A Phase III Randomized, Multicenter Trial Comparing Sirolimus/Tacrolimus with Tacrolimus/Methotrexate as Graft-Versus-Host Disease (GVHD) Prophylaxis After HLA-Matched, Related Peripheral Blood Stem Cell Transplantation

BMT CTN PROTOCOL 0402
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PROTOCOL SYNOPSIS – BMT CTN PROTOCOL 0402

A Phase III Randomized, Multicenter Trial Comparing Sirolimus/Tacrolimus with Tacrolimus/Methotrexate as Graft vs. Host Disease (GVHD) Prophylaxis After HLA-Matched, Related Peripheral Blood Stem Cell Transplantation

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Study Design: The study is designed as a Phase III, randomized, open label, multicenter, prospective, comparative trial of sirolimus and tacrolimus versus tacrolimus and methotrexate as GVHD prophylaxis after HLA-matched, related peripheral blood stem cell transplantation in patients with hematologic malignancies. Recipients will be stratified by transplant center and will be randomized to the sirolimus/tacrolimus or tacrolimus/methotrexate arms in a 1:1 ratio.

Primary Objective: The primary objective is to compare the rates of 114-day Grades II-IV acute GVHD free survival between patients in the two study arms from the time of randomization using an intent-to-treat analysis.

Secondary Objectives: Patients randomized to the two study arms will be compared for the following endpoints: time to neutrophil and platelet engraftment, the incidence of Grades III-IV acute GVHD, mucositis severity, time to first hospital discharge after transplantation, all infections, CMV reactivation and thrombotic microangiopathy incidence and VOD during the 100 days post-transplantation, malignant disease relapse and 1-year relapse-free and overall survival.

Eligibility Criteria: Eligible patients are between 2 and 60 years of age, have acute leukemia, myelodysplasia, chronic myeloid leukemia, adequate organ function, a serologic (or higher resolution) 6/6 Class I HLA-A and B and molecular Class II DRB1 matched sibling donor, and are able to give signed informed consent prior to enrollment.

Treatment Description: Patients will receive one of two conditioning regimens described in the protocol, at the discretion of the transplant physician. The transplant physician must choose among these regimens prior to GVHD prophylaxis assignment by randomization. Conditioning regimens will vary by center but at each center will be the same for patients randomized to either GVHD prophylaxis regimen. Stem cell donors will donate peripheral blood stem cells according to local institutional practices. Peripheral blood stem cells will not be manipulated or T-depleted prior to administration. Standard post-

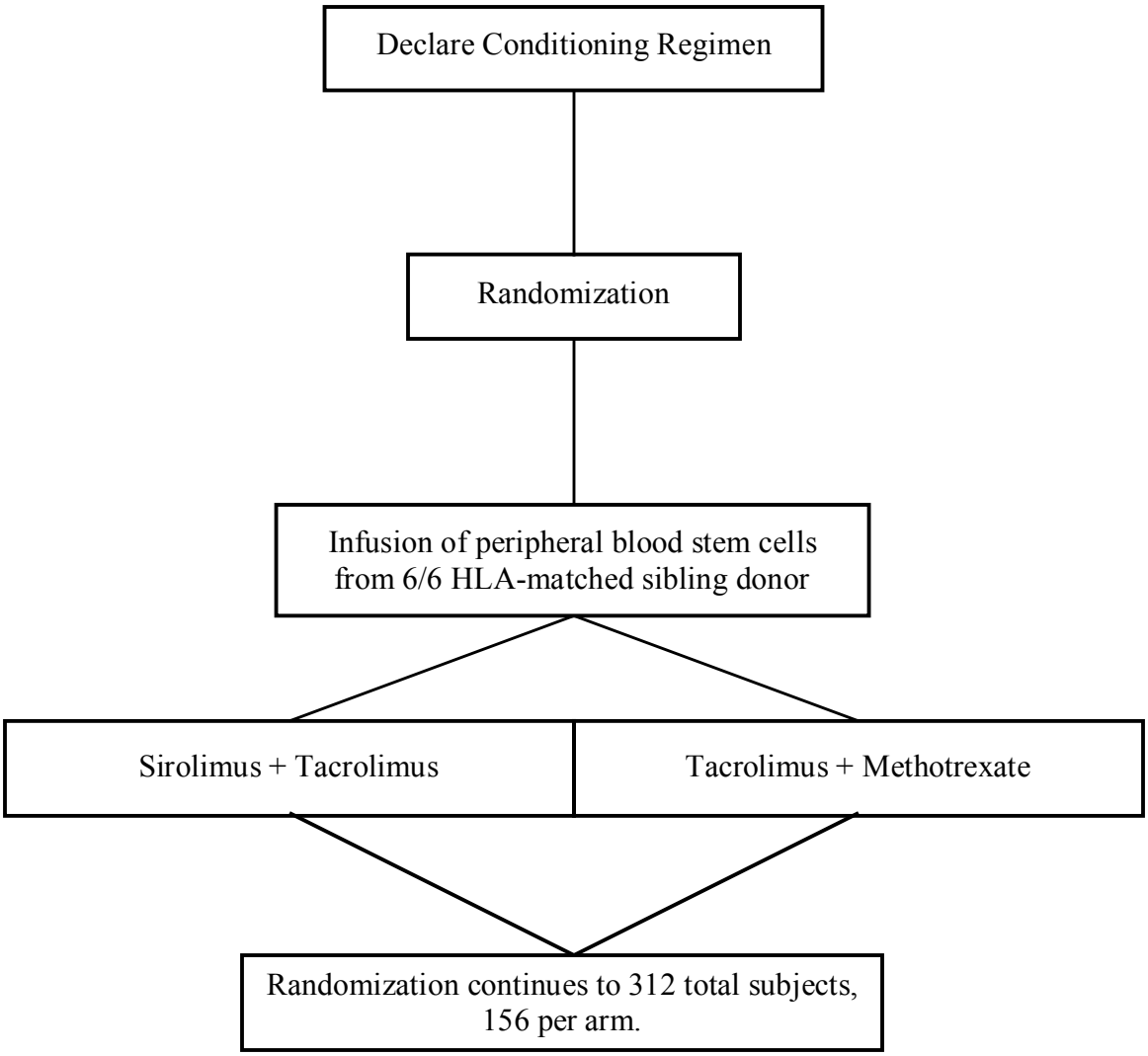
transplant care will be administered. Patients will be randomized to one of two GVHD prophylaxis regimens and will be followed for the endpoints of interest.

Accrual Objective: Patients who are candidates for transplantation of G-CSF-mobilized peripheral blood stem cells from HLA-matched, related donors will be targeted for accrual. Approximately 156 patients will be accrued per study arm (total of 312 patients).

Accrual Period: The estimated accrual period is three years.

Study Duration: Patients will be followed for 114 days post randomization for evaluation of the primary endpoint, with additional follow-up to two years after transplantation for evaluation of secondary endpoints.

TREATMENT SCHEMA



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CHAPTER 1

1. BACKGROUND AND RATIONALE

1.1. Allogeneic Stem Cell Transplantation

Hematopoietic stem cell transplantation (HSCT) is an accepted therapy for hematologic malignancies, marrow failure, and some congenital disorders. However, both transplant-related toxicity and graft-versus-host disease (GVHD) continue to be major problems that limit the general value of HSCT.

Transplant-related toxicity results from the conditioning regimen and agents used to control GVHD. Patient and donor age, disease remission status, number of prior chemotherapy regimens, comorbid diseases and many other factors influence short and long-term risks of transplant-related morbidity and mortality. Modifications to the chemotherapeutic and GVHD prophylaxis agents that reduce transplant-related toxicity would be a welcome improvement.

Acute GVHD results from a complex interaction of donor T cells and recipient tissues that involves recognition of major and/or minor histocompatibility antigens in an inflammatory milieu. Clinical injury is thought to derive from direct T cell injury through perforin/granzyme, Fas/FasL interactions, and the effects of inflammatory cytokines. The context of this recognition is a critical factor. HSCT recipients recently subjected to conditioning regimen toxicity and often having active infection present a pro-inflammatory milieu for T cell recognition and activation. This may in part be mediated through direct and indirect cytokine effects. It is reasonable to propose that drugs that result in additional tissue injury may contribute to GVHD. They also clearly increase transplantation-related morbidity.

Grade II-IV acute GVHD occurs in 35-40% of patients undergoing HLA-matched related donor stem cell transplantation[1]. Fifteen percent develop severe, Grade III-IV disease. Approximately 40% of patients with acute GVHD will have durable responses to corticosteroid therapy; there has been little change in this response rate over the past 20 years despite addition or substitution of other immunosuppressive drugs to GVHD treatment regimens[2]. The prognosis for the 60% of patients without long-lasting responses is poor[3]. A strategy that minimizes the incidence of GVHD, without other adverse effects, rather than improves treatment of established GVHD, seems more likely to improve overall survival after allogeneic transplantation.

1.2. GVHD Prophylaxis

GVHD can be prevented or decreased with a variety of pharmacologic agents[4] and non-pharmacologic techniques[5]. Early transplants were done using post-transplant methotrexate to prevent GVHD; in the 1980s cyclosporine was demonstrated to be superior to methotrexate and in 1986 the combined use of cyclosporine and methotrexate was shown to be superior to single agent prophylaxis[6]. Recently, other immunophilin inhibitors, such as tacrolimus, first used successfully to prevent rejection after solid organ transplantation, have been developed as

GVHD prophylactic agents because of favorable toxicity profiles in comparison with cyclosporine[7]. As monotherapy for prevention of GVHD after allogeneic transplantation, tacrolimus has demonstrated safety and efficacy in rodent studies[8] as well as in trials of human subjects[9].

Trials evaluating combination therapy as prophylaxis for GVHD in comparison with single agent therapy focused mainly on cyclosporine rather than tacrolimus. The Seattle group initially demonstrated the superiority of cyclosporine/methotrexate over cyclosporine alone for GVHD prophylaxis[6]. Since then, several trials have demonstrated the efficacy of adding a second agent to cyclosporine[10, 11, 12]. There are few studies evaluating single agent tacrolimus with tacrolimus-based combination therapy. However, in one small trial evaluating GVHD after peripheral blood stem cell transplantation, where GVHD rates may be higher than after bone marrow transplantation[13], no statistically significant increase in GVHD was noted in patients who received tacrolimus monotherapy in comparison with patients who received combination tacrolimus/methotrexate[14]. The addition of a third agent to established 2-drug GVHD prophylaxis regimens has not shown a benefit[15, 16].

Large Phase III studies comparing tacrolimus/methotrexate versus cyclosporine/methotrexate for both matched, related and unrelated donors have been performed. In the matched, related donor setting, 329 patients were randomized to receive either tacrolimus/methotrexate or cyclosporine/methotrexate. The incidence of Grade II-IV acute GVHD was 31.9% in the tacrolimus arm and 44.4% in the cyclosporine arm[17]. Similarly, in the unrelated donor study, the incidence of Grade II-IV acute GVHD was 56% among the 46 patients randomized to tacrolimus and was 74% among the 63 patients randomized to receive cyclosporine[18].

Despite data from retrospective database studies[19], prospective Phase II studies[20] and the data from two randomized controlled trials[17, 18], cyclosporine-based regimens remain the most common form of GVHD prophylaxis in the HLA-identical sibling and unrelated donor settings, however, the use of tacrolimus and tacrolimus-based regimens is increasing. Table 1.2 demonstrates current GVHD prophylaxis agent use, according to a recent Center for International Blood and Marrow Transplant Research (CIBMTR) analysis.

Table 1.2 – Current Use of GVHD Regimens, as Reported to the IBMTR

Regimen	Matched, Related Donor	Unrelated Donor
Methotrexate alone	4%	1%
Cyclosporine alone	6%	2%
Tacrolimus ± other	5%	6%
Cyclosporine ± other	6%	27%
Cyclosporine, Methotrexate ± other	42%	32%
Tacrolimus, Methotrexate ± other	24%	24%
T cell depletion ± other	10%	5%
Other	2%	4%

Methotrexate has a long history of use as a GVHD prophylaxis agent in HSCT. It has been shown to complement the effects of cyclosporine in preventing severe GVHD[6]. However, it also may increase the risk of other complications such as mucositis and hemorrhagic pneumonia/interstitial pneumonitis[21] and as a consequence, may increase the incidence of GVHD through tissue injury[22, 23]. Methotrexate is an antiproliferative agent and is associated with slower recovery of neutrophils after allogeneic transplantation. In a retrospective study of peripheral blood stem cell transplantation trials, the use of full-dose methotrexate was associated with a 2.5 day median delay in the time to neutrophil engraftment[24].

1.3. Sirolimus

Sirolimus (Rapamune®, Wyeth) is a naturally occurring compound originally isolated from a soil saprophyte (*Streptomyces hygroscopicus)* found uniquely on Easter Island (Rapa Nui). In addition to its immunosuppressive properties, sirolimus has antifungal, antiviral and antineoplastic properties.

Although structurally similar to calcineurin inhibitors, sirolimus binds uniquely to FK binding protein 12 (FKBP12) and then complexes with mTOR (mammalian Target of Rapamycin). Sirolimus does not interact with calcineurin or its downstream effectors. The sirolimus-FKBP12-mTOR complex inhibits several distinct biochemical pathways, resulting in a reduction in DNA transcription, DNA translation, protein synthesis and cell cycling, ultimately leading to T cell immunosuppression. Upstream pathways that interact with mTOR include the PTEN/pI3 kinase/Akt pathway and the Janus kinase pathway, which is important in mediating IL-2 driven signaling from the T cell receptor, among others[25]. Although the mechanisms are less clear, this compound appears to exert some of its immunosuppressive properties via inhibition of dendritic cell activity through a reduction in antigen uptake[26, 27], cellular maturation[28], intracellular signaling[29] and apoptosis induction[30, 31].

Sirolimus is widely used in solid organ transplantation. In renal transplantation, several randomized trials demonstrate that addition of sirolimus to an established immunosuppressive regimen is associated with improved allograft survival[32, 33] and long-term renal function[34]. Although not tested in a randomized fashion, there is ample evidence to suggest that the introduction of sirolimus to facilitate early calcineurin minimization after liver transplantation has lead to a reduction in adverse outcomes without increasing the graft rejection rate[35, 36, 37, 38].

Sirolimus has been used in combination with a variety of other immunosuppressive agents in the solid organ transplant setting. In vitro studies suggest that the combination of sirolimus and tacrolimus is more effective than the combination of sirolimus and cyclosporine in reducing memory T cell production, apoptosis induction and cytokine production[39]. When tested in a comparative fashion after renal transplantation, early results suggested fewer episodes of acute rejection and better preservation of renal function with sirolimus and tacrolimus versus sirolimus and cyclosporine[40, 41]. The use of combination immunosuppression with sirolimus in stem cell transplantation is attractive, since the drug has non-overlapping toxicities with calcineurin inhibitors and a different mechanism of action.

1.3.1. Preliminary Experience with Sirolimus in Stem Cell Transplantation

1.3.1.1. Therapy of established GVHD

In the only published report on therapy of established steroid-refractory acute GVHD, Benito *et al* described their experience treating 21 patients with sirolimus. Most patients were treated with 4-5 mg/m^2/day after an oral loading dose of 15 mg/m^2. Although the drug was active in this patient population (overall response rate 57%), the drug proved to be too toxic at the doses used. Five patients developed thrombotic microangiopathy and many others had reversible cytopenias[42].

The efficacy of sirolimus as therapy for chronic GVHD in combination with calcineurin inhibitors has been published in abstract only. Johnston *et al* described a 56% overall response rate when sirolimus was given with either tacrolimus or cyclosporine in patients with established chronic GVHD[43], while Couriel *et at* described a 68% response rate to the combination of sirolimus and tacrolimus in patients with steroid-refractory chronic GVHD[44].

1.3.1.2. Prophylaxis of GVHD

Unrelated Donor Transplantation

At the Dana-Farber Cancer Institute, 65 patients (median age 39.5 years, range 19-62) have undergone HLA-matched unrelated and single HLA-antigen mismatched related and unrelated bone marrow transplantation using the combination of sirolimus, tacrolimus and abbreviated methotrexate as GVHD prophylaxis. Presumably due to a reduction in methotrexate dose, rapid neutrophil and platelet engraftment was noted. The oral formulation of sirolimus was tolerated and patients maintained adequate serum levels during the trial (sirolimus 3-12 ng/mL, tacrolimus 5-10 ng/mL). Among the first 41 patients analyzed as part of a Phase II clinical trial, the incidence of Grade II-IV acute GVHD was 26% and the incidence of Grade III-IV acute GVHD was 13%. Overall, the combination was safe with only moderate transplant-related toxicity. Overall survival for the entire group of patients (n=65) at one year is 56% and at 2 years is 50% in this high-risk population[45].

Related Donor Transplantation

At the Dana-Farber Cancer Institute, over 50 patients (median age 41 years, range 18-59) have undergone HLA-matched, related peripheral blood stem cell transplantation using the combination of sirolimus and tacrolimus without methotrexate. The hypothesis tested in this trial was that the omission of methotrexate would not increase the rate of GVHD and would reduce transplant-related toxicity. To date, sirolimus has been well tolerated and oral administration has been effective in attaining and maintaining adequate serum drug levels. Among the first 38 patients followed for greater than 100 days, the median times to neutrophil and platelet engraftment were 14 days (range 11 to 17 days) and 13 days (range 10 to 47 days), respectively. Platelet engraftment occurred sooner in comparison with recent IBMTR analyses (13 vs. 18 days, unpublished data). The rates of Grade II-IV and III-IV acute GVHD were 16% and 5%, respectively, at 100 days. Transplant-related toxicity has been limited: 4 patients developed

severe veno-occlusive disease of the liver (11%) and no patient developed idiopathic pneumonia syndrome. CMV reactivation rates were low (<10%) and no patient developed an invasive fungal infection during the first 100 days after transplantation when prophylactic antifungal agents were not used. Thrombotic microangiopathy occurred in 4 patients (11%); it resolved in all cases when tacrolimus was discontinued. Transplant-related mortality at 100 days was 5%. Overall survival at 1 year is 72%[46], now with a median follow-up of 13 months. A summary of the major clinical results along with results from the randomized trial that compared tacrolimus/methotrexate with cyclosporine/methotrexate is found in Table 1.3.

Table 1.3 – Clinical Outcomes Associated With Alternate GVHD Prophylaxis Regimens After Matched, Related Allogeneic Stem Cell Transplantation

	Tacrolimus/ Sirolimus	**Tacrolimus/ Methotrexate**	**Cyclosporine/ Methotrexate**
Sample Size	38	165	164
Median Age (range)	41 (19-59)	40 (17-61)	40 (16-63)
% Male	50	56	61
Stem Cell Source	PBSC	BM	BM
Neutrophil Recovery (median days)	14	19	20
GVHD (%)			
Acute Grade II-IV	16	31.9	44.4
Acute Grade III-IV	5	13.3	17.1
Chronic	31	55.9	49.4
Survival (%)			
100-Day Treatment-Related Mortality	5	32	21
1 Year Relapse-Free Survival	69	47	58
1 Year Overall Survival	72	52	64

CHAPTER 2

2. STUDY DESIGN

2.1. Study Overview

This is a Phase III randomized, open label, multicenter clinical trial sponsored by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). The objective of the trial is to test the null hypothesis that there is no difference in acute GVHD-free survival at 100 days after peripheral blood stem cell transplantation using sirolimus and tacrolimus versus tacrolimus and methotrexate for GVHD prophylaxis. Conditioning regimens will vary by center but at each center will be the same for patients randomized to either GVHD prophylaxis regimen. Because randomization is expected to occur approximately 14 days prior to transplant, the primary endpoint of this trial is Grades II-IV acute GVHD-free survival at 114 days post-randomization. Secondary analyses will consider neutrophil and platelet recovery, time to hospital discharge, acute and chronic GVHD, relapse, infections, adverse events (mucositis, thrombotic microangiopathy and CMV reactivation) and overall survival. Accrual is anticipated for three years with a follow-up period of two years.

