

14. Costs and Reimbursements

Most of the visits for this research study are standard medical care for patients undergoing allogeneic transplants and will be billed to your insurance company. You and/or your health plan/insurance company will need to pay for some or all of the costs of standard treatment in this study.

You or your insurance will not be charged for optional blood samples for research on this study.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at http://cancer.gov/clinicaltrials/understanding/insurance-coverage. You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

15. Ethical Review

The ethical aspects of this research study have been reviewed and approved by [name of IRB].

16. Further Information

If you need more information about this study, or if you have problems while you are participating in this study, you can contact the study doctor or his/her staff. They can be reached at the telephone numbers listed here:

[Insert name and contact details]

17. Independent Contact

If you wish to speak to someone not directly involved in the study, or if you have any complaints about any aspect of the project, the way it is being conducted or any questions about your rights as a research participant, then you may contact:

[Insert appropriate contact details]

For questions about your rights while taking part in this study, call the _____*[name of center]* Institutional Review Board (a group of people who review the research to protect your rights) at _____ *(telephone number).*

18. CCR5 Donor Screening

Human Immunodeficiency Virus (HIV) uses a protein called CCR5 as a way to get inside of cells. A few people are naturally able to block HIV infection because their body does not make the CCR5 protein. As a result, HIV does not have a way to enter their cells and cause an infection.

Proteins are in every cell in our body and help keep the blood, skin and other parts of our bodies healthy. Every protein is made by a specific kind of gene. Every person has 2 copies of the gene that makes the CCR5 protein. One copy is from their mother and the other copy is from their father. Some people have changes to their genes that make the CCR5 protein. Another word for changes to a gene is mutation. Changes to the CCR5 gene means the body can't make the CCR5 protein. People who have 2 copies of the CCR5 gene mutation are called CCR5delta32homozygotes and they are naturally able to block HIV infection.

Very few people in the world have mutations to both copies of their CCR5 gene. If you are Caucasian with a family background from northern Europe, the chance is about 1 out of 100 people in finding a matched donor with 2 copies of the CCR5 gene mutation. If you do not have a northern European background, the chance is very, very small that you would find a donor who has 2 copies of the CCR5 gene mutation.

In one case, a person with 2 copies of the CCR5 gene mutation donated their blood-making cells for a transplant in a patient who had HIV and a blood cancer. Now the patient does not have any signs of HIV and does not need drugs to treat his HIV. We do not know if this will happen again, even if a donor has the CCR5 gene mutation

Besides the possible benefit of blocking HIV infection, some risks may come with a donor who has the CCR5 mutations. Research has shown that people with 2 copies of the CCR5 gene mutation may not fight off infections from West Nile virus (WNV) very well. WNV spreads through mosquito bites. Serious cases of WNV can cause a brain infection.

In addition to doing the standard tests to make sure a donor is a good match for you, we will also test possible donors to see if they have the CCR5 gene mutations. This testing will not slow down our search for your donor. We will let you know if we find a donor and if that donor has

the CCR5 mutations. At that point, you and your doctor will need to decide if you want to use a donor with or without the CCR5 mutations.

♦ ♦

Blood Samples for Research (optional)

Please note: This section of the informed consent form is about future research studies that will be done using blood samples from people who are taking part in the main study described above. You may give small blood samples for these future research studies if you want to. You can still be a part of the main study even if you say 'no' to giving blood samples for future research studies. You can say "yes" or "no" to giving blood samples for future research studies. Please mark your choice at the end of this section.

We would like to have one small (4 teaspoons or 17 mL) blood sample for future research. If you agree, this sample will be obtained pre-transplant. It will be kept and may be used in research to learn more about HIV, cancer and other diseases. Usually the blood can be drawn from your central venous catheter at the time of the other blood collections. If this is not possible, it will be taken from a vein. When the sample is given to investigators for research, no information about your name, address, phone number or other information that will let the researcher know who you are will be provided.

The samples collected for research purposes will be sent to the AIDS and Cancer Specimen Resource Repository. The samples will be labeled with unique codes that do not contain information that could identify you. A link to this code does exist. The link is stored at the Data and Coordinating Center for the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). The staff at the repository where your sample is being stored does not have a link to this code. Your research samples will continue to be stored at the ACSR Repository until they are used up for approved research.

DNA from your stored blood and tissue samples and your health information might be used in genome-wide association (GWA) studies for a future project either done or supported by the National Institutes of Health (NIH).

Genome-wide association studies are a way for scientists to identify genes involved in human disease. This method searches the genome for small genetic changes that are more common in people with a particular disease than in people without the disease. Each study can look at hundreds of thousands of genetic changes at the same time. Researchers use data from this type of study to find genes that may add to a person's risk of developing a certain disease. If your coded genetic and clinical information is used in such a study, the researcher is required to add the DNA test results and non-identifying information into a public research database. This public database is called the NIH Genotype and Phenotype Database and it is managed by the National Center for Biotechnology Information (NCBI). The NCBI will never have any information that would identify you, or link you to your information or research samples.

A new federal law (2009), called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and employers of 15 or more persons to discriminate against you based on your genetic information. Health insurance companies and group health plans may not request your genetic information that we get from this research. This means that they must not use your genetic information when making decisions regarding insurability. Be aware that this new federal law will not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

The research that may be done with your blood is not designed specifically to help you. It might help people who have HIV, cancer and other diseases in the future.

Reports about research done with your blood will not be given to you or your doctor. These reports will not be put in your health record. The research will not have an effect on your care.

Things to Think About: The choice to let us have blood samples for future research is up to you. No matter what you decide to do, it will not affect your care.

If you decide now that your blood can be kept for research, you can change your mind at any time. Just contact your study doctor and let him or her know that you do not want us to use your blood sample. Then any blood that remains will no longer be used for research.

In the future, people who do research on these blood samples may need to know more about your health. While the study doctor or others involved in running this study may give the researchers reports about your health, it will not give them your name, address, phone number, or any other information that will let the researchers know who you are.

Sometimes blood is used for genetic research (about diseases that are passed on in families). Even if your blood is used for this kind of research, the results will not be put in your health records.

Your blood will be used only for research and will not be sold. The research done with your blood may help to develop new products in the future.

Benefits: The benefits of research using blood include learning more about what causes HIV, cancer and other diseases, how to prevent them, and how to treat them.

Risks: The greatest risk to you is the release of information from your health records. We will do our best to make sure that your personal information will be kept private. The chance that this information will be given to someone else is very small.

Making Your Choice: Please read each sentence below and think about your choice. After reading each sentence, please indicate your choice below. If you have any questions, please talk to your doctor or nurse, or call our research review board at _____.

No matter what you decide to do, it will not affect your care.

Statement of Consent

The purpose of storing blood samples, the procedures involved, and the risks and benefits have been explained to me. I have asked all the questions I have at this time and I have been told whom to contact if I have more questions. I have been told that I will be given a signed copy of this consent form to keep.

I understand that I do not have to allow the use of my blood and for research. If I decide to not let you store research samples now or in the future, it will not affect my medical care in any way.

I voluntarily agree that a blood sample may be collected and that my blood and related information can be stored indefinitely by the BMT CTN and/or AIDS and Cancer Specimen Resource Repository for research to learn about, prevent, or treat health problems. I also understand that my DNA and health information may or may not be used in genome-wide association studies.

- ☐ I do agree to give a blood sample for research.
- ☐ I do not agree to give a blood sample for research.

____ ____________

Signature Date

♦ ♦

Health Insurance Portability and Accountability Act 1 (HIPAA1) Authorization to use and disclose individual health information for research purposes

A. **Purpose**:
As a research participant, I authorize the Principal Investigators and the researcher's staff to use and disclose my individual health information for the purpose of conducting the research study:

Allogeneic Hematopoietic Cell Transplant for Hematological Cancers and Myelodysplastic Syndromes in HIV-Infected Individuals

B. **Individual Health Information to be Used or Disclosed**:
My individual health information that may be used or disclosed to do this research includes:

- Demographic information (for example: date of birth, sex, weight)
- Medical history (for example: diagnosis, complications with prior treatment)
- Findings from physical exams
- Laboratory test results obtained at the time of work up and after transplant (for example: blood tests, biopsy results)

C. **Parties Who May Disclose My Individual Health Information**:
The researcher and the researcher's staff may collect my individual health information from:
[*List hospitals, clinics or providers from which health care information can be requested*]

D. **Parties Who May Receive or Use My Individual Health Information**:
The individual health information disclosed by parties listed in item c and information disclosed by me during the course of the research may be received and used by the following parties:

<u>Principal Investigator and the researcher's staff</u>

Dr. Joseph Alvarnas, Co-Principal Investigator

Dr. Richard Ambinder, Co-Principal Investigator

<u>Study Sponsors</u>

- National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI), both of the National Institutes of Health (NIH),
- Blood and Marrow Transplant Clinical Trials Network (BMT CTN), Data and Coordinating Center

1 HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information

- U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)
- U.S. government agencies that are responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments.

E. **Right to Refuse to Sign this Authorization**:
I do not have to sign this Authorization. If I decide not to sign the Authorization, I will not be allowed to participate in this study or receive any treatment related to research that is provided through the study.

My decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

F. **Right to Revoke**:
I can change my mind and withdraw this authorization at any time by sending a written notice to the Principal Investigator to inform the researcher of my decision.

If I withdraw this authorization, t he researcher may only use and disclose the protected health information already collected for this research study. No further health information about me will be collected by or disclosed to the researcher for this study.

G. **Potential for Re-disclosure**:
My individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected.

Examples include potential disclosures for law enforcement purposes, mandated reporting or abuse or neglect, judicial proceedings, health oversight activities and public health measures.

H. **This authorization does not have an expiration date.**

I have read and understood this Consent Form. The nature and purpose of the research study has been explained to me.

- I have had the chance to ask questions, and understand the answers I have been given. I understand that I may ask questions at any time during the study.
- I freely agree to be a participant in the study.
- I understand that I may not directly benefit from taking part in the study.
- I understand that, while information gained during the study may be published, I will not be identified and my personal results will stay confidential.
- I have had the chance to discuss my participation in this research study with a family member or friend.
- I understand that I can leave this study at any time, and doing so will not affect my current care or prevent me from receiving future treatment.
- I understand that I will be given a copy of this signed consent form.

_____ _____

Participant Name Date

_____

Signature Date

I certify that I have provided a verbal explanation of the details of the research study, including the procedures and risks. I believe the participant has understood the information provided.

_____

Name of Counseling Physician Date

_____

Signature of Counseling Physician Date

Pediatric Assent to Participate in Research

Study Title: Allogeneic Hematopoietic Cell Transplant for Hematological Cancers and Myelodysplastic Syndromes in HIV-Infected Individuals

Protocol: BMT CTN 0903 (AMC 080)

A. Why am I here?

We are inviting you to join our study because you have HIV (human immunodeficiency virus) and you need a transplant to treat your blood cancer.

