



**A Phase II/III Randomized, Multicenter Trial Comparing
Sirolimus plus Prednisone and Sirolimus/Calcineurin
Inhibitor plus Prednisone for the Treatment of
Chronic Graft-versus-Host Disease**

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**BMT CTN PROTOCOL 0801
Version 6.0**

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PROTOCOL SYNOPSIS – BMT CTN PROTOCOL 0801

A Phase II/III Randomized, Multicenter Trial Comparing Sirolimus plus Prednisone and Sirolimus/Calcineurin Inhibitor plus Prednisone for the Treatment of Chronic Graft-versus-Host Disease

Principal Investigators: Paul Carpenter, MB, BS
Mukta Arora, MD

Study Design: Combined Phase II/III, randomized, open label, multicenter, prospective comparative study of sirolimus plus prednisone versus sirolimus/calcineurin-inhibitor plus prednisone for the treatment of chronic GVHD.

Phase II Component:

A Phase II randomized trial of sirolimus + prednisone (Arm 1) versus sirolimus + calcineurin inhibitor + prednisone (Arm 2).

The intent is to enroll subjects at the start of initial therapy for chronic GVHD, or before their chronic GVHD is refractory to glucocorticoid therapy, or is chronically dependent upon glucocorticoid therapy and multiple secondary systemic immunosuppressive agents. Patients will be stratified by transplant center and will be randomized to an experimental arm of sirolimus + prednisone or the comparator arm of sirolimus + calcineurin inhibitor + prednisone in a 1:1 ratio.

Phase III Component:

The Phase II study above will proceed into the Phase III component of the protocol if the experimental arm is more efficacious than the comparator arm.

Thus, subjects that were enrolled on the Phase II will continue to be followed for the Phase III endpoints. Phase III accrual will then be completed by enrolling additional patients.

Primary Objective:

Phase II Component:

To estimate the proportion of subjects with complete or partial responses after 6 months of therapy in both study arms using an intention to treat analysis.

Phase III Component:

To compare the proportion of subjects with complete resolution of all reversible manifestations at 24 months after starting therapy in both study arms.

Secondary Objectives: **Phase II component estimates among patients receiving SRL versus SRL +CNI:** the percent reduction in the average daily dose of prednisone (or equivalent) by 6 and 12 months; the cumulative incidence of treatment failure at 1 year; the prevalence of active symptomatic chronic GVHD at 1 and 2 years; the cumulative incidence of discontinuation of all systemic immunosuppressive therapy at 1 and 2 years; the overall and cancer progression-free survival at 1 and 2 years; the candidate serum biomarkers of chronic GVHD at baseline, 2 months and 6 months; and, to evaluate NIH and other new response instruments in chronic GVHD.

Phase III component comparisons among patients receiving SRL versus SRL +CNI: percent reduction in the average daily dose of prednisone (or equivalent) at 6, 12, and 24 months; cumulative incidence of treatment failure at 1 and 2 years; prevalence of active symptomatic chronic GVHD at 1 and 2 years; cumulative incidence of discontinuation of all systemic immunosuppressive therapy at 1 and 2 years; overall and cancer progression-free survival at 1 and 2 years; candidate serum biomarkers of chronic GVHD at baseline, 2 months, and 6 months; and, to evaluate NIH and other new response instruments in chronic GVHD.

3-Year Assessments: All patients except those who are enrolled during the last 12 months of accrual will be evaluated at 3 years after beginning study therapy for the endpoints above under the Phase III component. Patients enrolled in the last 12 months will not complete 3 year assessments and will be excluded from the analysis.

Eligibility:

Inclusions:

Suitable candidates are patients with classic chronic or overlap syndrome (classic chronic plus acute) GVHD that meets NIH Consensus Working Group Guidelines in one of the following categories:¹

- a) Previously untreated (newly diagnosed) as defined by having received < 14 days of prednisone (or equivalent) before enrollment/randomization to study therapy.
- b) Previously treated but inadequately responding after \leq 16 weeks of initial therapy with prednisone and/or CNI \pm additional non-sirolimus agent (started at the time of chronic GVHD diagnosis).

Exclusions:

- a) Patients with late persistent acute GVHD or recurrent acute GVHD only.

- b) Inability to begin prednisone therapy at a dose of \geq 0.5 mg/kg/day (or equivalent).
- c) Receiving sirolimus for treatment of chronic GVHD (sirolimus for prophylaxis or treatment of acute GVHD is acceptable).
- d) Already receiving sirolimus (for prophylaxis or treatment of acute GVHD) with prednisone at \geq 0.25 mg/kg/day (or equivalent) \pm additional agents.
- e) Receiving therapy for chronic GVHD for more than 16 weeks.
- f) Invasive fungal or viral infection not responding to appropriate antifungal or antiviral therapies.
- g) Creatinine clearance $<$ 50 mL/min/1.73 m² or a serum creatinine based on the Cockcroft-Gault formula (adults) or Schwartz formula (age \leq 12 years).
- h) Inability to tolerate oral medications.
- i) Absolute neutrophil count $<$ 1500 per microliter.
- j) Requirement for platelet transfusions.
- k) Receiving any treatment for persistent, progressive or recurrent malignancy.
- l) Progressive or recurrent malignancy defined other than by quantitative molecular assays.
- m) Known hypersensitivity to sirolimus.