2.2. Hypothesis and Study Objectives

2.2.1. Primary Hypothesis

The primary hypothesis to be tested in this randomized, controlled trial is that the combination of sirolimus and tacrolimus (study group) will result in a 15% improvement in 114-day Grades II-IV acute GVHD-free survival from the time of randomization in comparison to the combination of tacrolimus and methotrexate (control group) after myeloablative HLA-matched, related donor allogeneic peripheral blood stem cell transplantation for acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML) or myelodysplastic syndrome (MDS).

2.2.2. Secondary Hypotheses

Secondary hypotheses to be tested in this clinical trial include:

- Stable neutrophil and platelet engraftment will occur sooner with study therapy;
- Hospital discharge will occur sooner in the study group;
- Incidence of Grades II-IV and III-IV of acute GVHD will be lower in the study group;
- Mucositis incidence and severity will be reduced with study therapy;
- Infectious events will be less frequent in the study group;
- CMV reactivation will be less frequent in the study group;
- Chronic GVHD incidence will be lower in the study group; and,

- Relapse-free and overall survival will be higher in the study group.

2.2.3. Primary Objective

The primary objective is to compare rates of 114-day Grades II-IV acute GVHD-free survival post randomization for HLA-matched, related donor allogeneic peripheral blood stem cell transplantation using two different GVHD prophylaxis regimens.

2.2.4. Secondary Objectives

Secondary objectives are to compare patients receiving sirolimus and tacrolimus with those receiving methotrexate and tacrolimus for:

1. Time to stable neutrophil and platelet engraftment after transplantation;
2. Incidences of Grades III-IV acute GVHD;
3. Mucositis severity;
4. Time to first hospital discharge;
5. Incidence of infections in the first 100 days post-transplantation;
6. CMV reactivation in the first 100 days post-transplantation;
7. Thrombotic microangiopathy incidence in the first 100 days post-transplantation;
8. VOD incidence in the first 100 days post-transplant;
9. Incidence of chronic GVHD;
10. Relapse of primary malignant disease in distinct disease subgroups; and,
11. 1-year relapse-free and overall survival.

2.3. Patient Eligibility for Randomization

2.3.1. Patient Eligibility for Randomization

Diagnoses to be included:

1. Acute Myelogenous Leukemia at the following stages:
   - First Remission
   - Second or subsequent remission

   Complete remission is defined as the absence of blasts in the peripheral circulation at the time of enrollment and < 5% blasts in the marrow within 28 days of enrollment.
2. Acute Lymphoblastic Leukemia at the following stages:
   - First Remission
   - Second or subsequent remission

   Complete remission is defined as the absence of blasts in the peripheral circulation at the time of enrollment and < 5% blasts in the marrow within 28 days of enrollment.

3. Acute Biphenotypic Leukemia at the following stages:
   - First remission
   - Second or subsequent remission

   Complete remission is defined as the absence of blasts in the peripheral circulation at the time of enrollment and < 5% blasts in the marrow within 28 days of enrollment.
4. Chronic Myelogenous Leukemia at the following stages:
   - First or Subsequent Chronic Phase:
     - Stable, not hematologic remission: blasts present in marrow and/or peripheral blood, but disease does not qualify as accelerated or blast phase
     - Hematological remission: no blast cells or precursor cells in the blood or marrow
     - Partial cytogenetic remission: Ph+ metaphases > 0% but < 35%
     - Complete cytogenetic remission: absence of Ph+ metaphases
   - Accelerated Phase - any one of the following symptoms:
     - WBC difficult to control (> 50 x 10^9/L despite therapy)
     - Rapid doubling of WBC (< 5 days)
     - 10% blasts in blood or marrow
     - 20% blasts and/or promyelocytes in blood or marrow
     - 20% basophils and/or eosinophils in blood
     - Anemia or thrombocytopenia unresponsive to standard treatment
     - Persistent thrombocytosis (> 1000 x 10^9/L)
     - Cytogenetic abnormalities in addition to Ph+
     - Increasing splenomegaly
     - Marrow fibrosis
5. Myelodysplastic syndromes at any of the following stages:
   - Refractory anemia
   - Refractory anemia with ringed sideroblasts
   - Refractory cytopenia with multilineage dysplasia
   - Refractory cytopenia with multilineage dysplasia and ringed sideroblasts
   - Refractory anemia with excess blasts-1 (5-10% blasts)
   - Refractory anemia with excess blasts-2 (10-20% blasts)
   - Myelodysplastic syndrome, unclassified
   - MDS associated with isolated del (5q)
   - Chronic Myelomonocytic Leukemia

Other eligibility criteria include:

1. 6/6 HLA-matched sibling, defined by Class I (HLA-A and B) serologic typing (or higher resolution) and Class II (HLA-DRBI) molecular typing willing to donate peripheral blood stem cells and meeting institutional criteria for stem cell donation. The donor must be medically eligible to donate stem cells according to individual transplant center criteria. Pediatric patients in whom a pediatric sibling donor is not anticipated to be a suitable leukapheresis candidate are not eligible.
2. Karnofsky ≥ 70% or Lansky performance status ≥ 70% for patients < 16 years old at time of registration.

3. Age range: 2.0 – 60.0 years at time of registration.
4. For children less than age 18, willingness and ability to take oral medications per the treating physician.
5. Signed informed consent.

2.3.2. Patient Exclusion Criteria

Patients will be excluded from this trial if they meet the following criteria:

1. Prior allogeneic or autologous transplants using any hematopoietic stem cell source.
2. Seropositive for the human immunodeficiency virus (HIV).
3. Uncontrolled bacterial, viral or fungal infections (currently taking medication and progression of clinical symptoms).
4. Pregnancy (positive serum β-HCG) or breastfeeding.
5. Renal function: Serum creatinine outside the normal range for age; or calculated creatinine clearance < 50 mL/min/1.72m^2.
6. Hepatic function: Most recent direct bilirubin, ALT or AST greater than two times the upper limit of normal for the laboratory.
7. Pulmonary disease: In adults, FVC or FEV1 < 60% predicted (corrected for hemoglobin). In children, overt hypoxemia as measured by an oxygen saturation < 92%.
8. Cardiac ejection fraction < 45% in adults and children, or < 26% shortening fraction in children.
9. Cholesterol > 500 mg/dL or Triglycerides > 500 mg/dL while being treated or not on appropriate lipid-lowering therapy.
10. Patient with prior history of allergy to sirolimus.
11. Patient requiring voriconazole at time of study registration.
12. Patients receiving another investigational drug unless cleared by the Protocol Officer or Protocol Chair.
13. Patients with prior malignancies except resected basal cell carcinoma or treated carcinoma in-situ. Cancer treated with curative intent > 5 years previously will be allowed. Cancer treated with curative intent < 5 years previously will not be allowed unless approved by the Protocol Officer or Protocol Chair.

2.4. Conditioning

Two groups of myeloablative conditioning regimens will be allowed:

1. Cyclophosphamide and Total Body Irradiation (CY-TBI) with a TBI dose of at least 1200 cGy of fractionated TBI*.
2. Etoposide and Total Body Irradiation (VP16-TBI) with a TBI dose of at least 1200 cGy of fractionated TBI.

2.4.1. Additional Drugs

Additional drugs, including anti-T cell antibodies, may not be used in the conditioning regimen.

2.4.2. Choice of Conditioning Regimen

Transplant centers may use different regimens for patients with different diseases as required by institutional protocol. However, the transplant center must declare before randomization what conditioning regimen will be used for each patient. The specified regimens will be used for the patient whether randomized to receive sirolimus/tacrolimus or tacrolimus/methotrexate.

2.4.3. Order of Administration of TBI and Cyclophosphamide

The order of administration of cyclophosphamide and TBI is at the discretion of the transplant center. Within each institution, all patients should receive the cyclophosphamide and TBI in the same order. If cyclophosphamide or VP16 is given last, there should be at least a one-day rest period before the peripheral blood stem cell infusion.

2.4.3.1. TBI administration

Fractionated TBI will be administered according to the institutional protocol. The Institution will also define radiation sources, dose rates, details of lung shielding and sites receiving boost radiation. TBI may be delivered from either linear accelerator or Cobalt sources. Lung shielding is preferred but not required during TBI.

2.4.3.2. Cyclophosphamide administration

Cyclophosphamide will be administered intravenously. Mesna is allowed, but not required.

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* The conditioning regimen, from which the Phase II data is derived, is comprised of Cytoxan 1800 mg/m^2 x 2 doses and TBI 14 Gy in 7 fractions with lung shielding.

2.5. Stem Cell Transplantation

2.5.1. Stem Cell Collection

HLA-matched sibling donors will undergo G-CSF mobilization according to local institutional practices. Peripheral blood stem cells will be collected by large volume apheresis according to local institutional guidelines. Plasma and red cell depletion are allowed for volume reduction or ABO incompatibility but any other form of graft manipulation (including ex-vivo T cell depletion) is not permitted.

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

Up to two leukapheresis procedures may be performed to obtain the minimum $CD34^+$ stem cell target. If, after two leukapheresis procedures, fewer than 2 x 10^6/kg $CD34^+$ stem cells have been collected, transplant centers will have the discretion to continue peripheral blood stem cell harvesting or to proceed to bone marrow harvesting to obtain sufficient stem cells. Regardless of the decision, subjects will be followed and analyzed with their treatment assignment group in an intent-to-treat manner.

If more than 10 x 10^6/kg $CD34^+$ stem cells are collected, the excess should either be discarded or cryopreserved for future use, but should not be administered to the patient.

2.5.2. Stem Cell Administration

Peripheral blood stem cells will be administered on Day 0 to all patients according to individual institutional guidelines after appropriate processing and quantification has been performed by the local laboratory. Stem cells are administered through an indwelling central venous catheter. If infusion occurs over two days, Day 0 is the day infusion is completed.

2.6. GVHD Prophylaxis

This is a randomized, controlled clinical trial comparing two GVHD prophylaxis regimens: sirolimus/tacrolimus and tacrolimus/methotrexate. All patients will be randomly assigned to one of the two GVHD prophylaxis regimens. GVHD prophylaxis begins on Day –3. Blinding will not be performed. All patients will be analyzed with their treatment assignment group (intent-to-treat analysis).

2.6.1. Sirolimus/Tacrolimus

2.6.1.1. Dosing

Adults: Sirolimus will be given 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.

Children: Children aged < 12.0 years OR weighing < 40.0 kg will be given an oral loading dose of sirolimus of 3 mg/m^2 followed by a daily oral dose of 1 mg/m^2, rounded to the nearest full milligram.

Adults and Children: Tacrolimus will be given at a dose of 0.02 mg/kg every 24 hours as a continuous intravenous infusion beginning on Day –3. An effort will be made to convert the tacrolimus to oral dosing at 2-3 times the total 24-hour intravenous dose, split into 2 doses given every 12 hours as soon as clinically feasible.

2.6.1.2. Monitoring

Levels of sirolimus and tacrolimus will be drawn at least twice per week while hospitalized, then weekly or monthly thereafter unless a change in medication (e.g. use of itraconazole) or renal function might result in an acute change in level. At that point levels will be measured as clinically indicated. Levels of sirolimus must be assayed by HPLC or HPLC-MS. ELISA or other immunoassays are not permitted. Tacrolimus level is measured according to local institutional practices.

2.6.1.3. Target serum levels and dose modifications

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.

The target serum level for tacrolimus is 5-10 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 < 5 ng/mL, it is suggested, but not required, that the dose of tacrolimus be increased by approximately 25% increments every 1-2 days, rounded to the nearest 0.5 milligram (when dosing is oral) until the target range is achieved. Conversely, for levels > 10 ng/mL, it is suggested, but not required, that the dose of tacrolimus be decreased by approximately 25% every 1-2 days until the target level is achieved. Alternatively, tacrolimus 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.

2.6.1.4. Tapering

Tapering of sirolimus and tacrolimus will begin at Day 100 in the absence of GVHD and disease relapse. The goal of tapering is the complete discontinuation of immunosuppressant medications by 6 months. Sirolimus and tacrolimus should be tapered in an alternating fashion every other week when clinically feasible. Similar reductions in dosages should be employed for each drug at each tapering event, with modifications at the end of the tapering schedule to allow near simultaneous discontinuation of sirolimus and tacrolimus. Sample tapering schedules for adults and children are found in Appendix A.

2.6.2. Tacrolimus/Methotrexate

2.6.2.1. Dosing

Dosing of tacrolimus is as described in Section 2.6.1.1 above.

Methotrexate will be given at a dose of 15 mg/m^2 on Day 1 after transplantation, and at a dose of 10 mg/m^2 on Days 3, 6 and 11 after transplantation. Attempts should be made to administer all four doses of methotrexate. Omission of any of the doses of methotrexate is at the treating physician's discretion, will not be considered a protocol violation, but will be recorded as a deviation from the planned GVHD prophylaxis regimen.

Leucovorin rescue may be given at the treating physician's discretion. Patients at risk for methotrexate toxicity include those with large fluid collections (ascites, pleural effusions) or with decreased renal function. A recommended leucovorin rescue schema can be found in Appendix D.

2.6.2.2. Monitoring

Levels of tacrolimus will be drawn at least twice per week while hospitalized, then weekly or monthly thereafter unless a change in medication (e.g. use of itraconazole) or renal function might result in an acute change in level. At that point levels will be measured as clinically indicated.

2.6.2.3. Target serum levels and dose modification

Target dosing and dose modifications for tacrolimus are as described in Section 2.6.1.3 above.

2.6.2.4. Tapering

Tapering of tacrolimus is as described in Section 2.6.1.4 above. Tapering should commence at Day 100 in the absence of GVHD and disease relapse. The goal of tapering is the complete discontinuation of immunosuppressant medications by six months. Tacrolimus should be tapered every other week when clinically feasible. Similar reductions in dosages should be employed at each tapering event, to allow complete discontinuation by six months.

2.7. Supportive Care

All supportive care will be given in keeping with BMT CTN Manual of Procedures (MOP) and local institutional practice. Supportive care should be administered in a similar fashion to subjects randomized to both arms of the study.

2.7.1. Growth Factors

Growth factors will not be used following transplant unless absolute neutrophil cell count (ANC) recovery to $0.5x10^9$/L has not occurred by Day +14. If by Day +14, the ANC is still < $0.5x10^9$/L or if, after initial recovery, the ANC drops below 1 x 10^9/L, G-CSF may be given per institutional guidelines.

2.7.2. Blood Products

Transfusion thresholds for blood product support will be consistent with BMT CTN MOP and standard institutional guidelines. All blood products will be irradiated. Patients who are CMV negative will receive CMV negative or filtered blood products from study entry.

2.7.3. Prophylaxis Against Infections

All patients will receive prophylaxis against bacterial, fungal and viral infections during the peri-transplant period according to the BMT CTN MOP. Prophylaxis must be the same for patients on both arms of the protocol. This will include:

1. Anti-bacterial prophylaxis: In keeping with the BMT CTN MOP and local institutional standards for allogeneic transplants. Prophylactic antibacterial antibiotics should be used for patients during the neutropenic (ANC < 500/mcL) period.
2. Pneumocystis carinii: Prophylaxis will start at the time of hospital discharge or on Day 30 post-transplant according to institutional practice. Prophylaxis should be continued according to institutional practices.
3. Antifungal therapy: Prophylaxis with fluconazole or other antifungal agents can be given as per local institutional guidelines. **Fluconazole and other azoles are expected to increase serum tacrolimus and sirolimus levels, therefore, dosages of sirolimus and tacrolimus should be adjusted accordingly. Due to extreme interactions with sirolimus, voriconazole is contraindicated during sirolimus therapy and should not**

be used by patients randomized to receive sirolimus. At this time, voriconazole is not considered standard antifungal prophylaxis and should not be used as prophylaxis in the tacrolimus/methotrexate arm; however, in the event of suspected or documented fungal infection, voriconazole may be used in this study arm if deemed clinically necessary. For guidelines on the simultaneous use of sirolimus and oral voriconazole, see Appendix H.

4. HSV/VZV: Prophylaxis will begin with conditioning therapy and continue up to the time of discharge. At that time, prophylaxis should continue as per institutional guidelines.
5. CMV: Monitoring and preemptive or prophylactic treatment strategy will be in accordance with the BMT CTN MOP and local institutional practice.

2.7.4. Intravenous Immune Globulin (IVIG)

IVIG administration will be left to local institutional standard practice.

2.7.5. Kepivance

Kepivance is allowed, but not recommended because mucositis assessment is a secondary endpoint to the protocol.

2.8. Description of Study Drugs

2.8.1. Administration and Storage

2.8.1.1. Sirolimus (Rapamune®)

Sirolimus will be provided by the BMT CTN for up to six months.

Similar to the calcineurin inhibitors, sirolimus has low oral bioavailability due to first pass metabolism by the liver and intestinal wall as well as poor absorption because it is countertransported in the gut lumen by the multidrug efflux pump, P-glycoprotein (P-gp). The median t_{max} value for the oral suspension is < 1 hour indicating that there is rapid absorption. However, systemic availability is ~14% in stable renal transplant patients. Total body clearance is 127 to 240 mL/hr/kg and is not related to dose. Half-life is 57 to 63 hours. It is extensively bound by blood cells and plasma proteins. It is excreted by hepatic and gut metabolism. In Phase III studies, whole blood trough levels for a 2 mg/day dose were 8.59 ± 4.01 ng/mL and correlate with the AUC (r^2=0.95). Only ~2% is excreted in the urine.

The mean bioavailability of sirolimus after administration of the tablet is about 27% higher relative to the oral solution. Sirolimus oral tablets are not bioequivalent to the oral solution; however, clinical equivalence has been demonstrated at the 2-mg dose level. Differences in absorption kinetics and bioavailability in renal transplant patients are shown in Table 2.8a

Table 2.8a – Sirolimus Pharmacokinetics

Formulation, 2 mg	$C_{max,ss}$ (ng/mL)	t_{max} (h)	AUC $_{0\rightarrow\infty}$ (ng•h/mL)	CL/F/WT (mL/h/kg)
Oral Solution	14.4 ± 5.3	2.12 ± 0.84	194 ± 78	173 ± 50
Tablet	15.0 ± 4.9	3.46 ± 2.40	230 ± 67	139 ± 63

Renal failure does not affect the excretion of sirolimus, but excretion is reduced in liver failure as shown in Table 2.8b.