B. Why are you doing this study?

We are doing this study because we want to learn how well hematopoietic cell transplant treats blood cancers in people like you who have HIV. The kind of transplant we would do for you would use blood or bone marrow from another person who matches you. This is called an allogeneic transplant.

This form gives you information to help you decide if you want to be in this study. You should read this form and ask any questions you have before agreeing to be in the study. It is up to you to decide if you want to be in the study.

C. What will happen to me?

Before enrolling on study:
Your doctor will check to see if you have a type matched donor for your transplant. You will have several tests to see if it is okay for you to be on this study.

Before the transplant:
You will have several tests done to check your organ function. These tests will check your heart, lungs, and brain. Most of these tests are X-rays or scans, questions, or blood tests. The doctors will look at the results of all these tests to make sure that it is okay for you to have a transplant.

You will also need to have several tests for research. We will ask if we can take some blood from you up to 4 times (10-47 mL or about 2-13 teaspoons) with a very small needle. Eight weeks before your transplant, we will also ask to take more blood from you (about 36 teaspoons) only if the HIV in your body does not show up in a standard test.

Preparation for the transplant:
Before the transplant, you will need to receive medicines so that your body can accept the new bone marrow cells. This is called a 'conditioning regimen.'

You will receive chemotherapy medicine before your transplant. Chemotherapy is a combination of strong drugs that work to destroy your cancer cells and get your body ready for transplant.

Post-transplant follow-up and care:
After the transplant you will continue to get medicines to help the donor cells grow. These drugs will also help lower the chance of getting graft-versus-host disease (GVHD). GVHD is a complication that happens when the donor's cells attack your body. You will receive one or more medicines to prevent GVHD. You will continue to receive these drugs for at least six months after the transplant.

You will be in the hospital for about four weeks after your transplant. You will be allowed to go home from the hospital when your doctor feels it is safe. After you go home you will need to return to visit your doctors so they can check your recovery. Your doctors will need to check your blood and bone marrow after the transplant to make sure the new blood cells are growing in your body. Your doctors will also do blood tests and other tests to make sure your organs are working well.

This study will last for 2 years and we will watch you carefully for side effects, fevers, infections and other problems.

You will also need to have several tests for research. We will take some blood from you up to 7 times (about 2-14 teaspoons each time) with a very small needle. Also, 13 months after your transplant, we will ask to take more blood from you (about 36 teaspoons) only if the HIV in your body still does not show up in a standard test.

Optional Test for Future Research

Between 1 and 3 weeks prior to your transplant, we will ask your permission to take some more blood (about 6 teaspoons of blood) from you to use for future research.

You don't have to be in this research. If you don't want to give blood samples for future research you can still be in the other parts of the study. Your care will not be changed if you decide not to give these blood samples for research purposes. Please mark your choice below (check only one box):

❑ I do agree to give a blood sample for research.

❑ I do not agree to give a blood sample for research.

D. Will it hurt?

When you have your blood taken with a needle, it may feel like a pinch. It will hurt for a minute and sometimes the place where the needle went might be red and sore. You might get a little bruise where the blood was taken but it goes away in a few days.

E. What are the risks of being in this study?

The drugs may cause a skin rash, hair loss, nausea and vomiting, diarrhea and infections. Your blood counts will fall and you may get fevers, infections or start bleeding. You may also get mouth sores. These are temporary and you will feel better as your new bone marrow grows.

Since you will not be able to fight infections while your new bone marrow is growing back, you may need to get antibiotics. You may also need to get blood transfusions since your new bone marrow will not be making new blood cells right away.

Even with medicines to prevent it, you may get GVHD. This can cause skin rash, vomiting, diarrhea, stomach pain, lung and liver problems, swelling of the hands and feet, dry eyes, stiff joints, and tiredness. These problems are usually mild but can become very serious and prolonged. Medicines are given to prevent GVHD during and after transplant. If GVHD occurs even after taking these medicines, other medicines will need to be started and hospital stays may be necessary. The medicines used to treat GVHD also have side effects. They can cause tiredness, depression, sleep problems and mood swings. They can also make you get severe infections very easily. Your doctors will do their best to make you feel better and keep you safe. Often this may require many hospital stays. However, it is important to understand that there is a small risk (about a 1 in 10 chance) that you may die as a result of one or more of the complications of unrelated donor transplantation.

F. Will the study help me?

You may or may not benefit from taking part in this study.

G. What if I have questions?

You can ask us any questions that you have about the study. If you forget to ask a question and think of it later, you can call me [*insert office number*]. You can also ask your question the next time you see me.

You can call the study office at any time to ask questions about the study.

H. Do I have to be in this study?

It is up to you if you want to participate in this research study. If you don't want to be in the study, you can tell us and your parent or guardian. Your doctor will not be upset or angry if you don't want to join. You can say yes now and change your mind at any time. If you leave the study you can still get medical care from your doctor and transplant center. You will be told about new information or changes in the study that may affect your health or your willingness to continue in the study.

I. Can the doctor who is the Principal Investigator withdraw me from this study?

You can be taken off the study (with or without your consent) for any of the following reasons:

- You need a medical treatment not allowed in this study
- The investigator decides that continuing in the study would be harmful to you
- You become pregnant and the study participation could be harmful to the fetus
- The study is cancelled by the Food and Drug Administration (FDA) or the National Institutes of Health (NIH)

J. Will my medical information be kept private?

We will do our best to make sure that the personal information in your medical record be kept private. However, we cannot guarantee total privacy. Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used.

You will receive a copy of this form. If you need more information about this study, ask the study doctor.

****

Minor's Assent

I have read the information in this consent form and have had the study explained to me. My questions have been answered to my satisfaction. I agree to participate in the study.

_____ _____
Signature of Minor Date

_____ _____
Print Name of Minor *Age of Minor*

Certification of Counseling Healthcare Professional
I certify that the nature and purpose, the potential benefits, and possible risks associated with participation in this study have been explained to the above individual and that any questions about this information have been answered.

_____ _____
Counseling Healthcare Professional Date

APPENDIX C

LABORATORY PROCEDURES

APPENDIX C

LABORATORY PROCEDURES

1. MICROBIAL TRANSLOCATION MARKERS: CHARACTERIZATION OF THE RATES, SPECTRUM, RISK FACTORS, AND OUTCOMES OF INFECTIONS

HCT-related risk factors for infection (time to engraftment, degree of mucositis, duration of neutropenia) and HIV-related (CD4, VL, HAART resistance) risk factors will be investigated. Specimens will be collected to allow the analysis of the relationship of microbial translocation markers to febrile neutropenia and infectious outcomes.

Background

Recent observations that persons with chronic HIV-1 infection and AIDS demonstrate increased microbial translocation (MT) from the gut compared to uninfected persons may be related to the massive depletion of intestinal CD4+ lymphocytes within weeks of HIV-1 acquisition (1). Our group also recently demonstrated that microbial translocation in HIV-HCV co-infection was strongly associated with more rapid progression of liver cirrhosis. A key finding was that serum endotoxin (LPS) level, a marker of microbial translocation, was higher in patients with more advanced fibrosis (2). The detection of microbial translocation in peripheral blood likely underestimates the higher quantities of microbial products that enter the portal venous circulation with resulting sequelae in the liver.

Previous studies have shown that HIV+ recipients of autologous stem cell transplant are at risk for early hepatic veno-occlusive disease (VOD) (3). VOD is a condition that is characterized by endothelial cell injury in the hepatic sinusoids leading to sinusoidal thrombosis and occlusion. LPS is known to have a procoagulant effect by inducing gene expression of several inflammatory mediators including the procoagulant protein, tissue factor (4). Therefore, a high level of circulating MT markers (such as LPS) at the time of transplant could be a risk factor for development of early hepatic VOD. Moreover, monitoring serum levels temporally to explore associations with liver associated morbidity including VOD, may provide insights into defining risk factors for VOD. The long-term goal of this approach is to provide a rational basis for targeted antimicrobial therapy to reduce microbial translocation in patients at higher risk of developing VOD.

Hypothesis

We hypothesize that increased microbial translocation in HIV infected recipients of induction chemotherapy may increase the risk for development of veno-occlusive disease after autologous or allogeneic stem cell transplant.

Study Objectives

We propose testing archived sera for microbial translocation markers from HIV-infected persons who have received allogeneic stem cell transplant. Microbial translocation marker levels will then be correlated with clinical outcome (hyperbilirubinemia, development of VOD, death) as patients are followed longitudinally.

Study Design
Clinical data that will be obtained at baseline will include age, gender, race, HCV RNA levels, HIV RNA levels, CD4+ lymphocyte count, AST, ALT, total bilirubin, antibiotic usage within the prior 6 weeks, antiretroviral therapy, MELD score, and a history of liver-related complications.

Microbial Translocation Measurements
Markers of microbial translocation that will be tested include plasma LPS, LPS-binding protein (LBP), soluble CD14 (sCD14), and the polyclonal IgM antibody directed toward the LPS core polysaccharide (EndoCAb IgM). LPS testing will be done using the *Limulus* Amebocyte Lysate (LAL) assay (Lonza), while LBP (Cell Sciences), sCD14 (R&D Systems), and EndoCAb IgM (Hycult Biotechnology) assays are all standard plate-based ELISA tests. Microbial translocation markers will be log-transformed to normalize the data, as has been done previously. Values of the LAL assay < 10 pg/mL are considered below the linear range of the assay and will be considered 5 pg/mL (mean of 0 and 10 pg/mL) for standard calculations.

Statistical Components
Association between outcome (hyperbilirubinemia, VOD, and death) at Days 7, 14 and 100 post-transplant and level of microbial translocation markers will be determined by nonparametric Mann-Whitney tests comparing the distribution of prior microbial translocation markers between patients with vs. without each outcome.

Samples Required

Pre-transplant

A 10 mL peripheral blood sample will be collected in an EDTA containing Vacutainer tube 1 week prior to conditioning as well as on Day -3 of the conditioning regimen.

Post-transplant

A 10 mL peripheral blood sample will be collected in an EDTA containing Vacutainer tube will be collected at Days 7, 14, and 100 post-transplant.

Samples Shipment

Transplant centers will ship whole blood tube by priority overnight FedEx on the day of collection to the project laboratory for processing and microbial translocation marker testing.

References

1. J. M. Brenchley et al., *Nature Medicine* 12, 1365-1371 (2006).
2. A. Balagopal et al., *Gastroenterology* 135, 226-233 (2008).
3. T Spitzer et al., *Biol of Blood and Marrow Transpl.* 14, 59-66 (2008).