Treatment Description:

Arm 1: Sirolimus + Prednisone

Arm 2: Sirolimus + Calcineurin Inhibitor + Prednisone

Prednisone is administered initially as a single early morning dose of 1 mg/kg/day [or equivalent (Adults: maximum dose 100 mg, for age $<$ 17 years, the use of adjusted body weight as per institutional guidelines should be considered to calculate doses for patients who weigh $>$ 110% of ideal body weight)].

If prednisone at a dose of 1 mg/kg/day (or equivalent) is contraindicated (e.g. poorly controlled diabetes, hypertension, osteoporosis, avascular bone necrosis, major mood disturbance) patients may begin prednisone between 0.5 -1 mg/kg/day.

Prednisone therapy continues at the initial dose until there is objective evidence of improvement in manifestations of chronic GVHD.

The initial taper of prednisone from the starting dose of 0.5-1 mg/kg/**every day** (or equivalent) is attempted within 2 weeks after the

first evidence of improvement in GVHD and takes place over 4-8 weeks to achieve a dose of 0.5-1 mg/kg/**every-other-day**.

Once an alternating-day prednisone (or equivalent) regimen is achieved, the dose of prednisone should be held constant for 10-12 weeks until all reversible manifestations of chronic GVHD resolve, after which a second taper is attempted. The tempo of this second taper may follow individual institutional guidelines but it is recommended that the extent of the taper be approximately calibrated to the magnitude of an individual patient's alternating-day prednisone dose. For example, a patient whose prednisone dose has been stable at 0.5 mg/kg/every-other-day may attempt to taper prednisone completely. However, a patient whose prednisone dose has been stable at 1.0 mg/kg/every-other-day is recommended first to taper over 4-8 weeks to 0.5 mg/kg/every-other-day, followed by 2-3 months of further observation before attempting a complete taper.

Calcineurin inhibitor therapy continues at a dose which achieves the following trough serum levels (HPLC/TMS):

- a) Tacrolimus: 5-10 ng/mL
- b) Cyclosporine: 120-200 ng/mL

Sirolimus therapy begins at 2 mg orally per day (1 mg/m^2 per day if $< 40 \text{ kg}$) to target a trough serum level of 3-12 ng/mL.

Supportive care will follow institutional guidelines that reflect reasonable standard practices appropriate to the patient with chronic GVHD as outlined by the Ancillary Therapy and Supportive Care Working Group Report of the NIH Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD.²

Accrual Objective:

Phase II Component: One hundred subjects will be randomized 1:1 across the Phase II study (50 per arm).

Phase III Component: If the Phase II is successful, then 100 patients from the Phase II study continue on the Phase III. Two hundred additional subjects will be randomized 1:1 across the Phase III (100 per arm) for a combined Phase II/III total of 300 subjects.

Accrual Period:

The estimated accrual period is 3 years for the Phase II, and 4 years for the Phase III.

Study Duration:

The estimated study duration is 3.5 years for the Phase II, and 6 years for the Phase III.

STUDY DESIGN SCHEMATIC

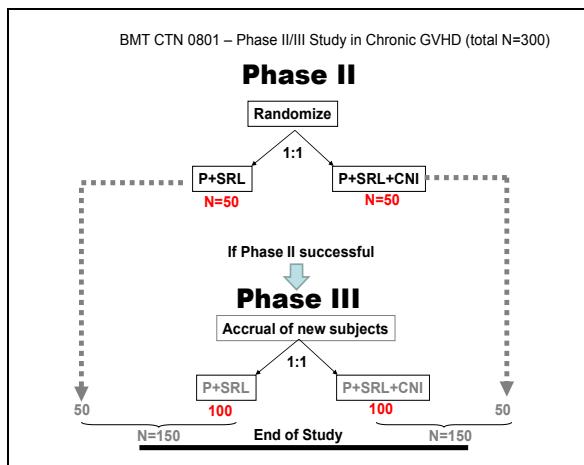


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CHAPTER 1

1. BACKGROUND AND RATIONALE

1.1. Chronic Graft-versus-Host Disease (cGVHD)

Fifteen percent to 80% of patients after allogeneic hematopoietic cell transplantation (HCT) develop chronic GVHD depending on stem cell source and donor-host factors.^{3, 4, 5, 6, 7, 8, 9} Therefore, it is not surprising that cGVHD is the major complication among patients who survive beyond 100 days. Standard immunosuppressive therapy (IST) for cGVHD using glucocorticoids with or without a calcineurin inhibitor has not changed for three decades despite the observation that most patients respond inadequately. If the total time to discontinue all systemic IST is measured as a surrogate for graft tolerance, it takes a median of 2-3 years from the onset of cGVHD therapy; varying according to stem cell source and other factors.¹⁰ Unfortunately, despite the association of cGVHD with reduced relapse of malignancy, the protracted duration of cGVHD makes it the leading cause of impaired immunity, compromised functional status, and late treatment-related deaths.^{11, 12, 13, 14, 15, 16} The protean and sometimes irreversible organ manifestations of cGVHD create a burden of symptoms that may negatively impact quality of life even after tolerance is achieved. New approaches to standard IST for cGVHD are needed to achieve early control of cGVHD manifestations and facilitate tolerance.