Table 2.8b – Sirolimus Pharmacokinetics in Hepatic Impairment

Population	$C_{max,ss}$ (ng/mL)	t_{max} (h)	AUC $_{0\rightarrow\infty}$ (ng•h/mL)	CL/F/WT (mL/h/kg)
Healthy Subjects	78.2 ± 18.3	0.83 ± 0.17	970 ± 272	215 ± 76
Hepatic Impairment	77.9 ± 23.1	0.84 ± 0.17	1567 ± 616	4 ± 62

Sirolimus oral solution: Sirolimus oral solution (bottles and foil pouches) should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). For dilution, the appropriate dose of sirolimus oral solution should be measured using an amber oral syringe. The dose should be added to a glass or plastic container that holds at least 60 milliliters (mL). Before taking the dose, it should be diluted with water or orange juice; IT SHOULD NOT BE DILUTED WITH GRAPEFRUIT JUICE. After mixing with the diluent, the dose should be taken immediately. The syringe should be discarded after one use. Sirolimus oral solution provided in bottles may develop a slight haze when refrigerated. If such a haze occurs allow the product to stand at room temperature and shake gently until the haze disappears. The presence of this haze does not affect the quality of the product.

Sirolimus tablets: Sirolimus tablets are available as white triangular shaped tablets marked "RAPAMUNE 1 mg" in bottles of 100 tablets or as cartons containing ten blister cards of ten tablets each. Sirolimus tablets should be stored at 20° to 25°C (68°-77°F). Cartons should be used to protect the blister cards and strips from light. Sirolimus tablets should be dispensed in a tight, light-resistant container.

2.8.1.2. Tacrolimus (Prograf®)

The pharmacokinetic parameters of tacrolimus have been determined following intravenous and oral administration in healthy volunteers, kidney transplant and liver transplant patients. Pharmacokinetic parameters for healthy volunteers are found in the Table 2.8c.

Table 2.8c – Tacrolimus Pharmacokinetics

			Parameters					
Population	N	Route, Dose	C_{max} (ng/mL)	t_{max} (hr)	AUC (ng*h/mL)	$t_{½}$ (hr)	Cl (L/hr/kg)	V (L/kg)
Healthy Volunteers	8	Iv (0.025 mg/kg/4hr)	---	---	598±125*	34.2±7.7	0.04±0.009	1.91±0.31
	16	po (5 mg)	29.7±7.2	1.6±0.7	243±73**	34.8±11.4	0.041±0.008***	1.94±0.53***
* AUC_{0-120} ** AUC_{0-72} *** Corrected for individual bioavailability								

Absorption of tacrolimus from the gastrointestinal tract after oral administration is incomplete and variable. The absolute bioavailability of tacrolimus was 17 ± 10% in adult kidney transplant patients (N=26), 22 ± 6% in adult liver transplant patients (N=17), and 18 ± 5% in healthy volunteers (N=16).

A single dose study conducted in 32 healthy volunteers established the bioequivalence of the 1 mg and 5 mg capsules. Another single dose study in 32 healthy volunteers established the bioequivalence of the 0.5 mg and 1 mg capsules. Tacrolimus maximum blood concentrations (C_{max}) and area under the curve (AUC) appeared to increase in a dose-proportional fashion in 18 fasted healthy volunteers receiving a single oral dose of 3, 7 and 10 mg. In 18 kidney transplant patients, tacrolimus trough concentrations from 3 to 30 ng/mL measured at 10-12 hours post-dose (C_{min}) correlated well with the AUC (correlation coefficient 0.93). In 24 liver transplant patients over a concentration range of 10 to 60 ng/mL, the correlation coefficient was 0.94. The rate and extent of tacrolimus absorption were greatest under fasted conditions. The presence and composition of food decreased both the rate and extent of tacrolimus absorption when administered to 15 healthy volunteers. The effect was most pronounced with a high-fat meal (848 kcal, 46% fat): mean AUC and C_{max} were decreased 37% and 77%, respectively; T_{max} was lengthened 5-fold. A high-carbohydrate meal (668 kcal, 85% carbohydrate) decreased mean AUC and mean C_{max} by 28% and 65%, respectively. In healthy volunteers (N=16), the time of the meal also affected tacrolimus bioavailability. When given immediately following the meal, mean C_{max} was reduced 71%, and mean AUC was reduced 39%, relative to the fasted condition. When administered 1.5 hours following the meal, mean C_{max} was reduced 63%, and mean AUC was reduced 39%, relative to the fasted condition. In 11 liver transplant patients, tacrolimus administered 15 minutes after a high fat (400 kcal, 34% fat) breakfast, resulted in decreased AUC (27 ± 18%) and C_{max} (50 ± 19%), as compared to a fasted state.

The plasma protein binding of tacrolimus is approximately 99% and is independent of concentration over a range of 5-50 ng/mL. Tacrolimus is bound mainly to albumin and alpha-1-acid glycoprotein, and has a high level of association with erythrocytes. The distribution of tacrolimus between whole blood and plasma depends on several factors, such as hematocrit, temperature at the time of plasma separation, drug concentration, and plasma protein

concentration. In a U.S. study, the ratio of whole blood concentration to plasma concentration averaged 35 ng/mL (range 12 to 67).

Tacrolimus injection must be diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection to a concentration between 0.004 mg/mL and 0.02 mg/mL prior to use. Diluted infusion solution should be stored in glass or polyethylene containers and should be discarded after 24 hours. The diluted infusion solution should not be stored in a PVC container due to decreased stability and the potential for extraction of phthalates. In situations where more dilute solutions are utilized (e.g., pediatric dosing, etc.), PVC-free tubing should likewise be used to minimize the potential for significant drug adsorption onto the tubing. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Due to the chemical instability of tacrolimus in alkaline media, tacrolimus injection should not be mixed or co-infused with solutions of pH 9 or greater (e.g., ganciclovir or acyclovir). Supplied as a sterile solution in 1-mL ampoules containing the equivalent of 5 mg of anhydrous tacrolimus per mL, in boxes of 10 ampoules. Store between 5°C and 25°C (41°F and 77°F). Tacrolimus capsules (1 and 5 mg) are stored at controlled room temperature, 15°C-30°C (59°F-86°F).

2.8.1.3. Methotrexate

Methotrexate sodium for injection should be reconstituted with an appropriate sterile, preservative free medium such as 5% dextrose solution, USP, or sodium chloride injection, USP. Reconstitute the 20 mg vial to a concentration no greater than 25 mg/mL. The 1 gram vial should be reconstituted with 19.4 mL to a concentration of 50 mg/mL. Reconstitute immediately prior to use. When high doses of methotrexate are administered by IV infusion, the total dose is diluted in 5% dextrose solution.

Each 20 mg and 1 g vial of lyophilized powder contains methotrexate sodium equivalent to 20 mg and 1 g methotrexate respectively. Store at controlled room temperature, 20°-25° C (68°-77° F); excursions permitted to 15°-30° C (59°-86° F). Protect from light.

2.8.2. Adverse Reactions

2.8.2.1. 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. However, the incidence of veno-occlusive disease of the liver was not noted to be higher than expected in trials of unrelated transplantation. 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.

2.8.2.2. Tacrolimus

There is a spectrum of well-described toxic effects of tacrolimus. The primary toxicities in large, controlled trials were reversible renal insufficiency (doubling of creatinine in 82%), hypertension requiring the use of antihypertensive medications (21%), hyperglycemia (12%), hypomagnesemia, hypokalemia, and neurologic toxicity. In addition there is an infusional toxicity from the cremaphor diluent. There is an increased risk of opportunistic infections and secondary malignancies. Some of these toxicities are recognized complications of transplantation.

2.8.2.3. 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.

2.8.3. Drug Interactions

2.8.3.1. Sirolimus

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

Drugs that may increase sirolimus blood concentrations include:

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

Due to extreme interactions with voriconazole, this drug is contraindicated during sirolimus therapy. It is recommended that drugs with known interactions with sirolimus be avoided when clinically feasible. When these medications are required, dose modifications of sirolimus may be required. For guidelines on the simultaneous use of sirolimus and oral voriconazole, see Appendix H.

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.

Care should be exercised when drugs or other substances that are metabolized by CYP3A4 are administered concomitantly with sirolimus. Grapefruit juice reduces CYP3A4-mediated metabolism of sirolimus and must not be used for dilution.

2.8.3.2. Tacrolimus

Tacrolimus is extensively metabolized by the cytochrome P-450 (CYP3A4) system.

Drugs that may increase tacrolimus blood concentrations include:

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

Drugs that may decrease tacrolimus concentrations include:

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

Due to extreme interactions with voriconazole, tacrolimus doses should be empirically lowered by 50% when concomitant therapy with voriconazole is initiated. It is recommended that drugs with known interactions with tacrolimus be avoided when clinically feasible. When these medications are required, dose modifications of tacrolimus may be required.

2.8.3.3. Methotrexate

Methotrexate is partially bound to serum albumin, and toxicity may be increased because of displacement by certain drugs, such as salicylates, phenylbutazone, phenytoin, and sulfonamides. Renal tubular transport is also diminished by probenecid; use of methotrexate with this drug should be carefully monitored.

Oral antibiotics such as tetracycline, chloramphenicol, and nonabsorbable broad-spectrum antibiotics, may decrease intestinal absorption of methotrexate or interfere with the enterohepatic circulation by inhibiting bowel flora and suppressing metabolism of the drug by bacteria. Penicillins may reduce the renal clearance of methotrexate; increased serum concentrations of methotrexate with concomitant hematologic and gastrointestinal toxicity have been observed with high and low dose methotrexate. Use of methotrexate with penicillins should be carefully monitored.

2.9. Management of Toxicities

Toxicities will be scored as per the NCI's Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (see http://ctep.cancer.gov/forms/CTCAEv3.pdf). Toxicities of the blood/bone marrow, gut, liver, and skin will be excluded from the common toxicity criteria and evaluated separately since they are likely to be affected by GVHD. GVHD severity will be determined clinically, however, biopsies of affected organs are strongly encouraged whenever possible.

2.9.1. GVHD

Specific therapy of GVHD will not be mandated by protocol, although it is recommended that only Grade II-IV GVHD be treated with systemic therapy. A recommendation for initial therapy of GVHD is methylprednisolone, 2 mg/kg/d IV (or an equivalent corticosteroid). If the patient responds to steroids, the steroid will be tapered as tolerated clinically, but with the intent of decreasing the dose by 10%/week. For steroid non-responders, treatment for GVHD will be per local institutional standards. Tacrolimus and sirolimus (in the appropriate treatment group) should be continued during treatment of acute GVHD.

Whenever possible, the diagnosis of GVHD should be confirmed with a biopsy of the involved organ and histological examination of a biopsy specimen by a pathologist experienced in the diagnosis of GVHD. Due to the early engraftment anticipated with the omission of methotrexate in the sirolimus arm, conditioning-related diarrhea may coincide with engraftment, therefore, sigmoidoscopy and biopsy are strongly encouraged to diagnose GVHD of the intestine. Diagnosis of isolated hepatic GVHD must be made by liver biopsy.

2.9.2. VOD

Treatment of veno-occlusive disease of the liver will be treated per local institutional standards. Participation in clinical trials is permissible. Tacrolimus and sirolimus (in the appropriate treatment group) should be continued during VOD therapy. Levels should be measured frequently in the context of hepatic and/or renal failure.

2.9.3. Idiopathic Pneumonia Syndrome/Diffuse Alveolar Hemorrhage

Treatment of idiopathic pneumonia syndrome/diffuse alveolar hemorrhage will be treated per local institutional standards. Participation in clinical trials is permissible. Tacrolimus and

sirolimus (in the appropriate treatment group) should be continued during idiopathic pneumonia syndrome/diffuse alveolar hemorrhage therapy.

2.9.4. Thrombotic Microangiopathy

Thrombotic microangiopathy will be defined according to BMT CTN guidelines. The diagnostic criteria include the concurrent occurrence of:

1- Red cell fragmentation on a manual differential (2 + schistocytes) with a negative Coombs test

and

2- LDH > normal

and either

3- Renal dysfunction (doubling of serum creatinine or a decrease > 50% in the measured creatinine clearance)

or

Neurological dysfunction unexplained by another etiology

The development of thrombotic microangiopathy should be managed conservatively. Tacrolimus should be discontinued from the GVHD regimen. At the treating physician's discretion, Mycophenolate Mofetil can be substituted for GVHD prophylaxis. Sirolimus should be held if serum levels are supratherapeutic and restarted once in the target range at an appropriate dose. The use of plasmapheresis and plasma exchange does not appear to be of significant clinical utility in the management of transplant-associated microangiopathy, but may be used at the discretion of the treating physician.

2.9.5. Hyperlipidemia

Increases in triglyceride and cholesterol levels as a result of prolonged exposure to sirolimus will be managed with dietary manipulations and HMG CoA inhibitors or other standard therapy as necessary to reduce levels to the normal range. Study drugs should be continued during therapy for hypercholesterolemia and hypertriglyceridemia unless the lipid levels are uncontrollable with standard therapy and are deemed to be a risk to the subject, per the treating physician.

2.9.6. Non-Engraftment

Engraftment will be considered to be three consecutive measurements ANC $\geq$ 500mcL over three or more days. If the ANC < 100 on Day 28, evaluation for graft failure will be initiated, including bone marrow aspiration/biopsy, cytogenetics and/or fluorescence in situ hybridization (FISH) if appropriate, and RFLP analysis if appropriate. Plans for a second stem cell infusion will be made depending on the results of the work-up.

2.9.7. Other Toxicities

All other associated toxicities that may be attributable to sirolimus will be managed symptomatically. If that is unsuccessful and the toxicity can be attributed to sirolimus, this drug will be discontinued.

CHAPTER 3

3. STUDY ENDPOINTS

3.1. Primary Endpoint

The primary endpoint is 114-day Grades II-IV acute GVHD-free survival from the time of randomization. All randomized patients will be analyzed for this endpoint. The event occurs at the first of either of two events: the development of Grade II-IV GVHD or death from any cause prior to 114 days from randomization.

3.2. Secondary Endpoints

3.2.1. Neutrophil and Platelet Engraftment

Neutrophil engraftment is defined as achieving an Absolute Neutrophil Count (ANC) > 500/mcL for three consecutive measurements on different days. The first of the three days will be designated the day of neutrophil engraftment. The ANC is calculated by the following formula:

ANC = Total WBC x (%Neutrophils + %Bands)

Platelet engraftment is defined as a platelet count > 20,000/mcL for three consecutive measurements over three or more days. The first of the three days will be designated the day of platelet engraftment. Subjects must not have had platelet transfusions during the preceding 3 days or in the following 7 days after the day of engraftment, unless the platelet transfusion is being given specifically to achieve a platelet threshold to allow an elective invasive procedure, such as a central catheter removal. The time to a platelet count > 100,000/mcL will be collected as well.

This endpoint will be evaluated through 100 days. All patients failing to meet the neutrophil engraftment target at this time will be considered to have non-engraftment.

3.2.2. Acute GVHD of Grades III-IV

Acute GVHD is graded according to the BMT CTN MOP (see Appendix E). The first day of acute GVHD onset at a certain grade will be used to calculate cumulative incidence curves for that GVHD grade (e.g., if the onset of Grade I acute GVHD is on Day 19 post-transplant and onset of Grade III is on Day 70 post-transplant, time to Grade III is Day 70). This endpoint will be evaluated through 114 days post-randomization.

3.2.3. Mucositis Severity

Mucositis severity will be scored per the modified OMAS scoring system (see Appendix F). Mucositis scores will be recorded three times per week (Monday, Wednesday and Friday) from

Day 0 to the shorter of Day 21 after transplantation or the time of discharge. Both peak and mean mucositis scores will be analyzed.

3.2.4. Time to First Hospital Discharge

The time from stem cell transplantation (Day 0) to the date of first hospital discharge will be recorded as the length of the initial hospital stay. Patients remaining in the hospital on Day 100 will be censored for this outcome.

3.2.5. Infections

Microbiologically documented infections will be reported by site of disease, date of onset, severity and resolution, if any. For definitions, see the BMT CTN MOP.

3.2.6. CMV Reactivation

CMV reactivation is defined as the presence of viral DNA level ≥ 2 pg/mL on two consecutive measurements using a viral hybrid capture or other suitable measurement technique.

3.2.7. Thrombotic Microangiopathy

The occurrence of thrombotic microangiopathy (according to the definition in Section 2.9.4) within the first 100 days after stem cell transplantation will be recorded. The first day of onset will be used for reporting purposes. The incidence of TMA will be compared between study groups using cumulative incidence curves.

3.2.8. VOD

VOD will be defined as the occurrence of VOD (based on the Baltimore Criteria for the diagnosis of VOD) in conjunction with other end-organ dysfunction.

Baltimore Criteria for the Diagnosis of VOD:

Jaundice (bilirubin ≥ 2 mg/dL)
and at least 2 of the following 3 clinical findings:

- Ascites
- Weight gain ≥ 5% above baseline weight (defined as weight on the first day of conditioning or the weight on the date of admission)
- Hepatomegaly (with pre-existing hepatomegaly there must be documentation by physical exam or imaging that liver size is increased over baseline)

Severe VOD is defined as meeting the above criteria and also demonstrating multi-organ failure, i.e., presence of one or both of the following:

- Renal dysfunction:
    - Serum creatinine ≥ 3 x value on the date of admission or ≥ 3 x lowest value during conditioning prior to SCT (whichever is lowest)

- Creatinine clearance or GFR ≤ 40% of admission value
- Dialysis dependence

- Pulmonary dysfunction:
  - Documentation of oxygen saturation ≤ 90% on room air or requirement for oxygen supplementation/ventilator dependence. Dysfunction must be attributable to fluid overload or mechanical impingement from abdominal distention or hepatic enlargement and not to an infection

3.2.9. Chronic GVHD

Chronic GVHD is scored according to the BMT CTN MOP. The first day of chronic GVHD onset will be used to calculate cumulative incidence curves.

3.2.10. Relapse of the Original Malignancy

Relapse of Malignancy - Testing for recurrent malignancy in the blood, marrow or other sites will be used to assess relapse after transplantation. For the purpose of this study, relapse is defined by either morphological or cytogenetic evidence of AML, ALL, CML, MDS or CMML consistent with pre-transplant features.

Minimal Residual Disease - Minimal residual disease is defined by the sole evidence of malignant cells by flow cytometry, or fluorescent in situ hybridization (FISH), or Southern blot, or Western blot, or polymerase chain reaction (PCR), or other techniques, in absence of morphological or cytogenetic evidence of disease in blood or marrow. Since the frequency of testing for minimal residual disease is highly variable among centers, and the sensitivity is highly variable among laboratory techniques, evidence of minimal residual disease will not be sufficient to meet the definition of relapse in the context of this study, even if transplant physicians will utilize the information to alter therapy. Data on tapering immunosuppression, infusing donor T cells, administering chemotherapy or biological agents to attempt reducing the tumor load will be captured in the case report forms.

Acute Leukemia - Relapse will be diagnosed when:

1. Leukemic blasts (> 25%) are documented in the blood or bone marrow after transplantation, or
2. Leukemic blasts > 5% and ≤ 25% are documented in the blood or bone marrow and supported by reappearance of cytogenetic abnormality, or
3. Leukemic blasts > 5% and ≤ 25% are documented in the blood or bone marrow on at least 2 occasions, or
4. There is leukemia detected at an extramedullary site.