4. Luyendik et al., *J Immunol.* 180, 4218-4226 (2008).

2. HIV SINGLE COPY PCR ASSESSMENT AND LATENT HIV RESERVOIR MEASUREMENTS: CHARACTERIZATION OF HIV INFECTION IN AIDS RELATED MALIGNANCIES

Background and Rationale

The effect of marrow ablative and reduced intensity chemotherapy on the HIV reservoir is not known, but the model of AIDS-related malignancies treatment does provide an opportunity to define changes which are associated with HCT. In the current study, we propose to characterize the HIV infection during and after such treatment of hematological malignancies and Myelodysplastic syndromes, by measurement of HIV load including the use of an assay which measures HIV at less than 1 copy per milliliter. In patients with no detectable viral load by standard clinical assays, this assay regularly detects low copy number viral RNA. This RNA is believed to be released from latency compartments established in hematopoietic cells.

Hypothesis

We hypothesize that treatment of AIDS-related malignancies using dose-intense or cytotoxic reduced-intensity therapy in patients will result in a substantial reduction in the size of the HIV reservoirs and thus in this very low level viremia. This study will represent the first application of this single copy PCR technique to such conditioning therapies with allogeneic hematopoietic stem cell transplant—although the approach has been used multi-institutional studies of antiretroviral therapies.

HIV Cellular Reservoir Analysis & Single-Copy HIV Assays

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-transplant 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.

Sample Requirements

Latent HIV Cellular Reservoir Analysis

Pre-transplant

Once the patient is consented and enrolled on the trial, a peripheral blood sample will be collected at approximately 8 weeks prior to initiation of ablative therapy for only those patients that have documented undetectable plasma HIV-1 by standard assay. A 180 mL

peripheral blood sample will be collected and placed in to eighteen (18) 10 mL EDTA containing, lavender-top Vacutainer tubes.

Post-transplant

A peripheral blood collection will be scheduled sometime after 12 months post-transplant, typically at 13 months post-HCT, for only those patients that are found to have undetectable plasma HIV-1 RNA by the standard assay performed at the clinical site on the 12 months sample. A 180 mL peripheral blood sample will be collected and placed in to eighteen (18) 10 mL EDTA containing, lavender-top Vacutainer tubes.

Sample Shipment
Transplant centers will ship whole blood tubes by priority overnight FedEx on the day of collection to the project laboratory for processing and testing.

Single-Copy HIV Assays (Plasma & PBMC)

Pre-transplant

A 30 mL peripheral blood sample will be collected in EDTA containing Vacutainer tubes 1-3 weeks and 1 week prior to initiation of ablative therapy.

Post-transplant

A 30 mL peripheral blood sample will be collected in EDTA containing Vacutainer tubes on Day 100, and at 6, 12, and 24 months post-transplant.

Sample Processing and Shipment
Transplant centers will ship whole blood tubes by priority overnight FedEx on the day of collection to the BMT CTN 0903-specific Central Processing Laboratory for sample plasma and PBMC aliquot processing and temporary sample storage. Available sample aliquots will be periodically shipped to the designated project laboratory for testing.

3. IMMUNE RECONSTITUTION ASSAYS (RESEARCH): IMMUNOPHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF IMMUNE RECONSITUTION

Background and Rationale:
Little is known about immune reconstitution following allogeneic hematopoietic cell transplantation to treat patients with hematologic malignancies in patients with HIV infection. Multiple variables including adaptive immune dysfunction and chronic viral infections raise the possibility that HIV+ patients will encounter more infectious complications compared to HIV- individuals following hematopoietic cell transplantation. It will therefore, be critical to perform correlative laboratory investigations examining adaptive and innate immune surveillance in this very unique setting. The use of multiparametric flow cytometry to identify specific adaptive and innate immune cell subsets, and use of available pathogen-specific epitopes should allow us to characterize the dynamics of immune reconstitution and identify first responses against HIV and other common pathogens post-ASCT.

Hypothesis:
We hypothesize that quantitative and qualitative differences in adaptive and innate immune reconstitution will correlate with clinical outcome in HIV+ patients who undergo allogeneic hematopoietic cell transplantation. Improved understanding of immune reconstitution in this very unique setting will lead to advances in the management of HIV+ patients with cancer and lead to novel approaches to improve long term survival of these patients.

Study Design:
Immunophenotypic Evaluation of T and NK Cell Subsets: To date no comprehensive multi time point study has been performed to evaluate reconstitution of adaptive immunity in HIV+ patients treated with peripheral blood hematopoietic cell transplantation. Examining the nature of adaptive and innate immune reconstitution will involve use of a multi panel flow cytometric assay to track changes in naïve, memory, activated and regulatory T cell subsets. Specifically, these studies will involve a detailed in vivo analysis of early, mid, early-late and late activation of B and T lymphocytes and characterization of T cell subsets including naïve, memory, Th1, Th2 and T regulatory cells. In addition we will evaluate maturation status of NK cells and their activation status. Multiparametric flow analysis will be performed on fresh peripheral blood samples collected at 60, 180 and 365 days post stem cell infusion.

Table 1 Multicolor T Cell Panel

Condition	FITC	PE	ECD	PC5	PC7
gate purity	CD3	CD56+ CD16	CD19	CD45	CD13
Activation	HLA DR	CD69	CD3	CD134	CD45
Med activation	CD8	CD49a	CD4	CD3	CD45
Naïve/memory cells	CD45RA	CD27	CD45RO	CD4	CD8
Naïve/memory cells	CD45RA	CD29	CD45RO	CD4	CD8
TH1/Th2	CD193	CD294	CD4	CXCR3	CD45
Th1/Th2	CD193	CD294	CD4	CD45	CCR4
T regulatory cells	CD8	CD127	CD4	CD25	CD45
TCR α/β and g/d	TCR $\gamma\delta$	TCR $\alpha\beta$	CD45	CD3	

Table 2 Multicolor NK Cell Panel

Condition	Flow Cytometric 5-color antigen panels				
Viability		7-AAD			
NK cell activation	CD45-PC7	CD3-FITC	CD158-PE	CD69-ECD	CD56+CD16-PC5
NK cell degranulation status	CD45-PC7	LAMP-1 CD107a+ CD107b - FITC	NKG2A CD159-PE	CD3-ECD	CD56+CD16-PC5
NK cell degranulation status	CD45-PC7	LAMP-3 CD63-FITC	NKG2D CD314-PE	CD3-ECD	CD56+CD16-PC5
NK Cell maturity status	CD45-PC7	CD3-FITC	CD16-PE	CD56-ECD	CD117-PC7

Samples of ACD (yellow top) and EDTA (lavender top) anticoagulant containing blood will be received at the project laboratory within 24h following collection and processed immediately upon receipt in the clinical flow cytometry laboratory. Prior to staining, all samples will be analyzed for viability using 7AAD method. Only samples with mononuclear cell viability $\geq$ 90% will be considered acceptable for further analysis. Viability of >95% is achieved in ACD tubes stored at room temperature for up to 48 hours. All samples will be stained using a PrepPlus2 automated staining system (Beckman Coulter) utilizing a five color whole blood staining technique with panels of directly conjugated monoclonal antibodies (see Tables 1 and 2) used in quantities that have been predetermined and standardized in our flow cytometric laboratory. Following 30 minutes of incubation at room temperature in the dark, red cells will be lysed using a Q-prep instrument and Coulter Lyse reagent according to manufacturer's recommendations. Samples will be analyzed on FC500 flow cytometer equipped with CXP software version 2.1 (all equipment and reagents by Beckman Coulter). Multiparametric

analysis will be performed with a gating strategy based on CD45 staining and light side scatter characteristics that allow adequate separation of lymphocyte, monocyte and myeloid cell populations. Detailed immunophenotypic characterization of the lymphocyte gate will be performed using Prism plot algorithm (Beckman Coulter). The results will be reported as percent of lymphocyte gate and as percent of total leukocytes analyzed. The results will also be reported as an absolute number of specific cell subset types per microliter of whole blood. Absolute cell number will be calculated based on duel platform method using percent of lymphocytes expressing specific immunophenotypic profile (derived from prism plot) and absolute number of lymphocytes derived from analysis of each whole blood sample using ActDIFF hematology analyzer (Beckman Coulter).

Functional Evaluation of Adaptive Immune Reconstitution: We will serially evaluate adaptive cellular immunity by tracking responsiveness to viral recall antigens. We will follow responsiveness to HIV as well as common viral pathogens like CMV and EBV. Studies will use pools of overlapping 15-18mer peptides from full-length viral proteins (JPT Technologies) encoded by CMV (pp65), EBV (BZLF1) and HIV (gag). Aliquots of frozen PBMCs will be thawed and enumerated. $1x10^6$ PBMCs will be cultured in the presence of individual viral pooled peptide preparations (or actin control pooled peptides, 1ug/ml final concentration) in the presence or absence of agonistic anti CD28 monoclonal antibody to control for costimulatory signals and provide an optimal condition for evaluation of responsiveness. Following overnight incubation (in presence of brefeldin A golgi plug reagent), a multi color flow cytometric evaluation will identify CD3/CD8 subsets that costain with IFNγ or CD107a (degranulation marker). Separate controls with 7AAD will evaluate PBMC viability. 1×10^5 CD3+ events will be collected using a FC500 flow cytometer and percentage of CD3/CD8+ T cells staining positive for IFNγ and or CD107a will be determined. Outcome of these bioassays will be correlated with immunophenotypic and clinical patient data.

Functional Evaluation of Innate Immune Reconstitution: Innate immune reconstitution studies will examine natural killer (NK) cell responsiveness to cytokines and to FcγRIIIa signaling. NK cell responsiveness assays will be performed on purified NK cells that will be isolated via rosette-sep immuno selection kits. To test cytokine responsiveness, $1x10^5$ NK cells will be incubated overnight in standard culture medium (supplemented with human AB serum) in interleukin 2 (IL-2 10nM) +/- IL-12 (10ng/ml). To evaluate FcγRIIIa responsiveness, 2 methods will be employed: (i) Immobilized IgG in presence or absence of IL12; and (ii) anti FcγRIIIa monoclonal antibody treatment +/- IL12. For FcgRIIIa signaling studies, 96-well flat-bottom plates will be coated with 100 μg/mL huIgG in cold PBS overnight at 4°C, washed with cold PBS, and then plated with immune cells (2×10^5 cells/well) +/- 10 ng/mL IL-12 (or saline control). At 24 and 48 hours cell-free culture supernatants will be harvested and analyzed for levels of IFN-γ by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN). NK cells will be checked for viability following the overnight pretreatment period and again at the conclusion of the experiment. The readout for the assay will be IFN-γ measured by ELISA or intracellular IFNγ and CD107a expression determined by flow cytometry (as in adaptive immune reconstitution studies) at 24 and 48 hrs. FcγRIIIa responsiveness can also be tested via use of anti FcγRIIIa mAb (clone 3G8) to cross link receptors +/- IL12 and IFNγ and CD107a detected via flow cytometry. Positive controls will include PBMCs collected from

immune-competent individuals, purified NK cells from immune-competent individuals incubated with IL2 + IL12, IL-15 + IL-18 and IL-15 + IL-21. We will correlate the outcome of these biologic studies with immunophenotypic studies examining NK cell subsets and clinical patient data.