A hypothesis driven choice of novel IST study agents has been problematic because cGVHD pathophysiology has been poorly understood. As a result, contemporary early therapy trials have been extremely limited. Most therapy trials have been restricted to single arm Phase II studies in steroid-refractory cGVHD and preliminary data exists for at least five commercially available therapies that have shown some activity.^{17, 18, 19, 20, 21} However, a renewed interest and understanding of the potential role of regulatory T cells (T_{regs}) in facilitating graft tolerance has generated enthusiasm for testing tolerance induction strategies that do not impede the expansion of T_{regs}. Traditional calcineurin-inhibitor (CNI) based therapy impedes T_{regs}²² and a subset analysis from one study suggested that survival was worse when progressive onset cGVHD was treated with cyclosporine plus prednisone compared to prednisone alone.²³ In contrast, a more recent therapy, sirolimus does not impede T_{regs}, making it a logical candidate to consider further.^{22, 24, 25, 26, 27} Therefore, this prospective randomized combined Phase II/III study will test two arms:

Arm 1:	Sirolimus + Prednisone
Comparator Arm (Arm 2):	Sirolimus + Calcineurin Inhibitor + Prednisone

1.2. Balancing Experimental Design Purity and Study Accrual

Eligibility criteria for this study (Section 2.3.1 and 2.3.2) were designed to avoid major heterogeneities in the resistance of individuals to IST yet should be compatible with the need to optimize timely accrual for early phase testing. Experimental therapy has generally been reserved for failed primary therapy despite the fact that time to discontinue IST for cGVHD usually exceeds several years. This trial encourages physicians to enroll patients with newly

diagnosed cGVHD but also allows the common practice of short term “testing” standard therapy and by subsequently allowing the inclusion of patients not responding to less than or equal to 16 weeks of initial therapy with prednisone \pm a CNI \pm an additional non-sirolimus agent.

1.3. Sirolimus (rapamycin) in GVHD

Sirolimus (rapamycin) is a naturally occurring macrolide that has immunosuppressive, antifungal, and antitumor properties. Although structurally similar to tacrolimus, sirolimus binds uniquely to FK binding protein 12 (FKBP12) and then complexes with mammalian target of rapamycin (mTOR) rather than with calcineurin. The FKBP12-mTOR complex affects upstream and downstream signaling that ultimately leads to T and B cell suppression.^{28, 29} Downstream there is reduced DNA transcription, translation, protein synthesis and cell cycling. Upstream interactions with mTOR include the PTEN/pi3 kinase/Akt and Janus kinase pathways, which importantly include the mediation of IL-2 driven signaling from the T cell receptor.³⁰ This contrasts with the calcineurin inhibitors, which more directly inhibit cytokine production, particularly IL-2. Sirolimus may also act by inhibiting antigen uptake, cellular maturation, intracellular signaling and induction of apoptosis in dendritic cells.^{31, 32, 33, 34, 35, 36}

Phase II data indicates activity of sirolimus in over one hundred patients with steroid-refractory cGVHD and overall response rates of 63% to 94%.^{17, 37, 38} [P. Carpenter unpublished] Sirolimus has also been reported to improve the efficacy of acute GVHD prophylaxis regimens although this did not extend to a reduced incidence of cGVHD.^{39, 40} In all these studies sirolimus was combined with a CNI and the side effect profile of combination therapy is now well delineated and generally manageable with close attention to therapeutic drug monitoring to avoid renal dysfunction that includes: thrombotic microangiopathy, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. Nephrotoxicity is rare when sirolimus is used without CNIs.

Of central interest to this study is the recent understanding that sirolimus might facilitate tolerance when used in CNI-free regimens via preferential expansion of T_{regs}.^{22, 41}

1.4. *FOXP3*⁺CD4⁺CD25⁺ T_{regs} in Chronic GVHD

Although IL-2 is important for the development and expansion of conventional effector T cells (T_{conv}), IL-2 is also critical for establishing and maintaining immune tolerance.^{42, 43, 44} The principal mechanism whereby IL-2 promotes immune tolerance appears to be via its roles in the preferential generation and maintenance of *FOXP3*CD4⁺CD25⁺ T_{regs} compared to T_{conv}. In one study, the frequency of CD4⁺CD25⁺ T_{regs} and median *FOXP3* values in patients with cGVHD was about half that of the non-cGVHD group but the suppressive function of these cells remains normal.⁴⁵ This reduction was thought to be physiologically relevant given the low naturally occurring frequency of T_{regs} in the blood of healthy individuals yet the critical role in controlling a wide range of immune responses. This is perhaps best illustrated by the clinical severity of the IPEX syndrome which is caused by specific mutations of *FOXP3* that result in a functional deficiency of T_{regs}.⁴⁶ Interestingly, sirolimus therapy was able to abrogate severe manifestations of the IPEX syndrome in 3 children.⁴⁷