Chronic Myelogenous Leukemia - Relapse will be diagnosed when:

1. Immature hematopoietic cells are persistently documented in the peripheral blood, or

2. There is myeloid hyperplasia in the bone marrow in the presence of a cytogenetic relapse, as defined by:

    a) 50% of the metaphases exhibit the characteristic 9;22 translocation with at least ten metaphases analyzed, or

    b) At least one metaphase exhibits the 9;22 translocation on each of two separate consecutive examinations at least one month apart, regardless of number of metaphases analyzed.

Myelodysplastic Syndrome - Relapse will be diagnosed when:

1. Reappearance of pre-transplant morphologic abnormalities, detected in two consecutive bone marrow specimens taken at least one month apart, or

2. Satisfying above criteria for evolution into acute leukemia, or

3. Reappearance of pre-transplant cytogenetic abnormalities in at least 50% of metaphases with at least ten metaphases examined, or

4. Reappearance of pre-transplant cytogenetic abnormality in at least two metaphases on each of two separate consecutive examinations at least one month apart, regardless of the number of metaphases analyzed.

3.2.11. Survival

Both overall survival and relapse-free survival will be assessed one and two years from the time of randomization. All patients will be assessed in an intention-to-treat fashion. Patients alive at the time of last observation will be censored.

CHAPTER 4

4. PATIENT REGISTRATION, RANDOMIZATION AND ENROLLMENT

4.1. Approaching Patients, Eligibility Screening and Obtaining Consent

Subjects will be approached for this study after the decision to proceed with transplantation is made and a suitable HLA-matched sibling identified. Transplant physicians will evaluate the patient eligibility for randomization onto this study (see Section 2.3). Eligibility criteria will be verified and ineligible patients will proceed off study and no further follow-up will be obtained Eligible patients willing to participate in the trial will sign an Institutional Review Board (IRB) approved consent form. Transplant center personnel will record the documentation of patient consent in EMMES AdvantageEDC^{SM} (Electronic Data Capture, an Internet-based data entry system) and patients will be registered through AdvantageEDC.

4.2. Transplant Protocol Registration

Before randomization occurs, the transplant center must state through AdvantageEDC which conditioning regimen will be used for the enrolled subject. Such a registration step will avoid potential biases that preferential use of a certain regimen on one treatment arm could confer to the study. At this stage, the transplant center will also verify that the patient is still a candidate for transplantation, and eligible for the trial.

4.3. Randomization

Once the subject is deemed eligible and has given written informed consent, and the transplant center has confirmed patient eligibility and registered the patient's conditioning regimen, randomization occurs. **Randomization should occur within 7 days of the planned initiation of conditioning therapy.** See Section 5.1.2 for further details on the randomization process.

4.4. Treatment Scheduling

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

4.5. Patient Evaluation

4.5.1. Recipient Evaluation

The patient pre-transplant evaluation must be completed within six weeks of conditioning for transplantation. This step is necessary because patient organ function, infection status and status

of malignancy may vary over time. This evaluation will protect patients with a new contraindication to transplant from initiating transplant therapy at an unsafe time.

4.6. Study Monitoring

A visit schedule based on transplant date is displayed for printing in AdvantageEDC and is shown in Table 4.6a.

Table 4.6a – Follow-Up Schedule

Study Visit	Target Day Post-Transplant
Baseline	≤ 28 days from conditioning
1 week	7 ± 2 days
2 week	14 ± 2 days
3 week	21 ± 2 days
4 week	28 ± 2 days
5 week	35 ± 2 days
6 week	42 ± 2 days
7 week	49 ± 2 days
8 week	56 ± 2 days
9 week	63 ± 2 days
10 week	70 ± 2 days
12 week	84 ± 2 days
100 day	100 ± 5 days
6 month	180 ± 14 days
9 month	270 ± 14 days
12 month	365 ± 14 days
24 month	730 ± 28 days

4.6.1. Patient Assessments

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

Table 4.6b – Patient Clinical Assessments

Study Assessments/ Testing	Base line	Days Post-Transplant																		
		7	14	21	28	35	42	49	56	63	70	77	84	91	98	100	180	270	365	730
History, Physical Exam, Weight, Height, Karnofsky/ Lansky Performance Status	X	X	X	X	X	X	X	X	X	X	X		X			X	X	X	X	X
CBC[1], Differential, Platelet Count, Blood Chemistries[2]	X	X	X	X	X	X	X	X	X	X	X		X			X	X	X	X	X
Infectious Disease Titers[3]	X																			
Serum Cholesterol and Triglyceride Levels	X				X				X							X	X	X	X	X
EKG, PFT, Chest X-ray	X																			
Bone Marrow Aspirate and Biopsy for Pathology and Cytogenetics	X															X			X	X
ß-HCG Serum Pregnancy Test (females only)	X																			
Sirolimus/Tacrolimus Level[4]		X	X	X	X	X	X	X	X	X	X		X			X	X	X	X	X
Mucositis Score[5]		X	X	X																
GVHD and Toxicity Assessments[6]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immune Reconstitution Assays					X											X	X		X	X
Whole Blood Sample[7]	X				X											X	X		X	XX

[1] CBC performed three times weekly from Day 0 until ANC > 500/mcL for three days and platelet count > 20,000/mcL after nadir, while hospitalized. CBC then performed weekly through 8 weeks and every other week through Day 100 post-transplant.

[2] Blood chemistries include: creatinine, bilirubin, alkaline phosphatase, AST, and ALT. Blood chemistries performed twice weekly until Day 28. Blood chemistries performed weekly after Day 28 until 12 weeks post-transplant.

[3] Infectious disease titers include: CMV, hepatitis panel (HepB SAb, HepB SAg, HepB Core Ab, HepC Ab), herpes simplex virus, varicella zoster virus, syphilis, HIV and HTLV ½ antibody.

[4] Sirolimus and Tacrolimus levels are drawn twice weekly while hospitalized, then weekly through week 10, every other week through 100 days post-transplant. Applicable only for patients receiving these drugs.

[5] Every Monday, Wednesday and Friday through Day 21 or hospital discharge. See Appendix F

[6] Morbidity assessments performed weekly until Day 100 post-transplant. GVHD assessments performed weekly until Day 98.

[7] Sample amount of 16 mL, or 8mL for patients < 12 years old. To be cryopreserved at the transplant center for future testing. The center may make further adjustments in blood volumes collected from patients less than 12 years old to meet the institution's Human Investigational Committee's Guidelines.

4.6.1.1. Pre-transplant evaluations

The following observations should be made < **six weeks before initiation of conditioning therapy**:

1. History, physical examination, height and weight.
2. Karnofsky/Lansky performance status.
3. CBC with differential and platelet count, creatinine, bilirubin, alkaline phosphatase, AST, ALT.
4. CMV antibody test, hepatitis panel (HepB SAb, HepB SAg, HepB Core Ab, HepC Ab), herpes simplex virus, varicella zoster virus, syphilis, HIV and HTLV1/2 antibody.
5. Serum cholesterol and triglyceride levels.
6. EKG.
7. Pulmonary function tests, including DLCO, FEV1, and FVC.
8. Bone marrow aspirates for pathology and cytogenetics.
9. Chest X-ray.
10. β-HCG for serum pregnancy test (females only).
11. Blood (16 mL, or 8 mL for patients < 12 years old) from recipient to be cryopreserved at the transplant center for future testing if consent obtained. This should be collected and processed per Appendix C. The center may make further adjustments in blood volumes collected from patients less than 12 years old to meet the institution's Human Investigational Committee's Guidelines.
12. Blood (16 mL, or 8 mL for patients < 12 years old) from donor to be cryopreserved at the transplant center for genetic testing if consent obtained. This should be collected and processed per Appendix C. The center may make further adjustments in blood volumes collected from patients less than 12 years old to meet the institution's Human Investigational Committee's Guidelines.

4.6.1.2. Post-transplant evaluations

The following observations should be made according to Table 4.6b:

1. History and physical exam to assess GVHD and other morbidity weekly through Day 100 post-transplant, then at 6, 9, 12 and 24 months. GVHD evaluation and grading to be in keeping with BMT CTN MOP.
2. Data on occurrence of infections and recorded as per the CTN MOP.
3. CBC at least three times a week from Day 0 until ANC > 500/mcL for 3 days and platelet count > 20,000/mcL for 3 days (while hospitalized only) after nadir reached. A manual differential must be performed. Thereafter CBC weekly until 10 weeks, every other week through 100 days, then at 6, 9, 12 and 24 months post-transplant.

4. Creatinine, bilirubin, alkaline phosphatase, ALT, AST, twice a week until hospital discharge and then weekly until 10 weeks, every other week through 100 days, then at 6, 9, 12 and 24 months post-transplant.
5. Tacrolimus and sirolimus levels twice a week until hospital discharge and then weekly until 10 weeks, every other week through 100 days, then at 6, 9, 12 and 24 months post-transplant (only when subject is using the medication).
6. Mucositis assessment three times per week (Monday, Wednesday and Friday) while hospitalized or until Day 21 (see Appendix F for modified OMAS scoring system).
7. Serum cholesterol and triglyceride level at months 1 and 2, 100 days, months 6, 9, 12 and 24 post-transplant.
8. Bone marrow aspirate and biopsy to pathology, aspirate and cytogenetics at 100 days, 12 and 24 months post-transplant.
9. Immune reconstitution blood samples at Days 28, 100, 180, 365 and 730 (see Appendix C).
10. Investigational future testing blood samples (16 mL, or 8 mL for patients < 12 years old) at Days 28, 100, 180, 365 and 730 if consent obtained (see Appendix C). The center may make further adjustments in blood volumes collected from patients less than 12 years old to meet the institution's Human Investigational Committee's Guidelines.

4.6.2. Case Report Forms

A description of the forms, the procedures required for forms completion and timeliness of submission can be found in the Data Management Handbook and User's Guide. Forms that are not received within the specified time are considered delinquent. Transplant centers can view submitted past due, and expected forms via AdvantageEDC. A missing form will continue to be requested either until the form is reported, or until an exception is granted.

4.6.3. Reporting Patient Deaths

Recipient death information must be reported to the BMT CTN Data Coordinating Center (DCC) within one business day of the event. Death after the patient has left the transplant center must be reported within one business day of the event notification to the transplant center. If the cause of death is unknown, it need not be recorded at the time of initial reporting. However, once the cause of death is determined, the form must be updated.

4.6.4. Reporting Serious Adverse Events

4.6.4.1. Patient SAEs

Reporting of patient serious adverse events (SAE) will be consistent with standard BMT CTN procedures. Unexpected, grades 3-5 adverse events (AEs) will be reported through an expedited AE reporting system via the web-based electronic data capture system, AdvantageEDC. Unexpected, grades 4-5AEs 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. Other SAEs will be tracked periodically as defined in the Form Submission Schedule, staged according to NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0. The Data and Safety Monitoring Board will receive summary reports of all adverse experiences on at least an annual basis.

4.6.5. CIBMTR Data Reporting

All transplant centers will be required to pre-register patients with the Center for International Blood and Marrow Transplant Research (CIBMTR) for all transplant patients whether or not they enroll in a BMT CTN Protocol. In addition, the transplant center must complete the CIBMTR Day 100 Report Form (including the Core, Graft and Disease Inserts) and CIBMTR Follow-up Form (including the Core and Disease Inserts) yearly for all patients enrolled in BMT CTN protocols. CIBMTR forms will be submitted directly to the CIBMTR at the times specified on the Form Submission Schedule.

CHAPTER 5

5. STATISTICAL CONSIDERATIONS

5.1. Study Design

The study is designed as a Phase III, randomized, open label, multicenter, prospective comparative study of sirolimus and tacrolimus versus tacrolimus and methotrexate as GVHD prophylaxis after HLA-matched, related peripheral blood stem cell transplantation in patients with hematologic malignancies. The target enrollment is 312 patients.

5.1.1. Accrual

It is estimated that three years of accrual will be necessary to enroll the targeted sample size. Both Core and non-Core Centers will enroll patients on this study. Accrual will be reported by race, ethnicity, gender, and age (pediatric patients will be defined as < 18 years).

5.1.2. Randomization

All patients will be randomized within 7 days prior to the initiation of conditioning therapy. Randomization will be performed in a 1:1 ratio using random block sizes for the two arms. Randomization will be stratified by transplant center.

5.1.3. Primary Endpoint

The primary endpoint is the Grades II-IV acute GVHD-free survival proportion at 114 days post-randomization. The primary analysis will be performed using the intent-to-treat principle so that all randomized patients will be included in the analysis. Death or the occurrence of Grade II-IV acute GVHD by Day 114 post-randomization will be considered events for this endpoint.

5.1.4. Primary Hypothesis

The primary null hypothesis of the study is that there is no difference between the GVHD-free survival rates for sirolimus and tacrolimus compared to tacrolimus and methotrexate.

$$H_{o:} \quad p_{st} = p_{tm}$$

$$H_{a:} \quad p_{st} \neq p_{tm}$$

5.2. Sample Size and Power Considerations

GVHD-free survival at 114 days post-randomization will be compared between the standard and experimental therapy arms using the standardized difference in the Kaplan Meier estimates of survival. The final analysis will be performed after all patients have been followed for a minimum of 114 days post-randomization. At this time point, all individuals will have been

completely observed for the primary outcome. Note that if there is no loss to follow up, the comparison of the Kaplan-Meier estimates is equivalent to the usual two-sample Z test of binomial proportions. However, the Kaplan Meier test will be used in the event of loss to follow up as well as in the interim analyses conducted with incomplete follow-up. The sample size of 156 patients per group is sufficient to maintain type I error of 5% across all planned interim analyses (see below) while providing 80% statistical power for a two-sided test to detect an increase in the proportion surviving without Grade II-IV GVHD at 114 days from 0.60 in the tacrolimus/methotrexate arm to 0.75 in the sirolimus/tacrolimus arm.

5.3. Interim Analysis and Stopping Guidelines

Interim analyses for efficacy will be conducted at times coincident with regularly scheduled meetings of the NHLBI-appointed Data and Safety Monitoring Board (DSMB) at approximately six month intervals. Monitoring of key safety endpoints will be conducted monthly, and if rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures.

5.3.1. Interim Analysis for Efficacy

Analyses will be performed as described below for the primary endpoint. Toxicity, adverse events, and other safety endpoints will be monitored regularly and reported to the DSMB at each interim analysis.

At the time of each interim analysis, the test statistic based on the Kaplan-Meier proportion will be compared to the critical value shown below. All patients randomized prior to the time of the interim analyses will be used to compute the Kaplan-Meier estimate. If the test statistic exceeds the critical value, the DSMB will discuss the continuation of the trial.

At each interim analysis time point, a two-sided test to detect either an increase or decrease in the proportion of patients surviving without Grade II-IV GVHD at 114 days will be performed, as described above for the final analysis. In order to preserve the over-all type I error rate at 5%, the critical value for the test statistic will be inflated above 1.96, the value that would be used if no repeated testing were used. Equivalently, the nominal p-value at which an observed difference is declared significant will be reduced below 0.05. The actual critical values and nominal p-values will be computed using statistical methods for group sequential testing with O'Brien Fleming boundaries.

As an example, Table 5.3a shows the critical values and nominal p-values for tests conducted every six months starting with the 9th month after the study opens to enrollment. The column labeled "followed to 114 days" shows the expected number of individuals who have reached the 114-day post-randomization follow-up time point, assuming uniform accrual over a three-year period. The fraction of patients followed to 114 days, as compared to a denominator comprised of the total sample of 312, quantifies the "statistical information" from which the critical values, nominal type I errors and cumulative type I error are computed.

Table 5.3a – Critical Values and Cumulative Type I Error

Calendar Time (Months)	Followed to 114 Days	Critical Value	Nominal Type I Error	Cumulative Type I Error
9	46	5.029	.0000	.0000
15	98	3.556	.0004	.0004
21	150	2.903	.0034	.0038
27	202	2.514	.0094	.0132
33	254	2.249	.0158	.0290
39.3 (Final)	312	2.053	.0210	.0500

In practice, the rate of accrual or timing of DSMB meetings may not be as anticipated. To permit necessary flexibility in scheduling interim analyses, the critical values will be recomputed to correspond to the actual available statistical information using the "use-function" approach of Lan and DeMets.

5.3.2. Operating Characteristics of the Design

Under the assumption that time to GVHD or death is exponentially distributed, the statistical power to reject the null hypothesis of equal 100-day GVHD-free survival is shown in Table 5.3b under a variety of scenarios, using the comparison of the Kaplan-Meier estimates. These simulation results assume uniform accrual of patients over three years. This table shows that the target sample size of 312 patients has 80% power to detect a 15% improvement in GVHD-free survival.

Table 5.3b – Power to Reject the Null Hypothesis under Various Scenarios

	Proportion Surviving GVHD-Free at Day 114		Power at Interim and Final Analyses By Month of Scheduled Analysis						
N	Standard	Experiment	9	15	21	27	33	39.3	Overall Power
312	.60	.60	.000	.001	.004	.011	.018	.021	.055
312	.60	.70	.000	.011	.057	.116	.144	.123	.451
312	.60	.75	.001	.044	.177	.248	.207	.125	.802
312	.60	.80	.003	.136	.358	.293	.133	.049	.972

* from simulation with 10,000 replications, assuming exponential time to failure

5.3.3. Guidelines for Safety Monitoring

The cumulative incidences of thrombotic microangiopathy and severe veno-occlusive disease (VOD) at 100 days post-transplant will be monitored separately for each arm. Death without each type of toxicity will be considered as a competing risk for that event. Refer to Section 3.2 for definitions of these safety monitoring endpoints. Monitoring will be performed monthly until

enrollment to that treatment arm is closed. Each month, the null hypothesis that the cumulative incidence of thrombotic microangiopathy is less than or equal to 10% will be tested against the alternative that it is greater than 10%. Similarly, the cumulative incidence of severe VOD will be tested against a null value of 10%. Since the busulfan/cyclophosphamide conditioning regimen was removed early in the study due to toxicity, only patients who receive TBI-based conditioning regimens will be included in the safety monitoring analysis for severe VOD.

A truncated Sequential Probability Ratio Test (SPRT) for a binomial outcome will be used for each endpoint, as described in greater detail below. Note that in the absence of censoring, the binomial proportion of patients experiencing the toxicity prior to death is equal to the cumulative incidence. This sequential testing procedure conserves type I error across all of the monthly examinations for a single toxicity endpoint, but not across the multiple safety endpoints or two treatment arms. Thus for a single endpoint and study arm, the type I error is approximately 5%, and across both safety endpoints and both treatment arms, the study-wise type I error is < 20%.

The rationale for not conserving type I error across multiple safety endpoints and treatments is twofold. First, adjusting the size of the test for multiple comparisons would reduce statistical power to detect adverse outcomes, which seems imprudent. Secondly, the procedure is a guideline for requiring additional review by the Data and Safety Monitoring Board, and is not a formal "stopping rule" that would mandate automatic closure of study enrollment.