Correlative Evaluation of Immune Reconstitution Studies:
Immunophenotypic and functional immune reconstitution studies will produce quantitative and qualitative data that can be compared to multiple clinical and laboratory outcomes in this unique setting. Specifically:

1) Clinical outcome: Recovery of specific T and NK cell subsets and functional immune responsiveness in the above assays can be compared to (a) documented infections post transplant (bacterial, fungal, viral) CR; (b) lymphoma disease free survival; (c) overall survival.
2) Laboratory comparisons: immunophenotypic and functional immune reconstitution can be compared to the following laboratory correlates, such as: chronic viral infection/reactivation (EBV, CMV, HIV).

Statistical Components:
Immune reconstitution results will be summarized at each post-transplant time point using descriptive statistics. Individual T, B and NK cell subsets identified by flow cytometry will be reported as percent of total mononuclear cells and as absolute cell numbers per microliter of peripheral blood. Statistical evaluation of functional studies evaluating adaptive and innate immune reconstitution will be reported as percentage of mononuclear cell subsets staining positive for IFNγ or granzyme B relative to control conditions. Two sided T tests will be employed for evaluation of statistical significance with $p \leq 0.05$ considered significant.

Samples Required:
A total of 37 mL peripheral blood will be collected and placed into (1) four 8.5 mL-fill, ACD Vacutainer tubes (yellow top) tubes containing ACD anticoagulant solution, and (2) one 3 mL EDTA containing (lavender) Vacutainer tube 8 weeks, and 6 and 12 months post-transplant.

Sample shipment: Transplant centers will ship whole blood tubes by priority overnight FedEx on the day of collection to the project laboratory for immediate processing for flow cytometry and for procurement of viable cells for functional immunology assays.

Project Laboratory Sample Processing & Testing: Upon arrival, mononuclear cell count in whole blood will be determined and, depending on absolute mononuclear cell count, 1 – 2 tubes will be immediately processed for flow cytometry immunophenotyping panels. PBMC will be cryopreserved for innate and adaptive functional studies that will be performed in batch at a later date.

4. OPTIONAL RESEARCH SAMPLE FOR UNDEFINED FUTURE RESEARCH

Patients consenting to provide research samples to be submitted to The AIDS and Cancer Specimen Resource Repository for future undefined testing will have an additional baseline peripheral blood sample collected.

Samples Required

A 17 mL peripheral blood sample will be collected in two 8.5 mL-fill Vacutainer blood tubes (yellow) containing ACD solution A anticoagulant. Samples will be collected 1-3 weeks prior to initiation of ablative therapy.

Sample Shipment

Transplant centers will ship whole blood tubes by priority overnight FedEx on the day of collection to the Aids Cancer Research Specimen Repository (ACSR) Repository for sample aliquot processing and sample storage.

TABLE C-1: COLLECTION AND SHIPPING PROCEDURES AND SAMPLE COLLECTION SCHEDULE FOR PATIENT BLOOD SAMPLES FOR PROTOCOL-DEFINED RESEARCH TESTING

RESEARCH TOPIC	RESEARCH SAMPLE	TYPE OF SAMPLE	SAMPLE COLLECTION, PROCESSING AND STORAGE REQUIREMENTS	SAMPLE COLLECTION TIME POINTS	SHIPPING SPECIFICATIONS
Optimizing Diagnostics and Preventative Care for Infections	**Microbial Translocation Markers**	10 mL peripheral blood sample collected in an EDTA containing, lavender-top Vacutainer tube.	Gently mix blood with EDTA by inverting the tube 8-10 times. Store at room temperature while preparing to ship to project laboratory.	**Pre-transplant** Within 1 week prior to conditioning and Day -3 of conditioning **Post-transplant** Days 7, 14 and 100	Peripheral blood tubes will be shipped at ambient temperature on the day of collection, to the Project Laboratory by priority overnight FED EX delivery for processing and research testing. Guidelines for specimen handling and shipment to the Project Laboratory is detailed in the BMT CTN 0903 Laboratory Sample Guide.
Characterization of HIV Infection and Latent HIV Reservoirs	**HIV Single-Copy PCR (Plasma & PBMC)**	30 mL peripheral blood sample collected in three 10 mL EDTA containing, lavender-top Vacutainer tubes.	Gently mix blood with EDTA by inverting the tubes 8-10 times. Store at room temperature while preparing to ship to central sample processing laboratory.	**Pre-transplant** Within 1-3 weeks prior to initiation of conditioning Within 1 week prior to initiation of conditioning **Post-transplant** Day 100, and 6, 12 and 24 months	Peripheral blood tubes will be shipped at ambient temperature on the day of collection, to the BMT CTN 0903-specific Central Processing Laboratory by priority overnight FED EX delivery for plasma and PBMC processing, temporary storage and batched distribution directly to the project laboratory for testing.

RESEARCH TOPIC	RESEARCH SAMPLE	TYPE OF SAMPLE	SAMPLE COLLECTION, PROCESSING AND STORAGE REQUIREMENTS	SAMPLE COLLECTION TIME POINTS	SHIPPING SPECIFICATIONS
Characterization of HIV Infection and Latent HIV Reservoirs	**Latent HIV Reservoir Measurements**	180 mL peripheral blood sample collected in 18-10 mL EDTA containing, lavender-top Vacutainer tubes.	Gently mix blood with the EDTA anticoagulant by inverting the tube 8-10 times. Store at room temperature while preparing to ship to project laboratory.	**Pre-transplant** Once the patient is consented and enrolled on the trial, sample will be collected at approximately 8 weeks prior to initiation of ablative therapy for only those patients that have documented undetectable plasma HIV-1 by standard assay. **Post-transplant** Will be scheduled sometime at 13 months post-transplant, for only those patients that are found to have undetectable plasma HIV-1 RNA by the standard assay performed at the clinical site on the 12 month sample.	Peripheral blood tubes will be shipped at ambient temperature on the day of collection, to the Project Laboratory by priority overnight FED EX delivery for processing and research testing. Guidelines for specimen handling and shipment to the Project Laboratory is detailed in the BMT CTN 0903 Laboratory Sample Guide.
Immune Reconstitution Studies	**T/NK Immunophenotyping, Innate Immune Function, Adaptive Immune Function,**	37 mL peripheral blood sample will be collected in four 8.5 mL-fill ACD solution (yellow top) containing Vacutainer tubes **and** one 3 mL Vacutainer tube (lavender) containing EDTA.	Gently mix blood with the ACD anticoagulant by inverting the tube 8-10 times. Store at room temperature while preparing to ship to project laboratory	**Post-transplant** 8 weeks, and 6 and 12 months post-transplant	Peripheral blood tubes will be shipped at ambient temperature on the day of collection, to the Project Laboratory by priority overnight FED EX delivery for processing and research testing. Guidelines for specimen handling and shipment to the Project Laboratory is detailed in the BMT CTN 0903 Laboratory Sample Guide.

RESEARCH TOPIC	RESEARCH SAMPLE	TYPE OF SAMPLE	SAMPLE COLLECTION, PROCESSING AND STORAGE REQUIREMENTS	SAMPLE COLLECTION TIME POINTS	SHIPPING SPECIFICATIONS
(Optional) Investigational Future Research Sample	**Undefined Future Research**	17 mL peripheral blood sample collected in 2-8.5 mL ACD containing, yellow-top Vacutainer tubes.	Gently mix blood with ACD by inverting the tube 8-10 times. Store at room temperature while preparing to ship to the ACSR Sample Repository.	**Pre-transplant** 1-3 weeks prior to ablative therapy	Peripheral blood tubes will be shipped at ambient temperature on the day of collection, to the Aids Cancer Research Specimen Repository by priority overnight FED EX delivery for processing and research testing. Guidelines for specimen handling and shipment to the Project Laboratory is detailed in the BMT CTN 0903 Laboratory Sample Guide.

APPENDIX D

TREATMENT REVIEW COMMITTEE

APPENDIX D

TREATMENT REVIEW COMMITTEE

The treating institution will review patients with the Treatment Review Committee prior to the initiation of transplant therapy by a five member review panel composed of experts in HIV drug resistance, HIV care, pharmacology, transplant infectious diseases and oncology. Recommendations for transplant are based on data contained in the patient registration and pre-transplant forms, as well as in supporting documentation, which includes the following:

- 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, if applicable
- Current ARV Drug Regimen
- Current Concomitant Medications

The Committee will review available past and current HIV-1 genotype results for patients with detectable viremia so as to exclude patients with multi-drug resistant HIV-1 who are unlikely to achieve viral suppression on alternate antiretroviral therapy. The Committee will also advise with regard to various drug interactions anticipated during the course of transplantation. The Treatment Review Committee will include well-qualified investigators from the University of Pittsburgh and Johns Hopkins University and other institutions as appropriate. Review materials will be sent to the panel four weeks prior to initiation of transplant therapy to allow sufficient time for implementation of recommendations for the antiretroviral regimen.

APPENDIX E

SUGGESTED PROPHYLAXIS

APPENDIX E

SUGGESTED PROPHYLAXIS

Infectious prophylaxis for HIV patients 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. Patient tolerance (nausea, allergic reaction, G6PD or other considerations) may contraindicate a particular regimen. Choices in order of preference are: 1) TMP/SMX 1 DS daily; 2) TMP/SMX 1 SS 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: Patients on TMP/SMX do not require additional prophylaxis. If toxoplasma IgG is positive and TMP/SMX cannot be used patients 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 patients may be unable to take po medications in the peri-transplant period, PCR for toxoplasmosis should be checked at least weekly whenever prophylaxis must be held in toxoplasma IgG positive patients for more than a week and the CD4 count is less than 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 antiretroviral medications.)
5. HSV/VZV: One of the following regimens should be used for 12 months after HCT, or per institutional standard, unless the patient remains on immunosuppressive Acyclovir 400 - 800 mg bid, valaciclovir 500 mg bid, or famciclovir 500 mg po bid.
6. M. Avium Complex (MAC): If CD4 less than 50, Azithromycin suggested at 1200 mg q week or 600 mg twice weekly.
7. Hepatitis:
    a. Patients with positive hepatitis B surface antigen should be evaluated for viral DNA replication (viral load) by a quantitative PCR method before enrolling the patient 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. Patients should be maintained on anti-Hepatitis B treatment throughout chemotherapy, throughout the transplant, and at least 12 months after the transplant.

d. Patients with hepatitis-C infection may be enrolled on the trial providing the above Hepatic criteria are met. Anti-hepatitis C treatment with ribavirin and interferon alpha is recommended but not required to be eligible for the study.

e. Liver biopsy must be performed in patients with Hepatitis-B or C infections if the severity assessment of liver disease based on Child-Turcotte-Pugh (CTP) classification indicates all of the following criteria; Serum bilirubin $\geq$ 2, serum albumin $\leq$ 3.5, and INR $\geq$ 1.7

f. Patients with no pathologic evidence of irreversible chronic liver disease such as bridging necrosis and/or significant fibrosis can be eligible for the study.