1.4.1. Inhibition of T_{regs} by Calcineurin-dependent IL-2 Production

Several recent data suggest that it is time to reevaluate the role of calcineurin-inhibitors (CNIs) for the treatment of GVHD and induction of tolerance. Murine T_{regs} are able to suppress experimental acute GVHD and murine T_{regs} share functional characteristics with their human counterparts. Because IL-2 signaling is critical for the survival of FOXP3⁺ T_{regs} in vivo it is important to understand the implications of commonly used IST on T_{reg} function in vivo. Zeiser *et al* compared cyclosporine and sirolimus as IST in an MHC class I and II mismatch T cell depleted bone marrow transplant model with T cell add-back in a 1:2 ratio of T_{regs} /T_{conv}.²² Cyclosporine significantly reduced T_{reg} function in vivo as assessed by increased proliferation of T_{conv}, GVHD severity, and reduced survival. The reduced suppressor function of cyclosporine-exposed T_{regs} was IL-2 dependent and correlated with a reduced number of FOXP3⁺ T cells in vitro and in vivo, suggesting the critical importance of calcineurin-dependent IL-2 production. In contrast sirolimus did not inhibit the expansion of donor derived FOXP3 T_{regs}.²² This data is in keeping with other in vitro studies showing that sirolimus selectively expands murine T_{regs} as well as functional T_{regs} in both healthy and diabetic human subjects.^{48, 49} Both thymic generation and peripheral preservation of FOXP3 T_{regs} appear to be negatively regulated by cyclosporine and facilitated by sirolimus.²⁶ Interestingly, the frequency of CD4⁺CD25⁺ does not track with the total CD4⁺ cells suggesting that T_{regs} are an independently regulated subset among CD4⁺ cells.⁴⁵ The differential impact of sirolimus on T_{regs} compared to T_{conv} appears to result from reduced usage of the mTOR pathway in T_{regs} compared with T_{conv} and explains the synergistic effect of sirolimus and T_{regs} in GVHD protection. The mechanism is complex but appears to involve increased usage of the STAT5 pathway over the PI3K pathway in response to IL-2 and, together with sirolimus-induced mTOR inhibition, the differentiation of CD4 T cells is skewed toward a FOXP3-expressing phenotype.^{25, 45} In addition, activation of T_{conv} induces rapid down-regulation of PTEN which does not occur in T_{regs}.²⁵ T cell compartment targeted PTEN knock-out mice have T_{regs} that are rendered susceptible to mTOR inhibition.

1.5. Calcineurin-Inhibitor Free Immunosuppression

Data from a randomized primary therapy study in cGVHD²³ and recent empirical data from four centers suggest that it is not unsafe to discontinue a CNI in the treatment of cGVHD. The cGVHD study comparing cyclosporine plus prednisone to prednisone required 79 of the 145 patients who were randomly assigned to prednisone to discontinue cyclosporine shortly after beginning the study. The cumulative incidence of secondary therapy was not increased in the prednisone only arm arguing that removal of cyclosporine is safe. A subset analysis suggested that survival was worse when progressive onset cGVHD was treated with cyclosporine plus prednisone compared to prednisone alone which is of interest from the context that CNI-based therapy impedes T_{regs}.²² Previous randomized trials had also suggested that the incidence of cGVHD was not affected by early discontinuation of cyclosporine in patients who did not have acute GVHD on Day 60 after transplantation⁵⁰ or by prolongation of cyclosporine in patients who did not have cGVHD on Day 80 after transplantation.⁵¹ A combined total of approximately 30 patients from Stanford, MD Anderson, NIH/NCI, and FHCRC have received a cGVHD therapy change that involved stopping a CNI and adding sirolimus or, in a few cases, mycophenolate mofetil. CNIs were mostly discontinued abruptly or after a brief taper. Major flares of acute inflammatory GVHD manifestations were not observed.

1.6. Endpoints for Clinical Trials and Response

The cGVHD field has been hampered by difficulties in being able to objectively measure complete and partial responses to therapeutic interventions. However, short term endpoints like CR+PR are necessary because long term, albeit more objective endpoints like survival, or discontinuation of all systemic IST, are impractical for early phase testing. To begin to address this obstacle, the NIH Consensus Development Project for Clinical Trials in cGVHD has published study design concepts aimed at improving the quality of response data to allow objective evaluation of new cGVHD therapies.^{52, 53} Central to the design of response criteria is that measurements need only capture the most important cGVHD manifestations that reflect clinically relevant (functional) benefit for an individual patient. Measurements should avoid detecting trivial responses that arise from crossing thresholds defined by categorical scoring. Ideally, instruments should measure a meaningful change calibrated as a percentage of baseline abnormality or function, and based upon statistical considerations or clinical perception. Clinically meaningful changes might include the measurement of self-reported observations of the way a patient feels or functions before and after treatment. Organ manifestations that might not be reversible (dry eye, fasciitis, bronchiolitis obliterans with fibrosis) need to be handled differently from manifestations that are expected to fully reverse. Alternatively, potentially irreversible manifestations should be included only for progression. The Working Group recognized that central review of clinical grading and response may improve reproducibility in multi-center trials.

A detailed performance analysis of the newly designed GVHD response instruments will be possible from the extensive data that will be generated by comprehensive measurements taken at baseline and after study interventions. The goal will be to develop a set of more objective, clinically relevant response criteria that could be used in future studies.