The SPRT can be represented graphically. At each monthly interim analysis, the total number of patients enrolled is plotted against the total number of endpoints observed in those patients, (e.g., patients with thrombotic microangiopathy). The continuation region of the SPRT is defined by two parallel lines. Only the upper boundary will be used for monitoring each treatment arm to protect against poor 100-day incidences of rates of thrombotic microangiopathy. If the graph falls above the upper boundary, the SPRT rejects the null hypothesis, and concludes that there are more events of thrombotic microangiopathy than predicted by the observed number of patients enrolled on study. Otherwise, the SPRT continues until enrollment to the treatment arm reaches the target goal.

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_1 when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta_i)$. The tests to be used in this protocol were developed from the following SPRTs:

- A SPRT contrasting 10% versus 20% 100-day cumulative incidence of thrombotic microangiopathy, with nominal type I and II errors of 5.5% and 20%, respectively. The common slope of the parallel lines is 0.145 and the intercept for the upper boundary is 3.301.
- A SPRT contrasting 10% versus 20% 100-day cumulative incidence of severe VOD, with nominal type I and II errors of 5.5% and 20%, respectively. The common slope of the parallel lines is 0.145 and the intercept for the upper boundary is 3.301.

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 of 5.5% and closer to 5%.

Table 5.3c demonstrates the stopping points for various numbers of enrolled patients for illustration.

Table 5.3c – Sample Stopping Boundaries for SPRT

Number of Patients Enrolled, n	Stop if Number of Events in First n Patients is >= x	Number of Patients Enrolled, n	Stop if Number of Events in First n Patients is >=x
15	6	90	17
30	8	105	19
45	10	120	21
60	13	135	23
75	15	150	26

Note that because there is a lag from transplant to complete follow up (100 days), the stopping rule may be triggered before complete follow up is available. For example, there may be 6 events in the first 15 patients even though the first 15 patients do not all have 100 days of follow up. This implies that the plot of the number of patients transplanted against events observed in those patients may change for earlier accrual points as follow up is completed, and will therefore be updated each month.

The actual operating characteristics of the truncated test, shown in Table 5.3d, were determined in a simulation study that assumed uniform accrual of 156 individuals over a three-year time period. No assumptions are made about the time to event distribution for either the toxicity event of interest or the competing event of death prior to observing that toxicity. The monitoring rule is based only on the binomial outcome of whether a patient experienced the toxicity by 100 days and prior to death. Because the simulations only use a binomial outcome, the mean month stopped is calculated based on the accrual time of the patient triggering the stopping rule + 100 days for complete follow up. In practice, because of the potential for the stopping rule to be triggered prior to complete follow up of a particular patient, the actual mean month stopped may be earlier than shown in the simulation results. However, this is difficult to estimate because it is dependent on assumptions about the time to event distribution for both the toxicity event and the competing risk, as well as the dependence between them.

Table 5.3d – Operating Characteristics of Sequential Testing Procedure from a Simulation Study with 10,000 Replications

Thrombotic Microangiopathy

True 100-Day Rate	10%	15%	20%
Probability Reject Null	0.052	0.484	0.934
Mean Month Stopped	38.1	30.3	18.6
Mean # Endpoints in 100 Days	15.2	18.1	14.9
Mean # Patients Enrolled	151.4	120.3	73.9

Severe VOD

True 100-Day Rate	10%	15%	20%
Probability Reject Null	0.052	0.484	0.934
Mean Month Stopped	38.1	30.3	18.6
Mean # Endpoints in 100 Days	15.2	18.1	14.9
Mean # Patients Enrolled	151.4	120.3	73.9

For example, the testing procedure for thrombotic microangiopathy rejects the null hypothesis in favor of the alternative 5% of the time when the true 100-day incidence is 10%, and 93% of the time when the rate is 20%. When the true 100-day toxicity incidence is 20%, on average, the Data and Safety Monitoring Board will be consulted 18.6 months after opening, when 14.9 events have been observed in 73.9 patients.

5.4. Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, race/ethnicity, performance status, primary disease, risk status, donor age, donor gender, and donor ethnicity. Between group comparisons will be performed for continuous variables via a t-test and for categorical variables, via the chi-square test.

5.5. Analysis of Secondary Endpoints

1. **Time to stable neutrophil and platelet engraftment after transplantation**; cumulative incidence curves will be computed by treatment. A log-rank test will be used to compare the two arms. A Cox proportional hazards model will be fit to control for important prognostic variables.
2. **Time to Grades II-IV or Grades III-IV acute GVHD**; cumulative incidence curves will be computed by treatment. A log-rank test will be used to compare the two arms. A Cox proportional hazards model will be fit to control for important prognostic variables.
3. **Mucositis severity**; median (range) and maximal mucositis scores will be summarized by treatment arm and compared using a Mann-Whitney Wilcoxon test.

4. **Time to first hospital discharge**; cumulative incidence curves will be computed by treatment. A log-rank test will be used to compare the two arms. A Cox proportional hazards model will be fit to control for important prognostic variables.
5. **Incidence of infection**; cumulative incidence curves will be computed by treatment. A log-rank test will be used to compare the two arms. A Cox proportional hazards model will be fit to control for important prognostic variables.
6. **Incidence of CMV reactivation**; cumulative incidence curves will be computed by treatment. A log-rank test will be used to compare the two arms. A Cox proportional hazards model will be fit to control for important prognostic variables.
7. **Incidence of thrombotic microangiopathy**; cumulative incidence curves will be computed by treatment. A log-rank test will be used to compare the two arms. A Cox proportional hazards model will be fit to control for important prognostic variables.
8. **Time to chronic GVHD**; cumulative incidence curves will be computed by treatment. A log-rank test will be used to compare the two arms. A Cox proportional hazards model will be fit to control for important prognostic variables.
9. **Relapse of primary malignant disease in distinct disease subgroups**; cumulative incidence curves will be computed by treatment. A log-rank test will be used to compare the two arms. Also, a Cox proportional hazards model will be fit to control for important prognostic variables. All patients will be followed for a minimum of one year post-transplant for relapse.
10. **Relapse-free and overall survival**; Kaplan-Meier curves will be computed by treatment. A log-rank test will be used to compare the two arms. Also, a Cox proportional hazards model will be fit to control for important prognostic variables. All patients will be followed for a minimum of one year post-transplant for mortality and relapse.

Two-sided tests will be used throughout. A p-value of 0.05 or less will be considered statistically significant.

5.6. Safety Analysis

All entered patients will be included in the safety analysis:

1. **Serious Adverse Events**: All reported serious adverse events potentially associated with study drug will be carefully examined with respect to the severity and relationship to study drug. Adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events Version 3.0. The incidence for each reported study drug associated adverse event delineated in Section 2.8.2 will be presented for each group.
2. **Clinical Laboratory Tests**: Laboratory tests will be performed during the study. Descriptive statistics will be calculated for each laboratory test. Abnormal laboratory test results will be summarized in the report. Laboratory data will be cross-classified into below, within, and above the reference range according to their values at screening and during the study.

APPENDIX A

SAMPLE TAPERING SCHEDULES FOR SIROLIMUS AND TACROLIMUS

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SAMPLE TAPERING SCHEDULES FOR SIROLIMUS AND TACROLIMUS

EXAMPLE 1:

Starting doses: Sirolimus 4 mg qd
Tacrolimus 2 mg bid

Day 100	Sirolimus	3 mg	Tacrolimus	2 mg bid
Day 100 + 2 Weeks	Sirolimus	3 mg	Tacrolimus	1.5 mg bid
Day 100 + 4 Weeks	Sirolimus	2 mg	Tacrolimus	1.5 mg bid
Day 100 + 6 Weeks	Sirolimus	2 mg	Tacrolimus	1.0 mg bid
Day 100 + 8 Weeks	Sirolimus	1 mg	Tacrolimus	1.0 mg bid
Day 100 + 10 Weeks	Sirolimus	1 mg	Tacrolimus	0.5 mg bid
Day 100 + 12 Weeks	Sirolimus	0 mg	Tacrolimus	0.5 mg bid
Day 100 + 14 Weeks	Sirolimus	0 mg	Tacrolimus	0 mg bid

EXAMPLE 2:

Starting doses: Sirolimus 2 mg qd
Tacrolimus 3 mg bid

Day 100	Sirolimus	2 mg	Tacrolimus	2.0 mg bid
Day 100 + 2 Weeks	Sirolimus	1.5 mg	Tacrolimus	2.0 mg bid
Day 100 + 4 Weeks	Sirolimus	1.5 mg	Tacrolimus	1.0 mg bid
Day 100 + 6 Weeks	Sirolimus	1 mg	Tacrolimus	1.0 mg bid
Day 100 + 8 Weeks	Sirolimus	1 mg	Tacrolimus	0.5 mg bid
Day 100 + 10 Weeks	Sirolimus	1 mg qod*	Tacrolimus	0.5 mg bid
Day 100 + 12 Weeks	Sirolimus	1 mg qod	Tacrolimus	0 mg bid
Day 100 + 14 Weeks	Sirolimus	0 mg	Tacrolimus	0 mg

* 1 mg qod may be replaced by 0.5 mg qd for sirolimus, however, this formulation does not exist in capsule format. Caplets may be manually split.

APPENDIX B

INFORMED CONSENTS

- **Participant Consent**
- **Donor Consent**

IRB #

Informed Consent to Participate in Research

Principal Investigator Contact Information
(Insert contact information for PI at your site)

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

Introduction
This is a clinical trial, which is a research study to answer specific medical questions. The information from this study will help future patients. The Study doctor (the person in charge of the research) will explain the clinical trial to you. Clinical trials include only people who choose to join the study.

Please take your time to decide if you want to join this study. Some people find it helpful to talk about the study with their family and friends before they make a decision. It may also be useful to talk with your doctor and other people on your health care team about the study. If you have questions or want to know more about the study, you can ask them for more information.

You are asked to join this study because:

1. You have a disease that can be treated by a peripheral blood stem cell transplant; and,
2. You have a brother or sister who has agreed to be your donor.

Before you decide whether or not to join the study, please read the information below. Feel free to ask questions to understand your rights and protections. Participating in this study is your choice. If you decide not to be in this study**,** you and your doctor will discuss other treatment options.

Why is this study being done?
Stem cell transplantation is a standard therapy for acute and chronic leukemias and myelodysplastic disorders. A common problem that may occur after a stem cell transplant is a condition known as graft-versus-host disease (GVHD). The word "graft" refers to the donor blood cells that you will receive during your transplant. The word "host" refers to the person (in this case, you) receiving the cells. GVHD is a complication where the donor graft attacks and damages some of your (the transplant recipient's) tissues.

- GVHD can cause skin rash, intestinal problems such as nausea, vomiting, or diarrhea,
- It may also damage your liver and cause hepatitis or jaundice.
- GVHD may also increase your risks of infection.

The purpose of this study is to compare two combinations of medications to see which is better at preventing GVHD. These combinations of medications in this study are:

A- Sirolimus and tacrolimus

B- Methotrexate and tacrolimus.

Doctors want to know if combination A is better than combination B or if they give the same results. The study will help doctors make GVHD treatment choices for future transplant patients.

How many people will take part in the study?
About 312 people throughout the country will take part in this study. Each study group will have 156 patients.

What will happen if I take part in this research study?
Before you begin the study -- You will need to have several exams, tests or procedures to find out if you can be in the study. These exams, tests or procedures are part of regular cancer care and may be done even if you do not join the study. If you have had some of them recently, they may not need to be repeated. This will be up to your doctor with the study.

- Medical history and physical exam, including height and weight
- Blood tests
- Urine tests
- Heart function tests
- Lung tests, including a Pulmonary Function Test (PFT)
- Bone marrow biopsies and aspirates

- If you are a woman able to have children, a pregnancy test will also be performed, using a blood sample. If you are pregnant, you will not be able to take part in this study.

Before your transplant, your doctor will choose from one of two conditioning regimens. The conditioning regimen prepares your body for transplant. It uses treatments such as chemotherapy and radiation to destroy the cancer cells and the cells that make up your immune system. Your doctor will decide which conditioning regimen you will receive before you join one of the study groups.

During the study **--** If the exams, tests and procedures show that you can be in the study, the next step is for you to be randomized into one of the study groups described below. Randomization means that you are put into a group by chance, similar to the flip of a coin. A computer program will place you in one of the study groups. Neither you nor your doctor can choose the group you will be in.

You will begin your conditioning chemotherapy and/or radiotherapy several days prior to the transplant. The treatments to prevent GVHD will begin three days before transplantation for both study groups.

A. Sirolimus/tacrolimus group:

- You will take a sirolimus pill by mouth once per day.
- Tacrolimus will be given **by intravenous infusion (through your vein)** every day while in the hospital.
- Before you leave the hospital the tacrolimus will be changed to a pill that you will take by mouth.
- These medications will be continued for at least 100 days from the time of transplantation.
- The amount of drug given will slowly be decreased and eventually stopped. This process occurs over several months.

B. Methotrexate/tacrolimus group:

- Tacrolimus will be given **by intravenous infusion (through your vein)** every day while in the hospital.
- Before you leave the hospital the tacrolimus will be changed to a pill that you will take by mouth.
- You will take methotrexate on 4 separate days. The methotrexate will be given by intravenous infusion on the 1st, 3rd, 6th and 11th days after your transplant.
- The tacrolimus will be continued for at least 100 days from the time of transplantation.

- The amount of drug given will slowly be decreased and eventually stopped. This process occurs over several months.

Peripheral blood stem cells from your donor will be given to you 3 days after starting the GVHD treatments.

Following the transplant, you will have the following **standard** tests and evaluations:

- Medical history
- Physical examination, including height and weight
- Blood tests
- Urine tests

You will have the following **study** tests performed:

- Blood tests to measure the levels of sirolimus and tacrolimus
- Blood tests to measure your cholesterol and triglycerides (fat) levels
- Oral exams to measure any side effects that occur in your mouth and throat (mucositis)

You will be asked to return to the transplant center for regular follow up care. The standard tests and study tests will be done at that time.

How long will I be in the study?
You will be in the study for up to two years. You will need to take medication for at least 3 months and possibly longer depending on how long it takes to gradually take you off of the medication. You will also need to answer questions about your medical health at regular times for up to two years.

Follow-up for your transplant will last as long as you require care. However, we would like to keep track of your medical condition for the rest of your life by contacting you and the doctor providing your regular medical care by phone or mail once a year. Keeping in touch with you and checking on your condition every year helps us look at the long-term effects of the study and transplantation in general. Many transplant centers include this type of long-term follow-up as part of their regular medical care. It is not necessary for you to agree to follow-up for longer than two years to participate in this study.

Can I stop being in the study?
Yes. You can decide to stop at any time. Tell your doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop safely.

It is important to tell your doctor if you are thinking about stopping so any risks from the medications can be evaluated. Another reason to tell your doctor that you are thinking about stopping is to discuss what follow-up care and testing could be most helpful for you.

Can the Principal Investigator withdraw me from the study?
You can be taken off the study (with or without your consent) for any of the following reasons:

- You do not qualify to be in the study because you do not meet the study requirements. Ask your doctor if you would like more information about this.
- You need a medical treatment not allowed in this study.
- The investigator decides that continuing in the study would be harmful to you.
- The study treatments have a bad effect on you.
- You become pregnant and the study treatment could be harmful to the fetus.
- You are unable to keep appointments or take study drugs as directed.
- Other study-specific reasons; for example, if the dose of study drug you are taking has been found to be unsafe.
- The study is cancelled by the Food and Drug Administration (FDA) or the National Institutes of Health (NIH).

What side effects or risks can I expect from being in the study?
Everyone taking part in the study will be watched carefully for any side effects. However, doctors don't know all the side effects that may happen. Side effects may be mild or very serious. Your health care team may give you medicines to help lessen side effects. In some cases, side effects can be serious, long lasting, or may never go away.

You should talk to your study doctor about any side effects that you have while taking part in the study.

Potential Side Effects and Risks from Study Drugs

Sirolimus and Tacrolimus	
Likely (10-25%)	**Less Likely but Serious (< 10%)**
• Elevation of blood lipids (fats, including cholesterol and triglycerides) • Mild reduction in blood counts, including platelets, red cells (anemia) and white blood cells • Low blood potassium • Muscle aches and pains • Fluid retention • High blood pressure • Tremor • Swollen gums • Increased facial hair	• Lung inflammation • Reversible kidney damage, which may lead to acute kidney failure and require hospitalization, possibly dialysis and may lead to permanent kidney failure • Thrombotic Microangiopathy, a syndrome comprised of abnormal kidney function and destruction of red blood cells and platelets. Temporary dialysis may be required for the kidney failure • Neurological dysfunction, including a decreased level of consciousness, coma, blindness and seizures • Veno-occlusive disease, which causes severe damage to the liver

Methotrexate and Tacrolimus	
Likely	**Less Likely but Serious**
• Delay in the time it takes for your blood counts to recover. This may increase the risk of infection and bleeding after transplantation, may increase the number of transfusions required during the transplantation and may prolong your hospital stay. • Worsening of mucositis (inflammation of the lining of the mouth, throat and stomach) • Low blood potassium • Muscle aches and pains • Fluid retention • High blood pressure • Tremor • Swollen gums • Increased facial hair	• Lung inflammation • Reversible kidney damage, which may lead to acute kidney failure and require hospitalization, possibly dialysis and may lead to permanent kidney failure • Thrombotic Microangiopathy, a syndrome comprised of kidney dysfunction and destruction of red blood cells and platelets. Temporary dialysis may be required for the kidney failure • Neurological dysfunction, including a decreased level of consciousness, coma, blindness and seizures

These side effects usually get better completely after you stop taking the drugs, but some long-term problems, such as kidney damage, may occur.

A standard combination of medications to prevent GVHD is the combination of tacrolimus and methotrexate. We are trying to determine if the combination of sirolimus and tacrolimus is as effective as tacrolimus and methotrexate. Because this is a comparative trial to study how to prevent GVHD, there is a risk that you will receive a less effective GVHD prevention program than the standard combination. Even though this study is trying to prevent GVHD from developing, there is a chance that you will still develop GVHD, no matter which combination you receive. All study participants who develop GVHD will be treated for GVHD. The treatment you receive will be up to your doctor.

Refer to Attachment A for additional risks and toxicities for all transplant patients.

Are there benefits to taking part in the study?
There may or may not be direct benefits to you from participating in this study. We hope that information gathered in this trial will benefit future transplant patients.

What other choices do I have if I do not take part in the study?
If you do not want to join this study, you should know your other options. These options include:

- Bone marrow transplantation
- Peripheral Blood Stem Cell Transplantation using standard treatment (but not included in study)
- No transplant
- Other chemotherapy
- No therapy for your cancer with care to help you feel comfortable

You should know about your treatment choices before you decide if you will take part in this study.

What are the costs of taking part in this study?
You and/or your insurance company will pay all standard care relating to your stem cell transplant.

You will not be billed for any tests or procedures that are only for research.

You will not be paid to be in this study.

Sirolimus will be provided free of charge by Wyeth, Inc for at least 6 months. At that time, you or your insurance carrier will responsible for the costs of this medication. Methotrexate and tacrolimus will be the responsibility of you or your insurance carrier.

The companies that make the drugs used in this study did not plan or design this clinical trial. They will also not have a part in analyzing the results of 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.