APPENDIX F

PHARMACOKINETIC DRUG-DRUG INTERACTIONS WITH ANTIRETROVIRAL MEDICATIONS

APPENDIX F

PHARMACOKINETIC DRUG-DRUG INTERACTIONS WITH ANTIRETROVIRAL MEDICATIONS

Preferred Antiretroviral Regimens for Patients Undergoing Allogeneic Hematopoietic Cell Transplant

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)

Tier 3: Boosted-PI based therapy (interruption of therapy may be required, see Table F-1)

Summary of Antiretroviral Agents and Drug-Drug Interactions

1. **Protease Inhibitors [PI]** (amprenavir, atazanavir, darunavir, fosamprenavir, indinavir, lopinavir/ritonavir, nelfinavir, ritonavir, saquinavir, tipranavir)
    a. Dosing
        i. All protease inhibitors (except nelfinavir) are typically given in combination with ritonavir to reach optimal pharmacokinetic targets
    b. Toxicity
        i. All protease inhibitors can cause gastrointestinal intolerance, hyperglycemia, lipohypertrophy, osteonecrosis, hepatotoxicity and hyperlipidemia.
    c. Metabolism
        i. Protease inhibitors are metabolized hepatically via cytochrome P450 co-enzymes, especially CYP3A4
    d. Drug Interactions
        i. When given in combination with ritonavir, all protease inhibitors are potent CYP3A4 inhibitors
        ii. Ritonavir
            1. Substrate for CYP1A2 (minor), 2B6 (minor), 2D6 (major), 3A4 (major)
            2. Inhibits CYP 2C8 (strong), 2C9 (weak), 2C19 (weak), 2D6 (strong), 2E1 (weak), 3A4 (strong)
            3. Induces CYP1A2 (weak), 2C8 (weak), 2C9 (weak), 3A4 (weak)

2. **Non-Nucleoside Reverse Transcriptase Inhibitors [NNRTI]** (delavirdine, efavirenz, nevirapine, etravirine)
    a. Dosing
        i. Efavirenz is the preferred agent in this class, nevirapine should be used with caution due to toxicity

b. Toxicity
    i. Class adverse effects include, rash, hepatotoxicity and gastrointestinal intolerance
c. Metabolism
    i. 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)

3. **Nucleoside Reverse Transcriptase Inhibitors [NRTI]** (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zidovudine)
    a. Dosing
        i. Zidovudine (AZT) should not be used in patients receiving hematopoietic cell transplantation
    b. Toxicity
        i. Adverse effects of the class include lactic acidosis, peripheral neuropathy and hepatic steatosis
    c. Metabolism
        i. 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

4. **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

TABLE F-1: SUMMARY OF ANTIRETROVIRAL DRUG-DRUG INTERACTIONS BASED ON CONDITIONING REGIMEN TO BE USED

Conditioning Regimen	Metabolism (Primary Site)	Interaction Potential* (Predicted Effect)	Recommendation/Evidence
Flu/Bu • Fludarabine • Bulsulfan	**Fludarabine (Liver):** • Rapid dephosphorylation to 2-fluoro-ara-A, then phosphorylated to 2-fluoro-ara-ATP intracellularly **Busulfan (Liver):** • Metabolized hepatically; substrate of CYP3A4	**High** Concomitant administration of ritonavir-boosted PIs may increase the levels of busulfan. NNRTIs may decrease levels of busulfan. **Strength of evidence: LOW**	**Raltegravir-Based:** May continue HAART as tolerated **NNRTI-Based:** May continue HAART as tolerated **PI-Based:** Regimens including ritonavir should be discontinued a minimum of 48 hours prior to the administration of myeloablative regimens containing busulfan Antiretroviral therapy should not be resumed until, at least, 48 hours after the last dose of busulfan and once the patient is able to consistently tolerate oral medications RIC with Flu/Bu has been given to one patient who was concomitantly receiving unboosted atazanavir and two patients receiving efavirenz (1) Flu/TBI was given successfully with concomitant efavirenz-based HAART in two patients (2)
Flu/Mel • Fludarabine • Melphalan	**Fludarabine (Liver):** • Rapid dephosphorylation to 2-fluoro-ara-A, then phosphorylated to 2-fluoro-ara-ATP intracellularly **Melphalan (Blood):** • Chemical hydrolysis in the plasma to inactive metabolites	**Low** The effects of ritonavir-boosted PIs on levels of fludarabine are unknown. Efavirenz has been given in combination with fludarabine. **Strength of evidence: MODERATE**	**Raltegravir-Based:** May continue HAART as tolerated **NNRTI-Based:** May continue HAART as tolerated **PI-Based**: The potential for interactions with ritonavir-boosted PIs or NNRTIs is low. Antiretroviral therapy should only be continued if the benefits clearly outweigh the risks. Flu/Mel has been given successfully as a reduced-intensity conditioning regimen in a patient on lopinavir/ritonavir, tenofovir, and lamivudine (3) Flu/TBI was given successfully with concomitant efavirenz-based HAART in two patients (2)

Conditioning Regimen	Metabolism (Primary Site)	Interaction Potential* (Predicted Effect)	Recommendation/Evidence
Cy/TBI • Cyclophosphamide • Total Body Irradiation	**Cyclophosphamide (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	**High** Concomitant administration of ritonavir may increase the levels of busulfan. Complex interactions exist between ritonavir and cyclophosphamide or acrolein. Cyclophosphamide levels may be increased when combined with protease inhibitors, but the active metabolites may be decreased. 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%. **Strength of evidence: LOW**	**Raltegravir-Based:** May continue HAART as tolerated **NNRTI-Based:** Regimens including NNRTIs should be used with caution and frequent monitoring due to the increase in toxic metabolites caused by CYP450 induction **PI-Based:** Regimens including ritonavir should be discontinued a minimum of 48 hours prior to the administration of myeloablative regimens containing cyclophosphamide. Antiretroviral therapy should not be resumed until, at least, 72 hours after the last dose of cyclophosphamide and once the patient 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.
RIC = Reduced intensity conditioning

TABLE F-2: EXPERIENCE WITH DRUG-DRUG INTERACTIONS IN PATIENTS RECEIVING AUTOLOGOUS STEM-CELL TRANSPLANTATION

Study	Conditioning Regimen	Antiretroviral Regimen	Interactions/Toxicity
Krishnan (6) ASCT, n=20	BCNU, VP-16, Cy (n=17) or TBI, VP-16, Cy (n=3)	*PI-based (n=15) NNRTI-based (n=5)	• Only 9/20 patients tolerated ARV throughout transplantation • 19/20 had abnormal LFTs • ARV resumed by 2 months after ASCT in most • 10/17 survivors required a change in ARV due to virologic failure • One patient died of regimen-related toxicity at Day +22
Spitzer (7) ASCT, n=20	Dose-reduced Bu, Cy Bu: 14 mg/kg (PO) or 11.2 mg/kg (IV) Cy: 120 mg/kg	*PI-based (n=20) *(Ritonavir and AZT not included)*	• One patient died of regimen-related toxicity at Day +33 (VOD) • No grade III or IV nausea or vomiting • Number of patients who discontinued ARV is unknown
Re (8) ASCT, n=10	BEAM	*NA	• 7/10 patients had gastrointestinal toxicity and 2/10 had hepatic toxicity • 3/10 patients stopped ARV after BEAM due to toxicity
Balsalobre (9) ASCT, n=68	BEAM and variates (n=65) TBI based regimens (n=3)	NA	• HAART continued in 95% of patients, but withdrawn in 22.5% • Of the patients who discontinued HAART, mucositis was the most common reason
Serrano (10) ASCT, n=11	BEAM (n=10) or BEAC (n=1)	*NNRTI-based (n=8) PI-based (n=2)	• No patients died from treatment related complications • No apparent interference between HAART and conditioning regimens was observed • No patients were concomitantly receiving AZT

*HAART continued through conditioning.
ASCT = Autologous Stem-Cell Transplantation; AZT = Zidovudine

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TABLE F-3: PHARMACOKINETIC INTERACTIONS FOR GVHD PROPHYLAXIS AND ANTIRETROVIRALS

I. Tacrolimus

a. Standard Dose
   i. IV: 0.03 mg/kg every 24 hours as a continuous IV infusion to begin 3 days prior to transplant (based on IBW)
   ii. PO: 0.12 mg/kg/day divided into 2 doses (Convert to oral administration when patient is able to tolerate)

b. Target Trough Level
   i. 5 – 15 ng/mL

Concomitant Medication	Effect on Tacrolimus	Mechanism	Recommendation
Protease Inhibitors (boosted) • Atazanavir/ritonavir (ATZ/r) • Fosamprenavir/ritonavir (fAPV/r) • Indinavir/ritonavir (IND/r) • Lopinavir/ritonavir (LPV/r) • Saquinavir/ritonavir (SQV/r) • Tipranavir/ritonavir (TPV/r)	↑↑↑ Tacrolimus Levels [1-9] May result in severe and prolonged tacrolimus toxicity.	Potent inhibition of CYP3A4 and P-glycoprotein	Hold tacrolimus upon initiation of ARV and monitor trough levels daily Based on level: • **< 5 ng/mL:** Administer 1 mg (PO) or 0.25 mg (IV)* • **5-15 ng/mL:** Administer 0.5 mg (PO) or 0.125 mg (IV)* • **>15 ng/mL:** HOLD tacrolimus until next level Once dosing is stable, tacrolimus levels may be monitored according to local guidelines. Most patients are maintained on 0.5 – 1 mg/week.
Protease Inhibitors (unboosted) • Atazanavir (ATZ) • Nelfinavir (NFV)	↑↑ Tacrolimus Levels [2, 6-7, 10-11] May result in severe and prolonged tacrolimus toxicity.	Strong inhibition of CYP3A4	Hold tacrolimus upon initiation of ARV and monitor trough levels daily Based on level: • **< 5 ng/mL:** Administer 1 mg (PO) or 0.25 mg (IV)* • **5-15 ng/mL:** Administer 0.5 mg (PO) or 0.125 mg (IV)* • **>15 ng/mL:** HOLD tacrolimus until next level Once dosing is stable, tacrolimus levels may be monitored according to local guidelines.
NNRTIs • Efavirenz (EFV) • Nevirapine (NVP)	↓ Tacrolimus Levels [6]	Strong induction of CYP3A4	Continue tacrolimus at the same dose and monitor trough levels daily. Based on level: • **< 5 ng/mL:** Increase tacrolimus daily dose by 25% • **5-15 ng/mL:** No adjustment • **>15 ng/mL:** Decrease tacrolimus daily dose by 25% Full induction effects may take up to two weeks. Daily tacrolimus doses are substantially increased for most patients with co-administration of NNRTIs. Efavirenz is a more potent inducer of CYP3A4 than is nevirapine.