CHAPTER 2

2. STUDY DESIGN

2.1. Study Overview

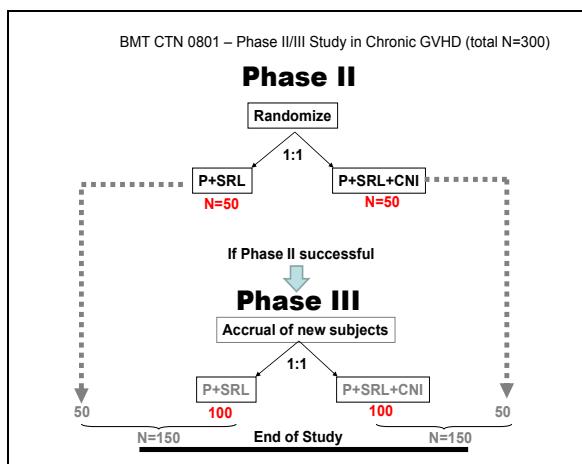
Combined Phase II/III, randomized, open label, multicenter, prospective comparative study of sirolimus plus prednisone versus sirolimus/calcineurin-inhibitor plus prednisone for the treatment of chronic GVHD.

Phase II Component:

Phase II will be a randomized trial of Sirolimus (SRL) + Prednisone (PDN) vs SRL + calcineurin inhibitor (CNI) + PDN.

The intent is to enroll subjects at the start of initial therapy for chronic GVHD, or before their chronic GVHD is refractory to glucocorticoid therapy, or is chronically dependent upon glucocorticoid therapy and multiple secondary systemic immunosuppressive agents. Patients will be stratified by transplant center and will be randomized to an experimental arm of SRL + PDN [Arm 1] or the comparator arm of SRL + CNI + PDN [Arm 2] in a 1:1 ratio.

Each study arm will include prednisone (or equivalent) and sirolimus. The experimental arm will avoid a calcineurin inhibitor.



Phase III Component:

If the result of Phase II confirms the hypothesis that the CNI free Arm (SRL +PRN) appears to be superior to the CNI containing Arm (SRL +CNI +PDN) then the trial will proceed into the Phase III component. Thus, subjects that were enrolled on the Phase II will continue to be followed for the Phase III endpoints. Phase III accrual will then be completed by enrolling additional patients.

2.2. Hypothesis and Study Objectives

The primary purpose of the Phase II component is to compare a treatment regimen that contains sirolimus without a calcineurin inhibitor, to a comparator regimen of sirolimus with a calcineurin inhibitor. The goal is to determine if sirolimus plus prednisone is a sufficiently promising treatment regimen for further comparison in the Phase III trial.

The primary hypothesis is that avoidance of a calcineurin inhibitor in the treatment of chronic GVHD might facilitate the development of tolerance and improve the GVHD response rates at 6 months, and ultimately the 24 month complete response rates compared to immunosuppressive therapy regimens that contain a calcineurin inhibitor (the comparator arm, SRL + CNI + PDN).

2.2.1. Primary Objective

Phase II Component: The primary objective is to estimate in patients with recently diagnosed chronic GVHD the rates of complete plus partial responses at 6 months after randomization in both study arms using an intention to treat analysis.

Phase III Component: To compare the proportion of subjects with complete resolution of all reversible manifestations at 24 months after starting therapy in both study arms.

2.2.2. Secondary Objectives

Phase II Component:

1. To estimate the percent reduction in the average daily prednisone (or equivalent) dose by 6 and 12 months, among patients receiving SRL versus SRL + CNI.
2. To estimate the cumulative incidence of treatment failure at 1 year, among patients receiving SRL versus SRL + CNI.
3. To estimate the prevalence of active symptomatic chronic GVHD at 1 and 2 years, among patients receiving SRL versus SRL + CNI.
4. To estimate the cumulative incidence of discontinuation of all systemic immunosuppressive therapy at 1 and 2 years, among patients receiving SRL versus SRL + CNI.
5. To estimate overall and cancer progression-free survival at 1 and 2 years, among patients receiving SRL versus SRL + CNI.
6. To evaluate candidate serum biomarkers of chronic GVHD at baseline, 2 months, and 6 months, among patients receiving SRL versus SRL + CNI.
7. To evaluate NIH and other new response instruments in chronic GVHD.

Phase III Component:

1. To compare the percent reduction in the average daily dose of prednisone (or equivalent) at 6, 12, and 24 months, among patients receiving SRL versus SRL+CNI.

2. To compare the cumulative incidence of treatment failure at 1 and 2 years, among patients receiving SRL versus SRL+CNI.
3. To compare the prevalence of active symptomatic chronic GVHD at 1 and 2 years, among patients receiving SRL versus SRL+CNI.
4. To compare the cumulative incidence of discontinuation of all systemic immunosuppressive therapy at 1 and 2 years, among patients receiving SRL versus SRL+CNI.
5. To compare overall and cancer progression-free survival at 1 and 2 years, among patients receiving the SRL versus SRL+CNI.
6. To compare candidate serum biomarkers of chronic GVHD at baseline, 2 months, and 6 months, among patients receiving SRL versus SRL+CNI.
7. To evaluate NIH and other new response instruments in chronic GVHD.

3-Year Assessments:

All patients except those who are enrolled during the last 12 months of accrual will be evaluated at 3 years after beginning study therapy for the endpoints 1 through 5 listed above under the Phase III component (also see Section 3.2). Patients enrolled in the last 12 months will not complete 3 year assessments and will be excluded from the analysis.