What if I am injured as a result of being in this study?
In the event that this research activity results in an injury, treatment will be available including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to your insurance company. If you think you have suffered a research related injury, let the study physicians know right away. Unexpected side effects or accidents might result in your getting sicker than anticipated in the course of this treatment. All available medical care will be provided to you, but you and your insurance company (3rd party payer) are responsible for the costs of all such care. If you have any questions about study-related injuries, you may contact [insert name of person at institution] at [insert phone number].

What are my rights if I take part in this study?
Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in the study.

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

If you have questions about your rights as a study subject, you may call the Institutional Review Board (IRB) office at [insert phone number].

Will my medical information be kept private?
All necessary steps will be undertaken to avoid your being identified in any public presentations. However, the results of this study treatment may be published in scientific journals in the future, but no one patient (including you) will be identified. Information concerning your transplant course may be reviewed or transmitted to national and international transplant registries, including the Center for International Blood and Marrow Transplant Research (CIBMTR) and the National Marrow Donor Program (NMDP), to the Food and Drug and Administration (FDA), Data Coordinating Center of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), the EMMES Corporation (which is helping to coordinate this study) and to other authorized study organizations. However, you will not be identified by name in publications or reports coming from such groups or review.

Information related to or resulting from your stem cell transplant will be reported to the CIBMTR. The CIBMTR is a voluntary organization of basic and clinical scientists working together in an effort to gather information on results of stem cell and marrow transplants. This information is used to guide clinical decisions and identify ways to improve transplant outcomes. Scientific data or medical information (not identifiable with you) that could be useful to others may be presented at meetings and/or published in medical journals.

HIPAA[1] authorization to use and disclose individual health information for research purposes

a. Purpose: As a research participant, I authorize the Principal Investigator and the researcher's staff to use and disclose my individual health information for the purpose of conducting the research study entitled *A Phase III Randomized, Multicenter Trial Comparing Sirolimus/Tacrolimus with Tacrolimus/Methotrexate as GVHD Prophylaxis After HLA-Matched, Related Peripheral Blood Stem Cell Transplantation.*

b. Individual Health Information to be Used or Disclosed: My individual health information that may be used or disclosed to conduct this research includes: demographic information (e.g., age, date of birth, sex, weight), medical history (e.g., diagnosis, complications with prior treatment), physical examination findings, and laboratory test results obtained at the time of work up and after transplantation (e.g., blood tests, biopsy results).

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

c. Parties Who May Disclose My Individual Health Information: The researcher and the researcher's staff may obtain 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:

- Principal Investigators and the researcher's staff, including Dr. Corey Cutler and Dr. Joseph Antin, Study Chairpersons and staff/laboratories at Dana Farber Cancer Institute
- Staff/laboratories identified in the protocol for the evaluation of other laboratory samples
- National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI), both of the National Institutes of Health (NIH), study sponsors
- Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Data and Coordinating Center
- 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 responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments.
- Others:

_____

_____

_____

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 research-related treatment that is provided through the study. However, 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, the 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. DNA from your stored blood sample might be used in genome-wide association (GWA) or pharmacogenomics 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. Pharmacogenomics studies are similar genetic tests but look specifically at genes related to how the body breaks down medications.

i. If your coded samples are used in such a study, the researcher is required to add your test results and sample information into a shared, 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.

j. This authorization does not have an expiration date.

****

About Using Blood for Research

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 six small (3 teaspoons or 16 mL) blood samples for future research. Patients less than 12 years old will provide 1½ teaspoons or 8 mL). If you agree, these samples will be obtained at the time other blood samples are drawn on 6 occasions pre-transplant and on Day 28, 100, 180, 365 and 730. They will be kept and may be used in research to learn more about GVHD, 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 National Heart, Lung, and Blood Institute (NHLBI) sample repository in Maryland. 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 samples will be stored at this repository until the samples have been used for the research tests or until the end of the study. Any research performed on the samples must first be approved by an advisory panel at the NHLBI.

The research that may be done with your blood is not designed specifically to help you. It might help people who have GVHD, 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 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.

- ❑ Yes, I agree to have small blood samples drawn for future research.
- ❑ No, I do not agree to have small blood samples drawn for future research.

_____ _____
Signature *Date*

****

If you have any questions about this study, you may contact the study Principal Investigator listed on the first page of this form.

If you have questions about your rights as a research participant, you may contact (INSERT CONTACT INFORMATION)

IRB #

CONSENT AND ASSENT INSTRUCTIONS

CONSENT: Subjects 18 years and older must sign on the subject line below. For subjects under 18, consent is provided by the Legally Authorized Representative.

ASSENT: Is required for subjects under the age of 18, using the Assent Section on the following page.

I have been informed about this study's purpose, procedures, possible benefits and risks. I have been given the chance to ask questions. My questions have all been answered satisfactorily. I understand that I can ask other questions at any time.

I voluntarily agree to take part, or to allow my child to take part, in this study.

By signing this consent form, I have not given up any of the legal rights that I (my child) otherwise would have as a subject in a research study.

_____ _____
Subject's Signature Date

If you are not the subject, please print your name_____
and indicate one of the following:

_____	The subject's parent	_____	The subject's guardian
_____	A surrogate	_____	A durable power of attorney
_____	A proxy	_____	Other, please explain:

_____ _____
Legally Authorized Representative Signature Date

As a representative of this study, I have explained the purpose, the procedures, the benefits, and the risks that are involved in this research study:

_____ _____
Signature of person conducting informed consent Date

ASSENT SIGNATURES: For subjects under the age of 18 years.

Assent of Minor

I have been told what I will be asked to do if I am in this study. I have been told that I don't have to be in this study. I may quit the study at any time, and no one will be mad at me. I have had a chance to discuss the study and ask questions. My questions have been answered. I agree to be in the study and do what I am asked to do so long as I continue in the study.

_____ _____ _____

Signature of Minor Date Age (years)
Study Personnel

I have explained the purposes, procedures, and risks involved in this research study in detail to:

_____

Print name(s) of Parents/Authorized Consenting Party, and

who in my opinion _____IS/____IS NOT capable of assenting to participate in this study.

Print child's name

_____ _____

Signature of Person Conducting Assent Date

ATTACHMENT A

Additional Risks and Toxicities Related to the Standard Transplant Procedure

There are certain risks related to a peripheral blood stem cell (PBSC) transplant. There are risks from the medications and/or irradiation therapy you will receive as part of the conditioning for the transplant, and risks related to the transplant itself. Most of these risks and side effects are listed below, but they will vary from person to person. There may be more risks involved with transplantation, and there may be more side effects. Your doctor may give you medications to lessen some of the side-effects.

Risks Related to the Transplant Conditioning Regimen

You and your doctor will choose a conditioning regimen that may include some of the following therapies.

Cyclophosphamide (Cytoxan): This is a common medication used to treat cancer. This medication kills cancer cells by stopping them from growing. Cyclophosphamide may cause you to have diarrhea (loose stools), nausea (feeling sick to your stomach), vomiting (throwing up), short-term hair loss, short-term bladder problems, and, at times, bleeding from the bladder. A few patients may have bladder damage and bleeding for a longer time. You will be given large amounts of a sterile solution through your central line to protect your bladder. A bladder catheter (thin plastic tube) may be inserted into your bladder, if your physician thinks that it can help you. Cyclophosphamide slows the making of new red blood cells, white blood cells, and platelets. This causes a risk of infection and/or severe bleeding until the transplanted donor cells begin to work in you. You will get blood transfusions as needed. Cyclophosphamide also lowers your defense system. As a result, you may have more infections for several months after transplant. In a small number of patients, cyclophosphamide can damage the heart muscle causing heart failure. Sometimes cyclophosphamide causes abnormal heart function. If this occurs you may have shortness of breath and have fluids build-up in your body. This medication can also cause the lungs to become scarred. If scarring of the lungs occurs it will usually happen three to six months after you receive the medication. Scarring of the lungs can cause death. Cyclophosphamide can damage the male (testes) or female (ovaries) sex glands. In men, the number of sperm may be reduced but you would still be able to have intercourse. Women who are still menstruating may have irregular periods or may no longer have any periods. Whether you are a man or woman, this medication will likely greatly decrease your chances of being able to have a child. It is not known whether the use of cyclophosphamide will cause more side effects or problems with your health in the future.

Etoposide (VP-16): This medication disrupts the growth of cancer cells and destroys them. While taking etoposide you most likely will have diarrhea (loose stools), nausea (feeling sick to

your stomach), vomiting (throwing up), lower white blood cell count that increases your risk of infection, lower platelet count that increases your risk of bleeding, hair loss, stopping of menstrual periods in women, temporary reduced or no sperm production in men. Less likely side effects that you may experience are fatigue, sores in the mouth or on the lips, fever, rash, and loss of appetite. Rare side effects that you may experience are damage to your heart, which may result in a heart attack or heart failure. Your blood pressure may fall during the infusion of etoposide, and etoposide rarely causes numbness and tingling in the fingers and toes, in a condition termed, neuropathy. This may be reversible. Etoposide can rarely cause changes in your liver function.

Total Body Irradiation (TBI): TBI may cause you to have diarrhea (loose stools), nausea (feeling sick to your stomach), vomiting (throwing up), and painful swelling of the saliva gland for a few days. You may also experience short-term hair loss. TBI kills both sick and normal marrow, leading to a lack of red blood cells, white blood cells, and platelets. The short-term loss of these blood cells could cause you to become anemic, develop an infection, and/or bleeding. This will continue until the transplanted donor cells begin to work in you. You will get blood transfusions as needed. There is a risk that cataracts (cloudiness) may develop in your eyes. This may mean partial loss of vision, and you may need contact lenses or surgery to remove the cataracts. The TBI dose used will probably result in sterility (not being able to have children.) It is not known whether the use of TBI will cause more side effects or problems with your health in the future.

The conditioning regimens allowed by this protocol are likely to cause women to enter premature menopause. Men who receive these conditioning regimens are unlikely to be able to father children. However, there is no guarantee that this will happen to you, so you should discuss the need for birth control with your doctor. In any case, you are not protected from sexually transmitted diseases as a result of having these treatments.

Risks Related to the Infusion Peripheral Blood Stem Cells (PBSC)

The infusion of bone marrow or PBSC usually has few side effects. Rarely the infusion may cause a headache, chest pain or trouble breathing, a slight fever, or blood in the urine. Very rarely, it may cause an allergic reaction (which shows up as a severe drop in blood pressure and may cause loss of consciousness).

Risks Related to the Transplant Procedure

The following risks are not specifically related to any one medication or the transplanted donor cells, but they are risks that are a part of the transplant procedure.

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, irradiation therapy, or both. 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 medicines and irradiation 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 irradiation 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. 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. A form of kidney failure, called thrombotic microangiopathy may result in reversible or irreversible kidney damage, and may require temporary or permanent dialysis therapy.

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. Poor thyroid function can result in fatigue, weight gain, hair loss, depression, dry skin and dry hair; however, this is easily corrected by the medication. As a result of irradiation, 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, irradiation 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 you may have difficulty eliminating this fluid. Furosemide is a medication that is often given to help eliminate this excess fluid. This medication may cause hearing loss and loss of body chemicals such as potassium and sodium.

IRB #

Informed Consent to Participate in Research

Study Title: A Phase III Randomized, Multicenter Trial Comparing Sirolimus/Tacrolimus with Tacrolimus/Methotrexate as GVHD Prophylaxis After HLA-Matched, Related Peripheral Blood Stem Cell Transplantation

Principal Investigator Contact Information: (Insert contact information for PI at your site.)

Introduction
We invite you to take part in a research study sponsored by the National Institutes of Health (NIH) and the Blood and Marrow Transplant Clinical Trials Network (BMT CTN).

First, we want you to know that:

- Taking part in NIH research is entirely voluntary.
- You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled.
- You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with your family, friends, or your personal physician or other health professional.

Purpose
During the course of this study, we will attempt to learn about genetic factors that may have an influence on the risks of Graft-vs-Host Disease (GVHD) and long-term outcome after allogeneic stem cell transplantation. GVHD is an immune reaction that occurs after transplantation of stem cells from one individual (the donor) to another (the recipient). GVHD is a form of rejection of the recipient tissues (such as the skin, the intestinal tract and the liver) by the donor immune system. We are interested in studying the small variations or differences in genes, called polymorphisms or variants that could influence the immune system to cause GVHD. At this time, there are several candidate genes that may influence the incidence of GVHD. We would like to store your DNA (which will be isolated from your blood) to eventually study some of these genes. We invite you to participate in this study so that we can learn more about these genetic factors.

Procedures
Sixteen (16) milliliters of blood will be used for the genetic analysis. We ask that you submit a sample of no more than 16 mL, equivalent to three teaspoons. For donors less than 12 years old, a sample of no more than 8 mL, equivalent to 1½ teaspoons, will be requested. If you withdraw from the study, your samples will not be used for other research studies or tested further.

Clinical information (e.g., HLA typing) about you will be collected. The NIH will not have access to the names of the patients enrolled in this study. The clinical information will be coded and compared to the genetic analysis at a later date.

The samples collected for research purposes will be sent to laboratories that have contracts with the BMT CTN to conduct these research tests. They will be labeled with unique codes that do not contain information that could identify you. The link is stored at the Data Coordinating Center for the BMT CTN. The staff at the laboratories where your samples are being tested do not have a link to this code.

In the laboratory, we will isolate DNA from your blood. At a later date, we will test the DNA for the polymorphisms in your genes and in the genes in your stem cell recipient. The polymorphisms will be correlated with clinical outcomes after transplantation.

Your samples will be stored at these laboratories until the entire sample has been used for the research tests or until the end of the study. If any of your samples are leftover after the research studies are completed, these samples will either be destroyed or be sent to the National Heart Lung and Blood Institute (NHLBI) sample repository in Maryland. If your leftover samples are sent to the repository, they will be given an anonymous code. These leftover samples stored at the repository can never be linked to you. Any research performed on these leftover samples must first be approved by an advisory panel at the NHLBI.

If you agree to allow your blood to be kept for research, you are free to change your mind at any time. We ask that you tell [the Principal Investigator] at [address] in writing and let him/her know you are withdrawing your permission for your sample to be used for research. Any unused sample will be destroyed.

Alternatives
You may choose not to participate in this part of the study. The decision to participate in this study will not affect the care given to you by your physicians.

Risk and Discomfort
There is a small risk of an infection or fainting from the blood draw.

At no time will this information be made available to those not directly involved in the study without your written consent. Only the results of the proposed analysis will be collected, presented and published. At no time, will the name or an identifier of patients or stem cell donors be available to anyone except those conducting the study. The investigators who will conduct the genetic analysis will not have access to the names of the patients or donors enrolled in this study. The clinical information will be coded and compared to the genetic analyses to be performed at a later date.

Benefits
It is possible that the information from this study could be important for future transplant donors and recipients. However individual results will not be reported directly to you.

This study will increase our understanding of the factors that influence the risk for developing GVHD after stem cell transplantation. We hope that it will eventually contribute to improvements in prevention and treatment of GVHD. There may be no direct benefit to you or your stem cell recipient from this study. If there are any questions, we will attempt to answer them with the most recent information.

HIPAA[2] AUTHORIZATION TO USE AND DISCLOSE INDIVIDUAL HEALTH INFORMATION FOR RESEARCH PURPOSES

1. Purpose: As a research participant, I authorize the Principal Investigator and the researcher's staff to use and disclose my individual health information for the purpose of conducting the research study entitled *A Phase III Randomized, Multicenter Trial Comparing Sirolimus/Tacrolimus with Tacrolimus/Methotrexate as GVHD Prophylaxis After HLA-Matched, Related Peripheral Blood Stem Cell Transplantation.*

2. Individual Health Information to be Used or Disclosed: My individual health information that may be used or disclosed to conduct this research includes: Demographic information (e.g., age, date of birth, sex, weight), medical history, physical examination findings, and genetic test results.

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

3. Parties Who May Disclose My Individual Health Information: The researcher and the researcher's staff may obtain my individual health information from (*list hospitals, clinics or providers from which health care information can be requested):*

   _____

   _____

   _____

4. Parties Who May Receive or Use My Individual Health Information: The individual health information disclosed by parties listed in item 3 and information disclosed by me during the course of the research may be received and used by the following parties:

   - Principal Investigators and the researcher's staff, including Dr. Corey Cutler and Dr. Joseph Antin, Study Chairpersons and staff/laboratories at Dana Farber Cancer Institute
   - National Heart, Lung and Blood Institute (NHLBI) and National Cancer Institute (NCI), both of the National Institutes of Health (NIH), study sponsors
   - Blood and Marrow Transplant Clinical Trials Network (BMT CTN), data coordinating center
   - 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 responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments.

5. 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 research-related treatment that is provided through the study. However, my decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

6. 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, the 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.

7. 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.

8. DNA from your stored blood sample might be used in genome-wide association (GWA) or pharmacogenomics 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. Pharmacogenomics studies are similar genetic tests but look specifically at genes related to how the body breaks down medications.

   If your coded samples are used in such a study, the researcher is required to add your test results and sample information into a shared, 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.

9. 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.

10. This authorization does not have an expiration date.

IRB #

OTHER PERTINENT INFORMATION

1. **Confidentiality.** When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or other authorized people.

2. **Policy Regarding Research-Related Injuries.** The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. **Payments.** The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health.

4. **Problems or Questions.** If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator at (xxx) xxx-xxxx.

5. **Consent Document.** Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM BELOW, A or B

A. Adult Patient's Consent.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

_____
Signature of Adult Patient & Date Signed

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM _____ THROUGH _____.

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study. (Attach NIH 2514-2, Minor's Assent, if applicable)

_____
Signature of Parent(s)/Guardian & Date Signed

If other than parent, specify relationship: _____

Child's Verbal Assent (if applicable).

The information in the above consent form has been adequately described to my child in language that my child can understand, and my child willingly agrees to participate in the study.

_____
Signature of Parent(s)/Guardian & Date Signed

_____
Signature of Investigator & Date Signed

_____
Signature of Witness & Date Signed

APPENDIX C

LABORATORY SAMPLES

APPENDIX C

LABORATORY SAMPLES

1. HLA TYPING

Before Transplantation: HLA typing will be performed for all patients and donors in American Society of Histocompatibility and Immunogenetics (ASHI)-approved laboratories designated by the transplant centers. HLA typing must be performed by serologic (or higher resolution) methods for HLA-A and B, and molecular typing for DRB1 consistent with NMDP standard procedures.

2. PATHOLOGY/CYTOGENETICS STUDIES

A bone marrow biopsy/aspirate is required from the patient within four weeks prior to the initiation of conditioning therapy. A bone marrow aspirate and biopsy with cytogenetics is required at Day 100 and 12 months and 24 months post transplant.

Pathology and cytogenetic studies will be conducted per institutional guidelines.

3. FLOW CYTOMETRY/IMMUNE RECONSTITUTION ASSAY

Flow cytometry on peripheral blood specimens from the patient will be performed at the following timepoints: Day 28, Day 100, Day 180, Day 365 and Day 730.
Tubes will be collected and shipped to:

Esoterix Clinical Trials Services
Attn: Shiela Rines
750 Walnut Avenue
Cranford, NJ 07016

Assays will be performed to determine the number of CD2, CD3, CD4, CD8, CD19, CD25, CD45Ro/Ra and CD56 cells.