Concomitant Medication	Effect on Tacrolimus	Mechanism	Recommendation
NNRTIs • Etravirine (ETV)	↓↓ Tacrolimus Levels (Thoeretical)	Potent induction of CYP3A4	Concomitant use of etravirine and tacrolimus should be discouraged.
NRTIs • Abacavir (ABC) • Didanosine (ddI) • Emtricitabine (FTC) • Lamivudine (3TC) • Stavudine (d4T) • Tenofovir (TDF) • Zidovudine (AZT, ZDV)	None [12]	N/A	No adjustments necessary. Monitor tacrolimus 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.
Integrase Inhibitor • Raltegravir (RLV)	None	N/A	No adjustments necessary. Monitor tacrolimus trough levels 2-3 times/week. Raltegravir based regimens may be preferred over PI- or 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 tacrolimus trough levels 2-3 times/week.
CCR5 Antagonist • Maraviroc (MVC)	None	N/A	No adjustments necessary. Monitor tacrolimus trough levels 2-3 times/week.

*To be administered in a one-time dose in response to the observed level.

II. Sirolimus

a. Standard Dose
   i. PO: 12 mg once on Day -3, then 4 mg daily as a single dose (administer on an empty stomach)

b. Target Trough Level
   i. 3 – 12 ng/mL

Concomitant Medication	Effect on Sirolimus	Mechanism	Recommendation
Protease Inhibitors (boosted) • Atazanavir/ritonavir (ATZ/r) • Fosamprenavir/ritonavir (fAPV/r) • Indinavir/ritonavir (IND/r) • Lopinavir/ritonavir (LPV/r) • Saquinavir/ritonavir (SQV/r) • Tipranavir/ritonavir (TPV/r)	↑↑↑ Sirolimus Levels [3, 8] May result in severe and prolonged sirolimus toxicity.	Potent inhibition of CYP3A4 and P-glycoprotein	Hold sirolimus upon initiation of ARV and monitor trough levels daily Based on level: • **< 3 ng/mL:** Administer 0.4 mg (PO) • **3 - 12 ng/mL:** Administer 0.3 mg (PO) • **>12 ng/mL:** HOLD sirolimus until next level Once dosing is stable, sirolimus levels may be monitored according to local guidelines.
Protease Inhibitors (unboosted) • Atazanavir (ATZ) • Nelfinavir (NFV)	↑↑ Sirolimus Levels [13] May result in severe and prolonged sirolimus toxicity.	Strong inhibition of CYP3A4	Hold sirolimus upon initiation of ARV and monitor trough levels daily Based on level: • **< 3 ng/mL:** Administer 0.4 mg (PO) • **3 - 12 ng/mL:** Administer 0.3 mg (PO) • **>12 ng/mL:** HOLD sirolimus until next level Once dosing is stable, sirolimus levels may be monitored according to local guidelines.
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.

Concomitant Medication	Effect on Sirolimus	Mechanism	Recommendation
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) • Zidovudine (AZT, ZDV)	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.
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 PI- or 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.

III. Cyclophosphamide

a. Standard dose[24]

i. 50 mg/kg IV over 1-2 hours is given on Day 3 post-transplant **(between 60 and 72 hours after marrow infusion)** and again on day 4 post-transplant (approximately 24 hours after first dose) (based on IBW)

Concomitant Medication	Effect on Cyclophosphamide (Cy)	Mechanism	Recommendation
Protease Inhibitors (boosted) • Atazanavir/ritonavir (ATZ/r) • Fosamprenavir/ritonavir (fAPV/r) • Indinavir/ritonavir (IND/r) • Lopinavir/ritonavir (LPV/r) • Saquinavir/ritonavir (SQV/r) • Tipranavir/ritonavir (TPV/r)	The conversion of Cy (a prodrug) to its active metabolites will be delayed, resulting in increased levels of Cy in the serum.	Inhibition of CYP3A4	Conversion of Cy to its active metabolites will be delayed, resulting in reduced efficacy of Cy. Certain metabolites may be increased or decreased. **Hold ritonavir-boosted regimens during GVHD prophylaxis.**
Protease Inhibitors (unboosted) • Atazanavir (ATZ) • Nelfinavir (NFV)	The conversion of Cy (a prodrug) to its active metabolites will be delayed, resulting in increased levels of Cy in the serum.	Inhibition of CYP3A4	Conversion of Cy to its active metabolites will be delayed, resulting in reduced efficacy of Cy. Certain metabolites may be increased or decreased. The concomitant use of unboosted PIs should be used with caution as Cy efficacy may be decreased. Consultation with pharmacokinetic team is required.
NNRTIs • Efavirenz (EFV) • Nevirapine (NVP)	Rapid conversion of Cy to its active metabolites results in decreased Cy concentrations and increase levels of active metabolites	Induction of CYP3A4 and CYP2B6	Dose reductions of Cy may be recommended to prevent toxicity. This has been shown to be effective with other CYP inducers, such as phenytoin.[25] Monitor patients closely for toxicity from active metabolites of cyclophosphamide.
NNRTIs • Etravirine (ETV)	Rapid conversion of Cy to its active metabolites results in decreased Cy concentrations and increase levels of active metabolites	Potent induction of CYP3A4 and CYP2B6	Avoid concomitant use due to the unpredictable interaction potential.
NRTIs • Abacavir (ABC) • Didanosine (ddI)	None	N/A	No adjustment necessary

Concomitant Medication	Effect on Cyclophosphamide (Cy)	Mechanism	Recommendation
NRTIs • Stavudine (d4T) • Zidovudine (AZT, ZDV)	None	N/A	No adjustment necessary
NRTIs • Lamivudine (3TC) • Emtricitabine (FTC) • Tenofovir (TDF)	None	N/A	No adjustments necessary
Integrase Inhibitor • Raltegravir (RLV)	None	N/A	No adjustments necessary.
Fusion Inhibitor • Enfuviritide (ENF, T20)	None	N/A	No adjustments necessary.
CCR5 Antagonist • Maraviroc (MVC)	None	N/A	No adjustments necessary.

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TABLE F-4: CONTINUATION OF ANTIRETROVIRAL THERAPY BASED ON ANTIFUNGAL PROPHYLAXIS REGIMEN

*Monitor azole drug levels routinely.

Antifungal Agent	Raltegravir Based Regimen	NNRTI Based Regimen	Boosted PI Based Regimen	Other
Voriconazole	No adjustments	↑ voriconazole dose to 400mg BID* ↓ efavirenz dose to 300mg daily.	Consider alternative antifungal agent; monitor voriconazole levels and increase dose based on level*	Consultation recommended, reviewed on a case by case basis
Posaconazole	No adjustments	Consider alternative antifungal agent; monitor posaconazole levels and increase dose as needed*	Consider alternative antifungal agent; monitor closely for PI toxicity*	Consultation recommended, reviewed on a case by case basis
Fluconazole	No adjustments	No adjustments. Monitor closely for hepatotoxicity.	Do not use doses >200mg/day	Consultation recommended, reviewed on a case by case basis
Echinocandins (Anidulafungin, Micafungin, Caspofungin)	No adjustments	No adjustments	No adjustments	Consultation recommended, reviewed on a case by case basis
Amphotericin B and derivatives	No adjustments	No adjustments	No adjustments	Consultation recommended, reviewed on a case by case basis†

TABLE F-5: DOSE ADJUSTMENTS FOR CONCOMITANT USE OF ANTIFUNGAL AGENTS AND IMMUNOSUPPRESSION

*Monitor immunosuppression levels routinely.

Antifungal Agent	Tacrolimus (FK) Cyclosporine (CY)	Sirolimus	Mycophenolate	Cyclophosphamide[28]
Voriconazole	↓ FK dose by 67%* ↓ CY dose by 50%*	Avoid concomitant use (see below) ↓ Sirolimus dose by 90%*	No adjustments	Avoid concomitant use (see below)
Posaconazole	↓ FK dose by 67%* ↓ CY dose by 25%*	Avoid concomitant use (see below) ↓ Sirolimus dose by 67%	No adjustments	Avoid concomitant use (see below)
Fluconazole	No adjustments*	No adjustments*	No adjustments	No adjustments (see below)
Echinocandins (Anidulafungin, Micafungin, Caspofungin)	No adjustments	No adjustments	No adjustments	No adjustments
Amphotericin B and derivatives	No adjustments	No adjustments	No adjustments	No adjustments

TABLE F-6: PHARMACOKINETIC INTERACTIONS BETWEEN ANTIFUNGAL, ANTIRETROVIRAL AND IMMUNOSUPPRESSIVE AGENTS

I. Voriconazole

a. Standard Dose
   i. IV: 6 mg/kg every 12 hours for 2 doses, then 4 mg/kg every 12 hours
   ii. PO: 4 mg/kg every 12 hours for 2 doses, then 200 mg twice daily

b. Target Trough Level
   i. 0.5 – 5 µg/mL

Concomitant Medication	Voriconazole Effect [24,25]	Mechanism	Recommendation
Calcineurin Inhibitors • Tacrolimus • Cyclosporine	↑↑ Calcineurin Levels	Moderate inhibition of CYP3A4	Upon initiation of voriconazole: • Decrease tacrolimus daily dose by 67% • Decrease cyclosporine daily dose by 50% Monitor daily trough levels of the calcineurin inhibitors and adjust doses as needed.
mTOR Inhibitors • Sirolimus	↑↑↑ Sirolimus Levels	Moderate inhibition of CYP3A4	**Voriconazole should not be used unless deemed absolutely clinically necessary**. If voriconazole must be added to control fungal infection, then following the 90% reduction in sirolimus dosing serum levels should be measured 24-48 hours later and then every 3-4 days until levels are stable and in the desired range. If voriconazole is given intravenously, or if voriconazole and sirolimus are not given together, these guidelines may not apply because the effect on bioavailability of sirolimus will be weaker.
Antimetabolites • Mycophenolate mofetil	None	N/A	No adjustments necessary.
Alkylating Agents • Cyclophosphamide	↑↑ Cyclophosphamide and 4-hydroxycyclophosphamide	Moderate inhibition of CYP3A4 and 2C19	Concomitant use is discouraged due to the accumulation of the toxic metabolite 4-hydroxycyclophosphamide[28]
Protease Inhibitors • Ritonavir (400mg BID)	↓↓↓ Voriconazole (Decreased by 66 – 80%)	Strong induction of CYP2C9 and 2C19	Contraindicated.
Protease Inhibitors (boosted) • Fosamprenavir/ritonavir (fAPV/r) • Indinavir/ritonavir (IND/r) • Lopinavir/ritonavir (LPV/r) • Saquinavir/ritonavir (SQV/r) • Tipranavir/ritonavir (TPV/r)	↓↓ Voriconazole (Decreased by 24 – 40%)	Strong induction of CYP2C9 and CYP2C19	Avoid concomitant use whenever possible. Monitor voriconazole trough levels bi-weekly and adjust as needed.