2.3. Patient Eligibility for Randomization

2.3.1. Patient Inclusion Criteria

Patients may be included in this trial if they meet all of the following criteria:

1. Suitable candidates are patients with classic chronic GVHD or overlap syndrome (classic chronic plus acute GVHD) that meets NIH Consensus Working Group Guidelines in one of the following categories:
 - a) Previously untreated (newly diagnosed) as defined by having received < 14 days of prednisone (or equivalent) before enrollment/randomization to study therapy.
 - b) Previously treated but inadequately responding after \leq 16 weeks of initial therapy with prednisone and/or CNI \pm additional non-sirolimus agent (started at the time of chronic GVHD diagnosis).
2. Patient or guardian willing and able to provide informed consent.
3. Stated willingness to use contraception in women of childbearing potential.
4. Stated willingness of patient to comply with study procedures and reporting requirements.

2.3.2. Patient Exclusion Criteria

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

1. Patients with late persistent acute GVHD or recurrent acute GVHD only.
2. Inability to begin prednisone therapy at a dose of ≥ 0.5 mg/kg/day (or equivalent).
3. Receiving sirolimus for treatment of chronic GVHD (sirolimus for prophylaxis or treatment of acute GVHD is acceptable).
4. Already receiving sirolimus (for prophylaxis or treatment of acute GVHD) with prednisone at ≥ 0.25 mg/kg/day (or equivalent) \pm additional agents.
5. Receiving therapy for chronic GVHD for more than 16 weeks.
6. Invasive fungal or viral infection not responding to appropriate antifungal or antiviral therapies.
7. Creatinine clearance < 50 mL/min/1.73 m² based on the Cockcroft-Gault formula (adults) or Schwartz formula (age ≤ 12 years):

$$\text{Adults: } eC_{Cr} (\text{mL/min}) = (140 - \text{age}) \times \text{mass} (\text{kg}) \times (0.85 \text{ if female}) / 72 \times \text{serum creatinine} (\text{mg/dL})$$

$$\text{Creatinine clearance (mL/min/1.73m}^2\text{)} = eC_{Cr} \times 1.73 / \text{BSA (m}^2\text{)}$$

$$\text{Children: } eC_{Cr} (\text{mL/min/1.73 m}^2) = k \times \text{height (cm)} / \text{serum creatinine (mg/dL)}$$

$$k = 0.33 \text{ (pre-term), } 0.45 \text{ (full term to 1 year old), } 0.55 \text{ (age 1-12 years)}$$

8. Inability to tolerate oral medications.
9. Absolute neutrophil count < 1500 per microliter.
10. Requirement for platelet transfusions.
11. Pregnancy (positive serum β -HCG) or breastfeeding.
12. Receiving any treatment for persistent, progressive or recurrent malignancy.
13. Progressive or recurrent malignancy defined other than by quantitative molecular assays.
14. Known hypersensitivity to sirolimus.

2.4. Stem Cell Transplantation

Patients may have received any type of transplant conditioning, any type of stem cell source and donors may be HLA-matched or mismatched.

2.5. Initiating, Monitoring and Adjusting Study Therapy

2.5.1. Sirolimus

2.5.1.1. Initiating sirolimus therapy (Arms 1 and 2)

Formulation	Route	Adult	Child < 17 yr
Rapamune®	Oral	2 mg daily	1 mg/m ² daily

Initial doses are calculated using actual body weight except for those patients who are greater than 100% of ideal body weight in which case calculation of dose using adjusted body weight is recommended.

There is generally no loading dose for patients with classic chronic GVHD. For patients with chronic GVHD and acute overlap syndrome, physicians may elect to administer a loading dose of 6 mg (3 mg/m² for children < 17 yr) followed by a daily maintenance dose of 2 mg.

2.5.1.2. Targeting sirolimus levels, dose adjustment and other considerations

The target serum level for **sirolimus** is **3-12 ng/mL**

Oral solution and tablets are available for incremental dosing. Dose adjustments are based upon clinical judgment of the managing physician after considering clinical toxicity, serum levels, GVHD, concomitant drug use and the rate of rise or decline of the serum level (see **Table 2.5b** and **“Sirolimus & CNI Therapy Physician Guide”**). Sirolimus blood levels will be measured by either HPLC or immunoassay.

Other Important Considerations:

- To minimize the variability of exposure to sirolimus, it should be taken at a consistent time of day and consistently with or without food.
- ***Sirolimus should not be taken within 4 hours after administration of Neoral® (or Gengraf®) cyclosporine oral solution and/or cyclosporine gelatin capsules*** because premarketing studies in healthy volunteers demonstrated 116%-512% elevations of mean Cmax and 148%-230% elevations in AUC when sirolimus oral solution or tablets were simultaneously administered with Neoral but not when the doses were separated by 4 hours.
- ***Grapefruit juice*** reduces CYP3A4-mediated metabolism of sirolimus and must not be administered with sirolimus or used for dilution.
- ***Extreme caution when subject is on voriconazole therapy (See Section 2.7.1).***
- ***Emesis:*** The sirolimus dose may be repeated within 15 minutes of a vomited dose.
- ***Impaired renal function*** does not mandate dosage adjustment.