Immunophenotype assays of lymphoid subsets and dendritic cells: Samples of peripheral blood will be obtained from patients at 28, 100, 180, 365 and 730 days post-transplant. Sample processing and testing should be performed as quickly after sample receipt as possible. Laboratory will perform and report to EMMES a complete blood count (CBC) and manual cell differential & morphology assessment on the EDTA containing samples submitted at each time point. Laboratory will calculate the absolute numbers of subsets of immune cells in the blood based upon the absolute number of leukocytes in the blood and the percentage of leukocytes that are T, B, NK, monocyte, or dendritic cell subsets as defined by multiparameter flow cytometry.

The flow cytometric analysis will be performed using a panel of flow cytometry assays, each containing multiple fluorescently labeled monoclonal antibodies. The FACS panel that will be used is shown below. Please note that all necessary controls are not shown in this table.

Flow Cytometry for Immune Reconstitution

Tube #	Class of Cells	Measured Blood Cell Cell Types	Subsets	Antibodies
1	Lymphoid Subsets	T cells	CD4 CD8 CD25+	anti-CD4(PerCP) anti-CD8 (FITC) anti-CD25 (PE) anti-CD3(APC)
2	Lymphoid Subsets	NK and γδ T cells	CD56+ γδ T cells	anti-CD56 (PE) anti-γδ TCR (FITC) anti-CD16 (PerCP) anti-CD3 (APC)
3	Lymphoid Subsets	CD8 T cell Subsets	Naïve and activated CD8 T cells	anti-CD8 (APC) anti-Ki67(FITC) anti-CD62L (PE) anti-CD45RA (TC)
4	Lymphoid Subsets	CD4 T cell Subsets	Naïve and activated CD4 T cells	anti-CD4 (APC) anti-Ki67(FITC) anti-CD62L(PE) anti-CD45RA (TC)
5	Lymphoid Subsets	B-cells & monocytes	CD5+ CD27 B cells CD14+ monocytes	anti-CD5 (PerCP) anti-CD27 (FITC) anti-CD14(PE) anti-CD19 (APC)
6	Dendritic Cell Subsets	DC subsets	DC1 and DC2 subsets	anti-lineage (FITC) anti-CD123(PE) anti-HLADR (PerCP) anti-CD11c(APC)
7	Isotype Controls	Isotype Ig	Non-specific staining	irrelevant (FITC) (PE) (PerCP) (APC) conjugates to murine monoclonal Ab

Laboratory will need to determine percent viability, the concentration of nucleated cells and will calculate and report the absolute numbers of subsets of immune cells per microliter of blood based upon the absolute number of leukocytes in the blood and the percentage of leukocytes that are T, B, NK, monocyte, or dendritic cell subsets as defined by multiparameter flow cytometry (see table below).

CD3+ CD3+/CD4+ CD3+/CD8+ CD3+/CD4+/CD8+ CD3+/CD4+/CD25+ CD3+/CD8+/CD25+ CD3+/□□TCR+ CD3+/□□TCR- CD3+/CD56+ CD3-/CD16+/CD56+ CD3-/CD16-/CD56+ CD4+/CD45RA+/CD62L+ CD4+/CD45RA+/CD62L+/Ki67+ CD4+/CD45RA+/CD62L- CD4+/CD45RA+/CD62L-/Ki67+	CD8+/CD45RA+/CD62L+ CD8+/CD45RA+/CD62L+/Ki67+ CD8+/CD45RA+/CD62L- CD8+/CD45RA+/CD62L-/Ki67+ CD19+ CD19+/CD5+ CD19+/CD27+ CD14+ DC1 (lineage – HLADR+ CD123- CD11c+) DC2 (lineage – HLADR+ CD123+ CD11c-) % Viability Total Nucleated Cell Count

4. INVESTIGATIONAL FUTURE TESTING

Blood samples (16 mL, or 8 mL for patients < 12 years old) will be collected from the patient for investigational future testing at baseline, Day 28, 100, 180, 365 and 730. The center may make further adjustments in blood volumes collected from patients less than 12 years old to meet the institution's Human Investigational Committee's Guidelines.

5. DONOR GENETIC STUDIES

Background

The science of allogeneic stem cell transplantation continues to evolve. Until recently, only a few donor characteristics, such as donor age, sex and ABO blood type, were known to influence transplant outcome. However, recently, a number of donor molecular characteristics have been identified that potentially influence recipient outcome. These donor characteristics are predominantly polymorphisms in previously described genes.

The gene polymorphisms fall into several distinct categories. Polymorphisms in cytokine genes (such as IL-6, IL-10 and others) have been linked to differences in acute[1-13] and chronic[14-16] GVHD outcome, and also to differences in host response to infectious agents after transplantation.[17,18] A second group of polymorphisms include genes not directly involved in the immune responses or regulation the immune response, but which are presented in the context of MHC I or II and are termed minor histocompatibility antigens.[19-23] Yet a third group of distinct polymorphisms are in the family of the Natural Killer (NK) cell receptor and ligand group, collectively termed the Killer Immunoglobulin-like Receptor (KIR) family, which have been linked to differences to both GVHD and relapse rates after transplantation.[24-28] There is a fourth group of genes only loosely related to the immune system within which polymorphisms can influence rates of GVHD.[29-33] Finally, genes involved in drug metabolism have been linked to toxicity and GVHD after allogeneic transplantation.[34, 35]

Current donor search strategies focus primarily on HLA typing, and then use ancillary factors such as age, sex, parity and CMV serostatus to select an appropriate donor. Recently, several investigators have suggested that search strategies that include donor genotype of cytokines and other relevant genes, be incorporated into search strategies.[36-38]

The purpose of this study is to collect donor samples for DNA and serum banking, from which studies of these polymorphisms can be performed. Since the results of the polymorphism analysis will be correlated with clinical results after transplantation, the samples will be stored at the time of stem cell donation, and will be analyzed at a later date, when transplant outcomes can be measured.

Research Plan

Prior to administration of G-CSF, donors will provide 16 mL (or 8 mL for donors less than 12 years old) of peripheral blood. The center may make further adjustments in blood volumes collected from patients less than 12 years old to meet the institution's Human Investigational Committee's Guidelines. The samples will be processed, stored under appropriate conditions, and shipped quarterly to the NHLBI repository. No analysis will occur until all samples have been received and pertinent research questions have been developed.

6. REPOSITORY INFORMATION

Standard procedures for collection, storage, and shipping of specimens will be followed according to the BMT CTN guidelines. Samples will be given a unique alphanumeric code that contains no personal identifiers. Transplant Center Coordinators will hold the link to the code. Laboratory staff will not have access to the link. All laboratory studies will be performed at laboratories under contract with the NMDP on behalf of the BMT CTN.

Frozen samples should be batched and shipped quarterly to the Repository in compliance with the shipping procedures specified in the BMT CTN MOP and the BMT CTN 0402 Laboratory Sample Information Guide:

Christina Demasco
NHLBI Repository
SeraCare BioServices
217 Perry Parkway
Gaithersburg, Maryland 20877
Phone: (301) 208-8100
Fax: (301) 208-8829
Email: nhlbi@bbii.com

SCHEDULE OF LABORATORY EVALUATIONS

Type of Sample	Method	Type of Storage	Dates Samples Obtained	Shipping Specifications	Test Location
HLA Typing	According to institutional practice	According to institutional practice	Prior to conditioning regimen.	N/A	Transplant Center
Pathology/ Cytogenetic Studies	According to institutional practice	According to institutional practice	Within four weeks prior to the initiation of conditioning therapy and on Day 100, 365 and 730 post-transplant.	N/A	Transplant Center
Flow Cytometry/ Immune Reconstitution	8.5 mL peripheral blood 8.5 mL Yellow Top ACD-A Anticoagulation Vacutainer (003 IR-Blood V)	No additional processing – do not centifuge.	Day 28, 100, 180, 365 and 730 post-transplant.	Shipped overnight on cold ice packs (4°C)	Esoterix Clinical Trials Services
	3 mL peripheral blood 3 mL Lavendar Top EDTA Anticoagulation Vacutainer (004 IR-Blood V)	No additional processing – do not centifuge.	Day 28, 100, 180, 365 and 730 post-transplant.	Shipped overnight on cold ice packs (4°C)	Esoterix Clinical Trials Services

Type of Sample	Method	Type of Storage	Dates Samples Obtained	Shipping Specifications	Test Location
Investigational Future Testing	10 mL peripheral blood (10 mL red top tube) OR 5 mL peripheral blood (6 mL red top tube) for patients < 12 years old[1]	Place upright in rack for 30 minutes. Centrifuge at 900 x g or 2100 rpm for 10 minutes. Extract serum, transfer 500 ul aliquots to 10 cryovials[2]. Store at -70° C.	Baseline (prior to the initiation of conditioning therapy) and on Day 28, 100, 180, 365 and 730 post-transplant.	Frozen shipment quarterly to Repository in compliance with shipping procedures specified in the BMT CTN MOP	TBD
	6 mL peripheral blood (7 mL lavender top tube) OR 3 mL peripheral blood (4 mL lavender top tube) for patients < 12 years old[1]	Centrifuge at 900 x g or 2100 rpm for 10 minutes within 30 minutes of collection. Extract plasma, transfer 500 ul aliquots to 6 cryovials[2]. Store at -70° C.	Baseline (prior to the initiation of conditioning therapy) and on Day 28, 100, 180, 365 and 730 post-transplant.	Frozen shipment quarterly to Repository in compliance with shipping procedures specified in the BMT CTN MOP	TBD

Type of Sample	Method	Type of Storage	Dates Samples Obtained	Shipping Specifications	Test Location
Donor Genetic Studies	10 mL peripheral blood (10 mL lavendar top tube) OR 5 mL peripheral blood (6 mL lavendar top tube) for donors < 12 years old[1]	Place upright in rack for 30 minutes. Centrifuge at 900 x g or 2100 rpm for 10 minutes. Extract pasma, transfer 500 ul aliquots to 10 cryovials. Store at -70° C. Resuspend cellular component in RBC lysis buffer, divide and transfer into 6 cryovials. Respin at 900 x g or 2100 rpm for 10 minutes. Discard buffer and freeze pellet at -70° C immediately.	Prior to administration of G-CSF	Frozen shipment quarterly to Repository in compliance with shipping procedures specified in the BMT CTN MOP	TBD

Notes:
[1] The center may make further adjustments in blood volumes collected from patients less than 12 years old to meet the institution's Human Investigational Committee's Guidelines.
[2] Use half of the amount of cryovials for patients less than 12 years old.

Appendix C **Reference List**

(1) Cavet J, Middleton PG, Segall M et al. Recipient tumor necrosis factor-alpha and interleukin-10 gene polymorphisms associate with early mortality and acute graft-versus-host disease severity in HLA-matched sibling bone marrow transplants. Blood. 1999;94:3941-3946.

(2) Cavet J, Dickinson AM, Norden J et al. Interferon-gamma and interleukin-6 gene polymorphisms associate with graft-versus-host disease in HLA-matched sibling bone marrow transplantation. Blood. 2001;98:1594-1600.

(3) Cullup H, Dickinson AM, Jackson GH et al. Donor interleukin 1 receptor antagonist genotype associated with acute graft-versus-host disease in human leucocyte antigen-matched sibling allogeneic transplants. Br J Haematol. 2001;113:807-813.

(4) Hattori H, Matsuzaki A, Suminoe A et al. Polymorphisms of transforming growth factor-beta1 and transforming growth factor-beta1 type II receptor genes are associated with acute graft-versus-host disease in children with HLA-matched sibling bone marrow transplantation. Bone Marrow Transplant. 2002;30:665-671.

(5) Karabon L, Wysoczanska B, Bogunia-Kubik K, Suchnicki K, Lange A. IL-6 and IL-10 promoter gene polymorphisms of patients and donors of allogeneic sibling hematopoietic stem cell transplants associate with the risk of acute graft-versus-host disease. Hum Immunol. 2005;66:700-710.

(6) Lin MT, Storer B, Martin PJ et al. Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. N Engl J Med. 2003;349:2201-2210.

(7) Lin MT, Storer B, Martin PJ et al. Genetic variation in the IL-10 pathway modulates severity of acute graft-versus-host disease following hematopoietic cell transplantation: synergism between IL-10 genotype of patient and IL-10 receptor beta genotype of donor. Blood. 2005;106:3995-4001.

(8) MacMillan ML, Radloff GA, Kiffmeyer WR et al. High-producer interleukin-2 genotype increases risk for acute graft-versus-host disease after unrelated donor bone marrow transplantation. Transplantation. 2003;76:1758-1762.

(9) Middleton PG, Taylor PR, Jackson G, Proctor SJ, Dickinson AM. Cytokine gene polymorphisms associating with severe acute graft-versus-host disease in HLA-identical sibling transplants. Blood. 1998;92:3943-3948.

(10) Mlynarczewska A, Wysoczanska B, Karabon L, Bogunia-Kubik K, Lange A. Lack of IFN-gamma 2/2 homozygous genotype independently of recipient age and intensity of conditioning regimen influences the risk of aGVHD manifestation after HLA-matched sibling haematopoietic stem cell transplantation. Bone Marrow Transplant. 2004;34:339-344.

(11) Nordlander A, Uzunel M, Mattsson J, Remberger M. The TNFd4 allele is correlated to moderate-to-severe acute graft-versus-host disease after allogeneic stem cell transplantation. Br J Haematol. 2002;119:1133-1136.

(12) Stark GL, Dickinson AM, Jackson GH et al. Tumour necrosis factor receptor type II 196M/R genotype correlates with circulating soluble receptor levels in normal subjects and with graft-versus-host disease after sibling allogeneic bone marrow transplantation. Transplantation. 2003;76:1742-1749.

(13) Takahashi H, Furukawa T, Hashimoto S et al. Contribution of TNF-alpha and IL-10 gene polymorphisms to graft-versus-host disease following allo-hematopoietic stem cell transplantation. Bone Marrow Transplant. 2000;26:1317-1323.

(14) Bogunia-Kubik K, Mlynarczewska A, Wysoczanska B, Lange A. Recipient interferon-gamma 3/3 genotype contributes to the development of chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Haematologica. 2005;90:425-426.

(15) Cullup H, Dickinson AM, Cavet J, Jackson GH, Middleton PG. Polymorphisms of interleukin-1alpha constitute independent risk factors for chronic graft-versus-host disease after allogeneic bone marrow transplantation. Br J Haematol. 2003;122:778-787.

(16) Kim DH, Lee NY, Sohn SK et al. IL-10 promoter gene polymorphism associated with the occurrence of chronic GVHD and its clinical course during systemic immunosuppressive treatment for chronic GVHD after allogeneic peripheral blood stem cell transplantation. Transplantation. 2005;79:1615-1622.

(17) Bogunia-Kubik K, Mlynarczewska A, Jaskula E, Lange A. The presence of IFNG 3/3 genotype in the recipient associates with increased risk for Epstein-Barr virus reactivation after allogeneic haematopoietic stem cell transplantation. Br J Haematol. 2006;132:326-332.

(18) Seo KW, Kim DH, Sohn SK et al. Protective role of interleukin-10 promoter gene polymorphism in the pathogenesis of invasive pulmonary aspergillosis after allogeneic stem cell transplantation. Bone Marrow Transplant. 2005;36:1089-1095.

(19) Akatsuka Y, Warren EH, Gooley TA et al. Disparity for a newly identified minor histocompatibility antigen, HA-8, correlates with acute graft-versus-host disease after haematopoietic stem cell transplantation from an HLA-identical sibling. Br J Haematol. 2003;123:671-675.

(20) Goulmy E. Human minor histocompatibility antigens. Curr Opin Immunol. 1996;8:75-81.

(21) Kernan NA, Dupont B. Minor histocompatibility antigens and marrow transplantation. N Engl J Med. 1996;334:323-324.

(22) Kotzampasaki EM, Spyropoulou-Vlachou MS, Kalofoutis C et al. Minor histocompatibility antigen HA-1 and HPA-5 polymorphisms in HLA-identical related bone marrow transplantation. Transplant Proc. 2004;36:1735-1738.

(23) Miklos DB, Kim HT, Miller KH et al. Antibody responses to H-Y minor histocompatibility antigens correlate with chronic graft-versus-host disease and disease remission. Blood. 2005;105:2973-2978.

(24) Chen C, Huang SL. Killer cell inhibitory receptors involved in graft-versus-host disease and graft-versus-leukemia effect in allogeneic hematopoietic stem cell transplantation - review. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2004;12:236-243.

(25) Gagne K, Brizard G, Gueglio B et al. Relevance of KIR gene polymorphisms in bone marrow transplantation outcome. Hum Immunol. 2002;63:271-280.

(26) Beelen DW, Ottinger HD, Ferencik S et al. Genotypic inhibitory killer immunoglobulin-like receptor ligand incompatibility enhances the long-term antileukemic effect of unmodified allogeneic hematopoietic stem cell transplantation in patients with myeloid leukemias. Blood. 2005;105:2594-2600.

(27) Igarashi T, Wynberg J, Srinivasan R et al. Enhanced cytotoxicity of allogeneic NK cells with killer immunoglobulin-like receptor ligand incompatibility against melanoma and renal cell carcinoma cells. Blood. 2004;104:170-177.

(28) Schaffer M, Malmberg KJ, Ringden O, Ljunggren HG, Remberger M. Increased infection-related mortality in KIR-ligand-mismatched unrelated allogeneic hematopoietic stem-cell transplantation. Transplantation. 2004;78:1081-1085.

(29) Bogunia-Kubik K, Lange A. HSP70-hom gene polymorphism in allogeneic hematopoietic stem-cell transplant recipients correlates with the development of acute graft-versus-host disease. Transplantation. 2005;79:815-820.

(30) Holler E, Rogler G, Herfarth H et al. Both donor and recipient NOD2/CARD15 mutations associate with transplant-related mortality and GvHD following allogeneic stem cell transplantation. Blood. 2004;104:889-894.

(31) Middleton PG, Cullup H, Dickinson AM et al. Vitamin D receptor gene polymorphism associates with graft-versus-host disease and survival in HLA-matched sibling allogeneic bone marrow transplantation. Bone Marrow Transplant. 2002;30:223-228.

(32) Middleton PG, Norden J, Cullup H et al. Oestrogen receptor alpha gene polymorphism associates with occurrence of graft-versus-host disease and reduced survival in HLA-matched sib-allo BMT. Bone Marrow Transplant. 2003;32:41-47.

(33) Rozman P, Karas M, Kosir A et al. Are human platelet alloantigens (HPA) minor transplantation antigens in clinical bone marrow transplantation? Bone Marrow Transplant. 2003;31:497-506.

(34) Kalayoglu-Besisik S, Caliskan Y, Sargin D, Gurses N, Ozbek U. Methylenetetrahydrofolate reductase C677T polymorphism and toxicity in allogeneic hematopoietic cell transplantation. Transplantation. 2003;76:1775-1777.

(35) Robien K, Schubert MM, Bruemmer B et al. Predictors of oral mucositis in patients receiving hematopoietic cell transplants for chronic myelogenous leukemia. J Clin Oncol. 2004;22:1268-1275.

(36) Dickinson AM, Middleton PG, Rocha V, Gluckman E, Holler E. Genetic polymorphisms predicting the outcome of bone marrow transplants. Br J Haematol. 2004;127:479-490.