Concomitant Medication	Voriconazole Effect [24,25]	Mechanism	Recommendation
Protease Inhibitors (unboosted) • Amprenavir (AMP) • Atazanavir (ATZ) • Indivavir (IDV) • Nelfinavir (NFV) • Saquinavir (SQV)	↑ Protease Inhibitor Level	Weak inhibition of CYP3A4	Frequent monitoring of adverse effects.
NNRTIs • Efavirenz (EFV)	↓ Voriconazole (Decrease AUC by 77%) ↑ Efavirenz (Increase AUC by 44%)	Two way interaction via CYP3A4	Increase voriconazole dose to 400 mg twice daily and decrease efavirenz dose to 300mg daily. Monitor voriconazole trough levels bi-weekly and adjust as needed.
NNRTIs • Etravirine (ETV)	↑ Voriconazole (possible) ↑ Etravirine (possible)	Two way interaction via CYP3A4	Frequent monitoring of adverse effects. Monitor voriconazole trough levels bi-weekly and adjust as needed.
NNRTIs • Nevirapine (NVP)	↓ Voriconazole (possible) ↑ Nevirapine (possible)	Two way interaction via CYP3A4	Frequent monitoring of adverse effects. Monitor voriconazole trough levels bi-weekly and adjust as needed.
NRTIs • Abacavir (ABC) • Didanosine (ddI) • Emtricitabine (FTC) • Lamivudine (3TC) • Stavudine (d4T) • Tenofovir (TDF) • Zidovudine (AZT, ZDV)	None	N/A	No adjustments necessary.
Integrase Inhibitor • Raltegravir (RLV)	None	N/A	No adjustments necessary. Raltegravir based regimens may be preferred over PI- or 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.
CCR5 Antagonist • Maraviroc (MVC)	↑ Maraviroc Levels	N/A	Decreased maraviroc dose to 150 mg twice daily

II. Posaconazole

a. Standard Dose
   i. PO: 200 mg three times a day (5 mL of oral suspension given with a high-fat meal)
b. Target Trough Level
   i. ≥ 0.5 μg/mL

Concomitant Medication	Posaconazole Effect [24,26]	Mechanism	Recommendation
Calcineurin Inhibitors • Tacrolimus • Cyclosporine	↑↑ Calcineurin Levels	Moderate inhibition of CYP3A4	Upon initiation of posaconazole: • Decrease tacrolimus daily dose by 67% • Decrease cyclosporine daily dose by 25% Monitor daily trough levels of the calcineurin inhibitors and adjust doses as needed.
mTOR Inhibitors • Sirolimus	↑↑↑ Sirolimus Levels (Increase by 788%)	Moderate inhibition of CYP3A4	Contraindicated, use alternative antifungal therapy whenever possible.
Antimetabolites • Mycophenolate mofetil	None	N/A	No adjustments necessary.
Alkylating Agents • Cyclophosphamide	↑↑ Cyclophosphamide and 4-hydroxycyclophosphamide	Moderate inhibition of CYP3A4	Concomitant use is discouraged due to the accumulation of the toxic metabolite 4-hydroxycyclophosphamide[28]
Protease Inhibitors (boosted) • Fosamprenavir/ritonavir (fAPV/r) • Indinavir/ritonavir (IND/r) • Lopinavir/ritonavir (LPV/r) • Saquinavir/ritonavir (SQV/r) • Tipranavir/ritonavir (TPV/r)	↑ Protease Inhibitor Level	Weak inhibition of CYP3A4	Frequent monitoring of adverse effects. Consider alternative antifungal therapy. Monitor posaconazole trough levels bi-weekly and adjust as needed. Dose of posaconazole may be decreased with high level.
Protease Inhibitors (unboosted) • Amprenavir (AMP) • Atazanavir (ATZ) • Indivavir (IDV) • Nelfinavir (NFV) • Saquinavir (SQV)	↑ Protease Inhibitor Level	Weak inhibition of CYP3A4	Frequent monitoring of adverse effects. Atazanavir AUC increased by 268%.

Concomitant Medication	**Posaconazole Effect** [24,26]	**Mechanism**	**Recommendation**
NNRTIs • Efavirenz (EFV)	↓ Posaconazole (Decrease AUC by 50%)	Strong induction of CYP3A4 and UDP-G induction	Consider alternative antifungal therapy. Monitor posaconazole trough levels bi-weekly and adjust as needed. Dose may be increased with low level.
NNRTIs • Etravirine (ETV)	↑ Etravirine (possible)	Weak inhibition of CYP3A4	No adjustments necessary.
NNRTIs • Nevirapine (NVP)	↓ Posaconazole (possible) ↑ Nevirapine (possible)	Two way interaction via CYP3A4	Frequent monitoring of adverse effects. Monitor Posaconazole trough levels bi-weekly and adjust as needed.
NRTIs • Abacavir (ABC) • Didanosine (ddI) • Emtricitabine (FTC) • Lamivudine (3TC) • Stavudine (d4T) • Tenofovir (TDF) • Zidovudine (AZT, ZDV)	None	N/A	No adjustments necessary.
Integrase Inhibitor • Raltegravir (RLV)	None	N/A	No adjustments necessary. Raltegravir based regimens may be preferred over PI- or 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.
CCR5 Antagonist • Maraviroc (MVC)	None	N/A	No adjustments necessary.

III. Fluconazole

a. Standard Dose
   i. IV: 200 – 400 mg daily (Convert to oral administration when patient is able to tolerate)
   ii. PO: 200 – 400 mg daily

Concomitant Medication	Fluconazole Effect [24,27]	Mechanism	Recommendation
Calcineurin Inhibitors • Tacrolimus • Cyclosporine	↑ Calcineurin Levels	Weak inhibition of CYP3A4	Monitor daily trough levels of the calcineurin inhibitors and adjust doses as needed.
mTOR Inhibitors • Sirolimus	↑ Sirolimus Levels	Weak inhibition of CYP3A4	Monitor daily trough levels of the calcineurin inhibitors and adjust doses as needed.
Antimetabolites • Mycophenolate mofetil	None	N/A	No adjustments necessary.
Alkylating Agents • Cyclophosphamide	Minimal effect on cyclophosphamide	Minimal inhibition of 2C9	No adjustments necessary.[28]
Protease Inhibitors (boosted) • Fosamprenavir/ritonavir (fAPV/r) • Indinavir/ritonavir (IND/r) • Lopinavir/ritonavir (LPV/r) • Saquinavir/ritonavir (SQV/r) • Tipranavir/ritonavir (TPV/r)	None ↑ Tipranavir Levels	Weak inhibition of CYP3A4	No adjustments necessary. Fluconazole doses > 200 mg are not recommended with co-administration of TPV/r.
Protease Inhibitors (unboosted) • Amprenavir (AMP) • Atazanavir (ATZ) • Indivavir (IDV) • Nelfinavir (NFV) • Saquinavir (SQV)	None	N/A	No adjustments necessary.
NNRTIs • Efavirenz (EFV)	None	N/A	No adjustments necessary.
NNRTIs • Etravirine (ETV)	↑ Etravirine (possible)	N/A	No adjustments necessary.

Concomitant Medication	Fluconazole Effect [24,27]	Mechanism	Recommendation
NNRTIs • Nevirapine (NVP)	↑ Nevirapine (Increase AUC by 110%)	Weak inhibition of CYP3A4	Frequent monitoring of liver function tests as combination results in an increased risk of hepatotoxicity.
NRTIs • Abacavir (ABC) • Didanosine (ddI) • Emtricitabine (FTC) • Lamivudine (3TC) • Stavudine (d4T) • Tenofovir (TDF) • Zidovudine (AZT, ZDV)	None	N/A	No adjustments necessary.
Integrase Inhibitor • Raltegravir (RLV)	None	N/A	No adjustments necessary. Raltegravir based regimens may be preferred over PI- or 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.
CCR5 Antagonist • Maraviroc (MVC)	None	N/A	No adjustments necessary.

IV. Echinocandin Agents (Anidulafungin, Micafungin, Caspofungin)

a. Drug Interactions

i. Not metabolized through CYP co-enzymes. The potential for drug-drug interactions is low.

V. Aphotericin B and derivatives

a. Drug Interactions

i. Not metabolized through CYP co-enzymes. The potential for drug-drug interactions is low.

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APPENDIX G

KARNOFSKY AND LANSKY PERFORMANCE STATUS SCALES

APPENDIX G

KARNOFSKY AND LANSKY PERFORMANCE STATUS SCALES

KARNOFSKY SCALE ≥ 16 YEARS

Percentage	
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self; unable to carry on normal activity or do active work
60	Requires occasional assistance, but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled, hospitalization indicated. Death not imminent
20	Very sick, hospitalization necessary, active supportive treatment necessary
10	Moribund, fatal processes, progressing rapidly
0	Dead

LANSKY SCALE < 16 YEARS

Percentage	
100	Fully Active
90	Minor restriction in physically strenuous play
80	Restricted in strenuous play, tires more easily, otherwise active
70	Both greater restrictions of, and less time spent in, active play
60	Ambulatory up to 50% of time, limited active play with assistance/supervision
50	Considerable assistance required for any active play; fully able to engage in quiet play
40	Able to initiate quiet activities
30	Needs considerable assistance for quiet activity
20	Limited to very passive activity initiated by others (e.g., TV)
10	Completely disabled, not even passive play
0	Dead

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APPENDIX H

SEQUENTIAL PROBABILITY RATIO TEST USING FUTILITY BOUNDARY

APPENDIX H

SEQUENTIAL PROBABILITY RATIO TEST USING FUTILITY BOUNDARY

I. Background – The Sequential Probability Ratio Test

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

The usual measures of performance of such a procedure are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$, and of accepting H_0 when $\theta = \theta_1$, respectively, and the expected sample size $E(N \mid \theta_j) \equiv E_j(N)$. Wald and Wolfowitz showed that among all tests, sequential or not, for which $\Pr_0(\text{reject } H_0) \le \alpha$ and $\Pr_1(\text{reject } H_0) \le \beta$, and for which $E_j(N)$ are finite, j=0, 1, the SPRT with error probabilities α and β minimizes $E_0(N)$ and $E_1(N)$. If, in addition, $x1, x2, \ldots$ are independent and identically distributed (i.i.d.) with density function $f(x,\theta)$, with monotone likelihood ratio in $\tau(x)$, then any SPRT for testing θ_0 against θ_1 has non-decreasing power function.