- **Impaired hepatic function** should prompt consideration for sirolimus maintenance doses to be reduced but no dose adjustment of the loading dose is necessary. Maintenance doses of sirolimus may be reduced by approximately one third in patients with hepatic impairment (Child-Pugh Score of $\geq 7/15$ based on the sum of 1, 2 and 3 points respectively for each of: serum bilirubin in mg/dL ($< 2, 2-3, > 3$), serum albumin in mg/dL ($> 3.5, 2.8-3.5, < 2.8$), INR ($< 1.7, 1.71-2.2, >2.2$), hepatic encephalopathy (none, grade I-II, grade III-IV), or ascites (none, slight, moderate/refractory)).
- **Interchangeability of oral solution and tablets:** Two-milligram Rapamune Oral Solution is clinically equivalent to 2-milligram Rapamune oral tablets; hence, are interchangeable on a milligram to milligram basis. However, it is not known if higher doses of Rapamune Oral Solution are clinically equivalent to higher doses of tablets on a milligram to milligram basis (see Section 2.7.1).

2.5.1.3. Sirolimus adverse reactions

Most side-effects of sirolimus occur with prolonged use, especially when used in combination with CNIs like tacrolimus and cyclosporine. Sirolimus alone is not associated with neurotoxicity or nephrotoxicity because of its inability to inhibit calcineurin. Adverse reactions that resulted in rates of sirolimus discontinuation $> 5\%$ were increased creatinine, hypertriglyceridemia and thrombotic thrombocytopenic purpura.

TABLE 2.5a. SIROLIMUS TOXICITIES

	Common >20%	Occasional 5-20%	Rare <5%
Immediate Within 1-2 days of receiving drug	Headache (L), hypertension (L), nausea, diarrhea, immuno-suppression (L), fever, constipation	Chest pain, insomnia, dyspepsia, vomiting, dyspnea	Hypotension, asthma, increased cough, flu like syndrome, tachycardia, anorexia, hypersensitivity reactions (exfoliative dermatitis, angioedema)
Prompt Within 2-3 weeks, prior to the next course	Tremor (L), renal dysfunction, elevated creatinine/BUN, anemia, pain (abdominal, back, pain), hyperlipidemia , hypercholesterolemia, hypertriglyceridemia, hyperglycemia, peripheral edema , weight gain, arthralgia	Elevated LFTs (with elevated sirolimus levels), stomatitis, urinary tract infections, URIs, mild thrombocytopenia , leukopenia , hyper/hypokalemia (L), hypophosphatemia, rash, hives, pruritis, delayed wound healing or dehiscence (L) , hypomagnesaemia (L), proteinuria	Opportunistic infections, pleural and pericardial effusions, non-infectious pneumonitis or bronchiolitis-obliterans organizing pneumonia and pulmonary fibrosis, thrombosis, myalgias, increased risk of CNI-induced HUS/TTP/TMA (L)
Delayed Any time later during therapy, excluding the above conditions	Acne		Chronic renal dysfunction, renal tubular necrosis, CHF, ascites, arthrosis, bone necrosis, osteoporosis

	Common >20%	Occasional 5-20%	Rare <5%
Late Any time after completion of treatment			Lymphoproliferative disorders, skin malignancies
Unknown Frequency and Timing	Sirolimus was embryo/fetotoxic in rats at dosages of approximately 0.2 to 0.5; clinical doses were adjusted for body surface area. It is not known whether sirolimus is excreted in human milk.		

(L) Toxicity may also occur later.

2.5.1.4. Management of sirolimus 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>).

- **Hyperlipidemia:** In the Phase III studies, treatment of new-onset hypercholesterolemia with lipid-lowering agents was required in 42-52% of patients enrolled in the sirolimus arms. Concomitant administration of sirolimus and HMG-CoA reductase inhibitors (statins) and/or fibrates appeared to be well tolerated. However, statins or fibrates should only be administered with caution to patients being treated with sirolimus and a CNI, at doses which are reduced according to label recommendations. Treated patients should be monitored for the development of rhabdomyolysis. Sirolimus should be continued during therapy for hypercholesterolemia and hypertriglyceridemia unless the lipid levels are uncontrollable with standard therapy and are deemed to be at risk to the subject, per the treating physician.
- **Thrombotic Microangiopathy (TMA):** Studies in adult transplant patients have shown an increase in TMA from 4.2% when patients were treated with tacrolimus or cyclosporine alone compared to 10.8% in patients treated with the tacrolimus/sirolimus combination.⁵⁴ TMA may occur both in the setting of sirolimus and/or CNI levels being above the therapeutic range or when levels are within desired ranges. Although complete renal recovery occurred in 92% of these patients, the potential seriousness of this complication requires careful monitoring and early therapy.

To meet the definition for TMA a patient must have all of the following:

1. Increased percentage (> 4%) of schistocytes in the blood
2. De novo, prolonged, or progressive thrombocytopenia (platelet count < 50x10⁹/L or 50% or greater reduction from previous counts)
3. Sudden and persistent increase in LDH
4. Decrease in hemoglobin concentration or increased red blood cell transfusion requirement
5. Decrease in serum haptoglobin

The following criteria are supportive of this diagnosis:

1. Sudden and persistent increase in BUN and Creatinine
2. Neurological symptoms

TMA should be managed preventively and conservatively. Prevention involves monitoring and keeping CNI and sirolimus levels within the target ranges. When CNI and sirolimus levels are high, doses need to be held for hours to days until levels in the therapeutic range are measured. High levels should prompt closer scrutiny of the patient's laboratory studies for a drop in hematocrit, presence of schistocytes, rise in serum LDH, BUN or creatinine, or drop in haptoglobin and in platelet count. Any changes in these measures should warrant close scrutiny of the patient with tight control of levels of tacrolimus and sirolimus. **Immediate reporting of TMA to the Protocol Principal Investigators is required.**

If TMA develops then the CNI should be discontinued and one of the Protocol Principal Investigators should be contacted to discuss the case. If a systemic agent is substituted for the discontinued CNI, then treatment failure should be designated. *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.