(37) Kallianpur AR. Genomic screening and complications of hematopoietic stem cell transplantation: has the time come? Bone Marrow Transplant. 2005;35:1-16.

(38) Socie G, Loiseau P, Tamouza R et al. Both genetic and clinical factors predict the development of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Transplantation. 2001;72:699-706.

APPENDIX D

RECOMMENDED LEUCOVORIN RESCUE SCHEMA

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RECOMMENDED LEUCOVORIN RESCUE SCHEMA

12 hours after d+1 methotrexate	Leucovorin 15 mg/m^2 iv q6h x 3 doses Maximum single dose 30 mg
12 hours after d+3 methotrexate	Leucovorin 10 mg/m^2 iv q6h x 6 doses Maximum single dose 25 mg
24 hours after d+6, 11 methotrexate	Leucovorin 10 mg/m^2 iv q6h x 8 doses Maximum single dose 25 mg

Methotrexate levels are not reliable during leucovorin therapy.

APPENDIX E

CONSENSUS GVHD GRADING

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CONSENSUS GVHD GRADING

Organ Staging of GVHD

Stage	Skin	Liver	Gut
0	No rash due to GVHD	Bilirubin < 2 mg/dL	< 500 mL (5 mL/kg for patients under 12 years) diarrhea per day
1	Maculopapular rash < 25% of body surface [a]	Bilirubin 2-3 mg/dL[b]	500 to 999 mL diarrhea per day [c] (5.1-10 mL/kg for patients under 12 years), or persistent nausea with histologic evidence of GVHD in stomach or duodenum
2	Maculopapular rash 25-50% of body surface [a]	Bilirubin 3.1-6 mg/dL[b]	1,000 to 1,499 mL diarrhea per day (10.1-15.0 mL/ kg for patients under 12 years)
3	Maculopapular rash > 50% of body surface [a]	Bilirubin 6.1-15 mg/dL [b]	1,500 or more mL diarrhea per day [c] (more than 15.0 mL/kg for patients under 12 years of age)
4	Generalized erythroderma with bullous formation	Bilirubin > 15 mg/dL[b]	Severe abdominal pain with or without ileus

[a] Use "Rule of Nines" to determine extent of rash.
[b] Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented.
[c] Downgrade one stage if an additional cause of diarrhea has been documented.

Overall Clinical Grading of Severity of Acute GVHD

Grade	Degree of Organ Involvement
0	No Stage 1-4 of any organ
I	Stage 1-2 rash and no liver or gut involvement
II	Stage 3 rash, or Stage 1 liver involvement, or Stage 1 gut involvement
III	None to Stage 3 skin rash with Stage 2-3 liver involvement, or Stage 2-4 gut involvement
IV	Stage 4 skin rash, or Stage 4 liver involvement

Adapted from Thomas *et al.*, NEJM, 1975, pp. 895-90

APPENDIX F

MODIFIED OMAS

MUCOSITIS SCORING SYSTEM

SAMPLE CASE REPORT FORM

APPENDIX F

MODIFIED OMAS MUCOSITIS SCORING SYSTEM SAMPLE CASE REPORT FORM

Site: Protocol:
Subject ID: Date: Assessor's initials:

Site	Ulceration	Erythema	
Maxillary labial mucosa	Y N	Y N	NE*
Mandibular labial mucosa	Y N	Y N	NE
Right cheek	Y N	Y N	NE
Left cheek	Y N	Y N	NE
Right lateral & ventral tongue	Y N	Y N	NE
Left lateral & ventral tongue	Y N	Y N	NE
Floor of mouth	Y N	Y N	NE
Soft palate	Y N	Y N	NE

*not evaluable

Ability to eat: solids liquids NPO Mouth Pain Y N

WHO score:

0 Normal mucosa
1 Erythema & mouth pain
2 Ulceration & ability to eat solids
3 Ulceration & liquids only
4 Ulceration & NPO

APPENDIX G

HUMAN SUBJECTS

APPENDIX G

HUMAN SUBJECTS

Subject consent: Candidates for the study will be identified as described in Chapter 4 of the protocol. The Principal Investigator or his/her designee at each transplant center will contact the candidates and enroll them onto the study. The study coordinator at each center will provide the patient with information about the purpose of the study and obtain consent. The Network will provide a template of the consent form to each center. Each center will customize the template according to their local requirements and submit it for review by the local Internal Review Board (IRB). The DCC will verify the adequacy of the consent forms. Each center must provide evidence of IRB approval.

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

Participation of women, children, minorities and other populations: Women, children and ethnic minorities will be included in this study.

Accrual will be monitored within each center with the expectation that the enrolled patient population is representative of the transplanted patient population at each center. Representation will be examined by comparing gender, race, ethnicity and age distributions. Accrual of minority patients will be expected to be in proportion to the number of minority patients transplanted at each center. The DCC and NHLBI will discuss enrollment anomalies with the centers.

APPENDIX H

GUIDELINES FOR SIMULTANEOUS USE OF SIROLIMUS AND ORAL VORICONAZOLE

APPENDIX H

GUIDELINES FOR SIMULTANEOUS USE OF SIROLIMUS AND ORAL VORICONAZOLE

Background

Voriconazole is currently considered the best initial treatment of invasive aspergillus[1] and other invasive fungal infections such as those caused by *Fusarium* sp. and *Scedosporium apiospermum*[2]. However, the use of voriconazole in combination with sirolimus was contraindicated at the time of voriconazole approval due to a substantial increase in sirolimus drug exposure in healthy subjects because voriconazole inhibits several CYP isoenzymes[3]. This inhibition results in a sharp increase in sirolimus bioavailability; voriconazole increased sirolimus peak plasma concentration (C_{max}) and area under the plasma-concentration-time curve (AUC) by 6.6 fold and 11.1 fold, respectively [3, 4]. Recently the group at Dana-Farber Cancer Institute has demonstrated that co-administration of voriconazole and sirolimus is safe if a 90% reduction in sirolimus dosing is undertaken[5]. Moreover, Pfizer Inc. has now undertaken a series of pharmacokinetic studies in normal volunteers that confirms that a 90% dose reduction of sirolimus results in reliable levels of sirolimus in the target range[6]. The increase in bioavailability is due to transient inhibition of CYP enzymes in the intestinal mucosa. Inhibition of liver CYP enzymes will prolong the half-life but not increase the bioavailability of the drug. The effects of intravenous voriconazole are less pronounced and dose modifications are not required when the intravenous formulation is used. The effect of voriconazole on CYP is transient; therefore, if sirolimus and voriconazole are staggered there is a progressively less potent effect on sirolimus level. If the drugs are staggered by as much as 5 hours, there is no effect on bioavailability. If voriconazole and sirolimus are separated by 1, 2, 3, or 4 hours, the effect on bioavailability is less predictable.

Suggested Dose Adjustments

A 90% dose reduction of sirolimus is an effective strategy for the co-administration of sirolimus and oral voriconazole. Most adults receive sirolimus tablets, which have a minimum strength of 1 mg and which cannot be crushed or broken. However, there is a liquid formulation of sirolimus at a concentration of 1 mg/mL. An appropriate fractional dose can be drawn into a 1 mL syringe and swallowed directly. The liquid has an unpleasant taste that can be masked by mixing with juice or milk. Alternatively, empty gelatin capsules can be dispensed along with the liquid sirolimus. Because the drug is not in aqueous solution, the liquid can be placed in the capsule by the patient, family member or health care provider without causing dissolution of the gelatin. The capsule can then be taken orally. Blood levels of sirolimus should be measured after the dosage change. Changes in levels due to altered bioavailability should be apparent within 24-48 hours. There will also be a prolongation of sirolimus half-life that may require dose adjustments in the weeks following the initiation of voriconazole therapy. If voriconazole is stopped or changed to intravenous formulation, the dose of sirolimus should be increased by 90% and blood levels should be monitored.

Example: Patient is on 2 mg sirolimus daily. Voriconazole is taken at the same time as the sirolimus and the new sirolimus dose is 0.2 mg dispensed as 0.2 mL taken directly or placed in gelatin capsules.

Endnotes for Appendix H

[1] Herbrecht R, Denning DW, Patterson TF, Bennett JE, Greene RE, Oestmann JW, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med 2002; 347:408-415.

[2] Center for Drug Evaluation and Research, FDA. NDA 21-266 and 21-267, VfendTM (Voriconazole). 24 May 2002 [Accessed 21 August 2005]; Available from: http://www.fda.gov/cder/foi/appletter/2002/21266ltr.pdf; Perfect JR, Marr KA, Walsh TJ, Greenberg RN, DuPont B, de la Torre-Cisneros J, et al. Voriconazole treatment for less-common, emerging, or refractory fungal infections. Clin Infect Dis 2003; 36(9):1122-1131.

[3] Product Information: Vfend(R), voriconazole. Pfizer Inc, New York, NY, 2005.

[4] Abel S, Allan R, Fielding A. Effect of voriconazole on the pharmacokinetics of sirolimus (poster). In: 12th European Congress of Clinical Microbiology and Infectious Diseases; April 24-27, 2002; Milan, Italy; April 24-27, 2002.12.

[5] Marty FM, Lowry CM, Cutler CS, Campbell BJ, Fiumara K, Baden LR, Antin JH. Voriconazole and sirolimus co-administration following allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant, 2006, in press.

[6] Bharat Damle, Clinical Pharmacology Pfizer Global Clinical Research and Development, personal communication.

APPENDIX I

REFERENCES

APPENDIX I

REFERENCES

[1] Champlin RE, Schmitz N, Horowitz MM et al. Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. Blood. 2000;95:3702-3709.

[2] Weisdorf D, Haake R, Blazar B et al. Treatment of moderate/severe acute graft-versus-host disease after allogeneic bone marrow transplantation: an analysis of clinical risk features and outcome. Blood. 1990;75:1024-1030.

[3] Martin PJ, Schoch G, Fisher L et al. A retrospective analysis of therapy for acute graft-versus-host disease: secondary treatment. Blood. 1991;77:1821-1828.

[4] Simpson D. New developments in the prophylaxis and treatment of graft versus host disease. Expert Opin Pharmacother. 2001;2:1109-1117.

[5] Ho VT, Soiffer RJ. The history and future of T-cell depletion as graft-versus-host disease prophylaxis for allogeneic hematopoietic stem cell transplantation. Blood. 2001;98:3192-3204.

[6] Storb R, Deeg HJ, Whitehead J et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. N Engl J Med. 1986;314:729-735.

[7] Przepiorka D, Devine S, Fay J, Uberti J, Wingard J. Practical considerations in the use of tacrolimus for allogeneic marrow transplantation. Bone Marrow Transplant. 1999;24:1053-1056.

[8] Markus PM, Cai X, Ming W et al. Prevention of graft-versus-host disease following allogeneic bone marrow transplantation in rats using FK506. Transplantation. 1991;52:590-594.

[9] Fay JW, Wingard JR, Antin JH et al. FK506 (Tacrolimus) monotherapy for prevention of graft-versus-host disease after histocompatible sibling allogeneic bone marrow transplantation. Blood. 1996;87:3514-3519.

[10] Mrsic M, Labar B, Bogdanic V et al. Combination of cyclosporin and methotrexate for prophylaxis of acute graft-versus-host disease after allogeneic bone marrow transplantation for leukemia. Bone Marrow Transplant. 1990;6:137-141.

[11] Storb R, Deeg HJ, Farewell V et al. Marrow transplantation for severe aplastic anemia: methotrexate alone compared with a combination of methotrexate and cyclosporine for prevention of acute graft-versus-host disease. Blood. 1986;68:119-125.

[12] Tollemar J, Ringden O, Sundberg B et al. Decreased incidence of graft-versus-host disease in bone marrow transplantation recipients treated with a combination of cyclosporine and methotrexate. Transplant Proc. 1988;20:494-498.

[13] Cutler C, Giri S, Jeyapalan S et al. Acute and chronic graft-versus-host disease after allogeneic peripheral- blood stem-cell and bone marrow transplantation: a meta-analysis. J Clin Oncol. 2001;19:3685-3691.

[14] Reynolds C, Ratanatharathorn V, Adams P et al. Comparative analysis of tacrolimus/methotrexate versus tacrolimus in allogeneic peripheral blood stem cell transplants: Engraftment, GVHD, relapse, and survival outcomes. Blood. 1998;92:449a.

[15] Chao NJ, Snyder DS, Jain M et al. Equivalence of 2 effective graft-versus-host disease prophylaxis regimens: results of a prospective double-blind randomized trial. Biol Blood Marrow Transplant. 2000;6:254-261.

[16] Ross M, Schmidt GM, Niland JC et al. Cyclosporine, methotrexate, and prednisone compared with cyclosporine and prednisone for prevention of acute graft-vs.-host disease: effect on chronic graft-vs.-host disease and long-term survival. Biol Blood Marrow Transplant. 1999;5:285-291.

[17] Ratanatharathorn V, Nash RA, Przepiorka D et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. Blood. 1998;92:2303-2314.

[18] Nash RA, Antin JH, Karanes C et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. Blood. 2000;96:2062-2068.

[19] Horowitz MM, Przepiorka D, Bartels P et al. Tacrolimus vs. cyclosporine immunosuppression: results in advanced- stage disease compared with historical controls treated exclusively with cyclosporine. Biol Blood Marrow Transplant. 1999;5:180-186.

[20] Hiraoka A, Ohashi Y, Okamoto S et al. Phase III study comparing tacrolimus (FK506) with cyclosporine for graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation. Bone Marrow Transplant. 2001;28:181-185.

[21] Davies SM, Kollman C, Anasetti C et al. Engraftment and survival after unrelated-donor bone marrow transplantation: a report from the national marrow donor program. Blood. 2000;96:4096-4102.

[22] Ho VT, Weller E, Lee SJ et al. Prognostic factors for early severe pulmonary complications after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2001;7:223-229.

[23] Devine SM, Geller RB, Lin LB et al. The outcome of unrelated donor bone marrow transplantation in patients with hematologic malignancies using tacrolimus (FK506) and low dose methotrexate for graft-versus-host disease prophylaxis. Biol Blood Marrow Transplant. 1997;3:25-33.

[24] Cutler C, Antin JH. Omission of day+11 methotrexate after matched-related allogeneic peripheral blood stem cell transplantation does not increase the risk of graft-vs.-host disease. Biol Blood Marrow Transplant. 2002;8:85 [Abstract].

[25] Kirken RA, Wang YL. Molecular actions of sirolimus: sirolimus and mTor. Transplant Proc. 2003;35:S227-S230.

[26] Hackstein H, Taner T, Logar AJ, Thomson AW. Rapamycin inhibits macropinocytosis and mannose receptor-mediated endocytosis by bone marrow-derived dendritic cells. Blood. 2002;100:1084-1087.

[27] Monti P, Mercalli A, Leone BE et al. Rapamycin impairs antigen uptake of human dendritic cells. Transplantation. 2003;75:137-145.

[28] Hackstein H, Taner T, Zahorchak AF et al. Rapamycin inhibits IL-4--induced dendritic cell maturation in vitro and dendritic cell mobilization and function in vivo. Blood. 2003;101:4457-4463.

[29] Chiang PH, Wang L, Liang Y et al. Inhibition of IL-12 signaling Stat4/IFN-gamma pathway by rapamycin is associated with impaired dendritic [correction of dendritc] cell function. Transplant Proc. 2002;34:1394-1395.

[30] Woltman AM, de Fijter JW, Kamerling SW et al. Rapamycin induces apoptosis in monocyte-and CD34-derived dendritic cells but not in monocytes and macrophages. Blood. 2001;98:174-180.

[31] Woltman AM, van der Kooij SW, Coffer PJ et al. Rapamycin specifically interferes with GM-CSF signaling in human dendritic cells, leading to apoptosis via increased p27KIP1 expression. Blood. 2003;101:1439-1445.

[32] MacDonald AS. A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. Transplantation. 2001;71:271-280.

[33] van Hooff JP, Squifflet JP, Wlodarczyk Z, Vanrenterghem Y, Paczek L. A prospective randomized multicenter study of tacrolimus in combination with sirolimus in renal-transplant recipients. Transplantation. 2003;75:1934-1939.

[34] Flechner SM, Goldfarb D, Modlin C et al. Kidney transplantation without calcineurin inhibitor drugs: a prospective, randomized trial of sirolimus versus cyclosporine. Transplantation. 2002;74:1070-1076.

[35] Fairbanks KD, Eustace JA, Fine D, Thuluvath PJ. Renal function improves in liver transplant recipients when switched from a calcineurin inhibitor to sirolimus. Liver Transpl. 2003;9:1079-1085.

[36] Nair S, Eason J, Loss G. Sirolimus monotherapy in nephrotoxicity due to calcineurin inhibitors in liver transplant recipients. Liver Transpl. 2003;9:126-129.

[37] Cotterell AH, Fisher RA, King AL et al. Calcineurin inhibitor-induced chronic nephrotoxicity in liver transplant patients is reversible using rapamycin as the primary immunosuppressive agent. Clin Transplant. 2002;16 Suppl 7:49-51.

[38] McAlister VC, Gao Z, Peltekian K et al. Sirolimus-tacrolimus combination immunosuppression. Lancet. 2000;355:376-377.

[39] Koenen H, Michielsen E, Verstappen J, Fasse E, Joosten I. Superior T-cell suppression by rapamycin and FK506 over rapamycin and cyclosporine A because of abrogated cytotoxic T-lymphocyte induction, impaired memory responses, and persistent apoptosis. Transplantation. 2003;75:1581-1590.

[40] Ciancio G, Burke GW, Gaynor JJ et al. A randomized long-term trial of tacrolimus and sirolimus versus tacrolimus and mycophenolate mofetil versus cyclosporine (NEORAL) and sirolimus in renal transplantation. I. Drug interactions and rejection at one year. Transplantation. 2004;77:244-251.

[41] Ciancio G, Burke GW, Gaynor JJ et al. A randomized long-term trial of tacrolimus/sirolimus versus tacrolimus/mycophenolate mofetil versus cyclosporine (NEORAL)/sirolimus in renal transplantation. II. Survival, function, and protocol compliance at 1 year. Transplantation. 2004;77:252-258.

[42] Benito AI, Furlong T, Martin PJ et al. Sirolimus (rapamycin) for the treatment of steroid-refractory acute graft-versus-host disease. Transplantation. 2001;72:1924-1929.

[43] Johnston L, Shizuru J, Stockerl-Goldstein K et al. Rapamycin for treatment of chronic graft versus host disease. Biol Blood Marrow Transplant. 2002;8:87[abstract].

[44] Couriel D, Hicks K, Saliba R et al. Sirolimus (rapamycin) for treatment of steroid-refractory chronic graft versus host disease. Biol Blood Marrow Transplant. 2003;9:67[abstract].

[45] Antin JH, Kim HT, Cutler C et al. Sirolimus, tacrolimus, and low-dose methotrexate for graft-versus-host disease prophylaxis in mismatched related donor or unrelated donor transplantation. Blood. 2003;102:1601-1605.

[46] Cutler C, Kim HT, Hochberg EP et al. Sirolimus and Tacrolimus Without Methotrexate as Graft-vs.-Host Disease Prophylaxis after Matched, Related Donor Peripheral Blood Stem Cell Transplantation. Biol Blood Marrow Transplant. 2004; 10:328-336.