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

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

II. Sequential Probability Ratio Test for Binomial Random Variable Using Futility Boundary

The goal of the study is to assess feasibility and safety of allogeneic HCT in HIV-infected patients using 100-day NRM as the primary endpoint. To establish safety we want to show that 100-day NRM is lower than 45% with a targeted NRM probability of 15%. This is equivalent to testing H_0: $p \geq 0.45$ vs. H_a: $p \leq 0.15$. To guard against excessive non-relapse mortality, we wish to construct stopping guidelines using the ***futility boundary*** of an SPRT test of H_0: $p = 0.45$ vs. H_a: $p = 0.15$ with nominal Type I error $\alpha = .07$ and Type II error $\beta = .30$. The stopping boundary is reached when NRM is high and it is no longer likely that we would be able to reject the null hypothesis at the end of the study. Otherwise, the trial will continue on.

Let $Y_1, Y_2, \cdots, Y_n$ be the indicator of non-relapse mortality status at day 100 for patient *i* where $Y_i, i = 1, \cdots, n$, are *i.i.d.* Bernoulli random variables with probability *p*. Let $X = \sum_{i=1}^{n} Y_i$ be the total number of non-relapse deaths observed by day 100, then *X* is a binomial random variable with probability mass function $f(x;p) = \binom{n}{x} p^x (1-p)^{(n-x)}$. The SPRT is derived based on a simple null and alternative hypothesis H_0: $p = p_0 = 0.45$ vs. H_a: $p = p_1 = 0.15$ with $\alpha = .07$ and $\beta = .30$. Since the log-likelihood ratio for the binomial,

$$log[f(x;p_1)/f(x;p_0)] = xlog[p_1/p_0] + (n-x)log[(1-p_1)/(1-p_0)]$$

is a monotone function of *x*, the test has non-decreasing power function. Therefore, the SPRT is a one-sided level 0.07 test for a composite null hypothesis H_0: $p \geq 0.45$ versus a composite alternative hypothesis H_a: $p \leq 0.15$ with power $1 - \beta = .70$.

In this study, stopping only occurs when non-relapse mortality is too high that it is unlikely that the null hypothesis would be rejected at full recruitment. The SPRT pauses when the likelihood-ratio statistics,

$$L_n = \frac{f(x;p_1)}{f(x;p_0)} = \frac{{p_1}^x (1-p_1)^{(n-x)}}{{p_0}^x (1-p_0)^{(n-x)}} < B$$

or equivalently,

$$\log(L_n) = x * log\left(\frac{p_1}{p_0}\right) + (n-x) * log\left(\frac{1-p_1}{1-p_0}\right) < logB$$

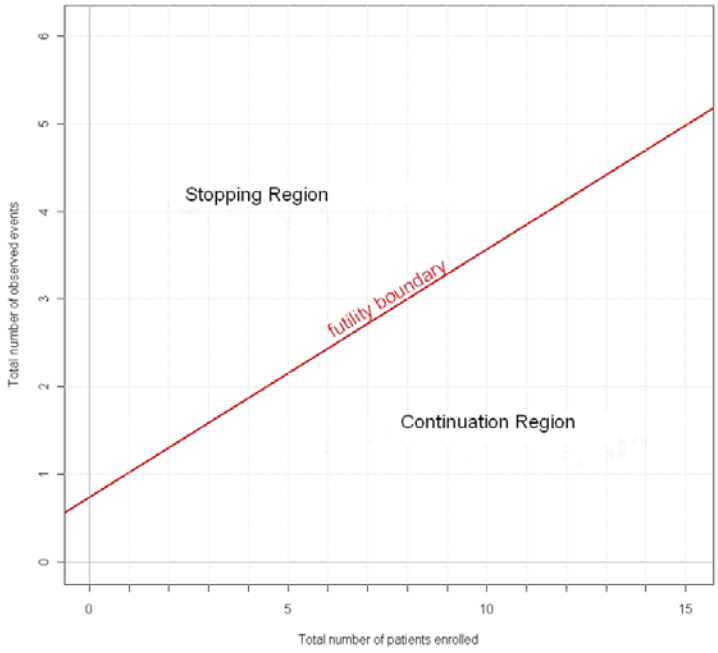
$$x * \left(log\left(\frac{p_1}{p_0}\right) - log\left(\frac{1-p_1}{1-p_0}\right)\right) < logB - n * log\left(\frac{1-p_1}{1-p_0}\right).$$

When $p_1 < p_0$, $log(p_1/p_0) - log\big((1-p_1)/(1-p_0)\big) < 0$. The stopping region is defined by

$$x > \frac{logB}{log\left(\frac{p_1}{p_0}\right) - log\left(\frac{1-p_1}{1-p_0}\right)} - n * \frac{log\left(\frac{1-p_1}{1-p_0}\right)}{log\left(\frac{p_1}{p_0}\right) - log\left(\frac{1-p_1}{1-p_0}\right)} = .738 + .283n.$$

The SPRT can be represented graphically by plotting the number of non-relapse deaths by day 100 (*x*) versus the number of patients enrolled in the study (*n*). The stopping boundary is represented by the line with the slope equals to .283 and an intercept of .738. The stopping region is the area above the stopping boundary and the continuation region is the area below the boundary. See figure H.1.

FIGURE H.1 SPRT STOPPING GUIDELINES USING FUTILITY BOUNDARY



The futility stopping guidelines derived from the SPRT are summarized in Table H.1.

TABLE H.1: FUTILITY STOPPING GUIDELINES FOR 100-DAY NRM

Number of patients enrolled (n)	SPRT stopping boundary (x)
2-4	2
5-7	3
8-11	4
12-15	5

The operating characteristics of the truncated SPRT with maximum sample size of 15 resulting from a simulation study with 10,000 replications are shown in Table H.2. The truncated SPRT test has Type I error of 8% and power of 83%.

TABLE H.2: OPERATING CHARACTERISTICS FOR FUTILITY STOPPING GUIDELINES FOR 100-DAY NRM

Non-relapse Mortality				
True 100-day rate	45%	35%	25%	15%
Probability stop and accept the null hypothesis	.92	.75	.47	.17
Mean month stopped	11.8	15.6	20.2	24.5
Mean # endpoints	2.7	2.9	2.8	2.0
Mean patients with 100 days follow up	6.0	8.2	10.8	13.4

APPENDIX I

IDENTIFICATION OF CCR5DELTA32 MUTATION HOMOZYGOUS DONORS

APPENDIX I

NMDP PROCEDURE FOR IDENTIFICATION OF CCR5DELTA32 MUTATION HOMOZYGOUS DONORS RELATED TO BMT CTN PROTOCOL 0903

Background

A recent case report by Hütter et al. (NEJM, 2009) documented the outcome of an allogeneic hematopoietic cell transplant (HCT) performed for the treatment of acute myeloid leukemia (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 the transplant, the recipient's antiretroviral 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 transplant (Hütter, personal communication).

The of BMT CTN 0903 Protocol Team would like to replicate these findings in the context of allogeneic HCT as proposed under Protocol 0903. The enrollment for the study is 15 patients. 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. As a consequence, it is expected that only subject enrolled on 0903 who are of European descent will have a reasonable likelihood of a suitably HLA-matched donor who also has CCR5delta32 homozygosity. It is estimated that of the 15 patients to be enrolled on study, approximately 2/3 or 10 patients will be of European descent. Of those 10 patients, approximately 7 will not have a sibling match. The search for an HLA well-matched donor with homozygosity will therefore be limited to this population. In order to understand how many patients would be expected to have sufficient donors to have a chance of having a CCR5delta32 homozygous donor, 30 consecutive Caucasian patients searching for a suitably matched donor were assessed for availability of potential 5/6 or 6/6 donors at HLA-A, -B, and –DRB1 using HapLogic 2. In that study, 3 of 30 patients had more than 200 potentially matched donors. Another 6 of 30 had at least 20 but less than 100 donors. Thus, of the 15 patients to be studied on 0903, approximately 1 patient is likely to have a suitably matched CCR5delta32 homozygous donor. Two other patients have a smaller chance of identifying such a donor, so it is estimated that 1-2 patients will be able to undergo HCT with such a donor.

It should be noted that the 0903 Protocol Team feels that a patient with multiple unrelated donors should be given the option to receive product from a well-matched unrelated donor even if a

sibling donor is available as long as logistical and financial barriers for such an HCT can be managed.

Guiding Principles for Donor Selection on BMT CTN 0903

1) The first priority for donor selection should be HLA matching such that an 8/8 non-homozygous donor, including a sibling, for example, would be selected over a 7/8 CCR5delta32 homozygous donor.
2) If a sibling donor and a well-matched CCR5delta32 homozygous donor are both available, then the transplant team and patient should decide together which donor is to be selected.

For Whom Will an Assessment of Donor CCR5delta32 Homozygosity Be Done?

1) An attempt to identify a suitably HLA-matched donor with CCR5delta32 homozygosity for a patient screened and enrolled on 0903 will take place when the following minimum conditions are met:
    a. There is a donor with at least a 50% chance of having a 5/6 match using HapLogic 2 or a 7/8 match (HLA-A, -B, -C, -DR) based on HapLogic 3 prediction when available
2) The assumption is that, in parallel with the search for a CCR5delta32 homozygous donor, the standard process for identifying a donor will be followed with typical NMDP and transplant center procedures (i.e. donors will be selected by HLA and non-HLA factors, other than CCR5delta32 mutation status).
3) The identification of a suitable unrelated donor **must not be delayed** by the process to identify a donor who is CCR5delta32 homozygous.

Overview of the Process for Identification of a CCR5delta32 Homozygous Donor

1) When a preliminary search for a patient on 0903 meets the criteria above, potential donors as defined by the HapLogic search and the matching criteria above will be contacted by the NMDP Member Services Donor Contact Team to confirm continued interest and availability to serve as a donor. The Member Services Donor Contact Team will also inform the potential donor that there is a research protocol associated with donation and that the research component will be explained by the CIBMTR Survey Research Group in a separate conversation.
2) For donors who are available and willing to donate, then the donor information will be passed to the CIBMTR Survey Research Group to inform the donor about BMT CTN 0903 and the goal of obtaining a CCR5 delta 32 homozygous donor. The donor will be asked to participate in CCR5delta32 screening and those who provide verbal agreement will be sent a buccal swab kit by mail with a consent form to sign and return and an instruction sheet with the swabs. The potential donor will return the buccal swab to the NMDP Sample Repository for logging and then batching to the CCR5 research testing laboratory. Donors will be informed that CCR5 testing is unlikely to reveal homozygosity and unless they are contacted specifically about homozygosity, then the result did not reveal homozygosity. If the donor is homozygous, s/he will be counseled about the significance of this finding, irrespective of whether that donor is ultimately chosen as the donor for an 0903 subject.

3) The testing laboratory will provide results to the Protocol Officer or his/her NMDP designee and the Protocol Officer or his/her NMDP designee will notify stakeholders as appropriate in the event a CCR5delta32 homozygous donor is identified (as well as all CCR5 homozygous donors as described above). Confirmatory CCR5 testing will be performed in CLIA-certified lab at the time of donor work-up.
4) The Protocol Officer and/or his/her NMDP designee will assist the NMDP teams with the process.

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APPENDIX J

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APPENDIX J

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