- **Hematological Toxicity:** It is recommended that the ANC, hematocrit and platelet counts will be monitored at the frequencies described in Table 2.5b, Section 2.5.1.3. A thorough investigation should be made to assess for possible causes of the cytopenia (recurrent disease, infection, drug effect other than sirolimus, TMA, autoimmune hemolytic anemia/thrombocytopenia, GVHD, late engraftment of platelets in cord blood transplant, etc.). If other causes have been ruled out and cytopenias are significant (ANC<500, platelets <20K) sirolimus doses may be decreased by 50%. If the cytopenia does not resolve after two weeks, sirolimus may be held. If counts improve, restart sirolimus at 50% of dose and titer to full dose as tolerated.
- **Other Toxicities:** Patients with toxicities such as lower extremity edema, arthralgias, and aphthous ulcers, should have a thorough assessment to rule out other causes of these symptoms (medications, heart failure, GVHD, etc.). If the toxicity is thought to be caused by sirolimus and is significant, sirolimus dosing may be decreased by 50%. If the symptoms do not stabilize, improve, or resolve after two weeks then sirolimus may be held. In some cases, **interstitial lung disease** has resolved upon discontinuation or dose reduction of sirolimus. The risk may be increased as the sirolimus concentration increases.

2.5.1.5. Sirolimus drug interactions

Sirolimus is a substrate for cytochrome CYP 3A4 and a P-glycoprotein (P-gp).

Drugs that may increase sirolimus blood concentrations include CYP 3A4, 5, or P-gp inhibitors:

- Calcineurin inhibitors: Simultaneous administration of cyclosporine soft gelatin capsules (Neoral®) results substantial increases in the sirolimus C_{max} and AUC. **This is avoided if administration of cyclosporine and sirolimus is taken 4 hours after administration of cyclosporine.**
- Calcium Channel Blockers: diltiazem, nicardipine, nifedipine, verapamil and amlodipine. Sirolimus should be monitored and a dose adjustment may be necessary.
- Triazole antifungal agents: fluconazole, itraconazole, clotrimazole, posaconazole, voriconazole, ketoconazole. The magnitude of increases sirolimus C_{max}, t_{max}, and AUC is such that **sirolimus should be administered cautiously together with fluconazole, itraconazole, or posaconazole, and with extreme caution if administered together with voriconazole.** If co-administration is unavoidable, then the dose of sirolimus should be greatly reduced at the time of initiation of the antifungal medication as recommended in Table 2.7a. and that there should be very frequent monitoring of trough concentrations of sirolimus in whole blood. Sirolimus concentrations should be measured upon initiation, during co-administration, and at discontinuation of antifungal treatment, with sirolimus doses adjusted accordingly.
- Macrolide antibiotics: clarithromycin, erythromycin, telithromycin, troleandomycin (but NOT azithromycin).
- Gastrointestinal prokinetics: cisapride, metoclopramide.

Drugs that may decrease sirolimus blood concentrations include CYP 3A4, 5, 7 or P-gp inducers:

- Anticonvulsants: carbamazepine, Phenobarbital, phenytoin.
- Antibiotics: Rifampin, rifampentine.
- Herbs: St. John's Wort (*Hypericum perforatum*).

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

2.5.2. Calcineurin Inhibitor (Tacrolimus or Cyclosporine)

2.5.2.1. Stopping calcineurin inhibitor therapy (Arm 1)

It is suggested that patients who are randomized to a CNI-free regimen and are already taking a CNI discontinue their CNI therapy by rapid taper over 1-2 weeks. The initial dose reduction should be at least 50% of the current dose.

2.5.2.2. Initiating calcineurin inhibitor therapy (Arm 2)

Formulation	Route	Adult	Child < 17 yr
Tacrolimus (Prograf®)	oral	0.01-0.02 mg/kg twice daily	0.05-0.08 mg/kg twice daily
Cyclosporine (Neoral® or Gengraf®)	oral	1-2 mg/kg twice daily	3.75 mg/kg twice daily

Initial doses are calculated using actual body weight except for those patients who are greater than 100% of ideal body weight in which case calculation of dose using adjusted body weight is recommended.

2.5.2.3. Targeting CNI levels, dose adjustment and other considerations

The target serum level for **tacrolimus** is **5-10 ng/mL**

The target serum level for **cyclosporine** is **120-200 ng/mL**

Dose adjustments are based upon clinical judgment of the managing physician after considering clinical toxicity, serum levels, GVHD, concomitant drug use and the rate of rise or decline of the serum level. A reasonable approach that incorporates these principles into a management algorithm is suggested in **Table 2.5b. and “Sirolimus & CNI Therapy Physician Guide”** which attempts to target steady state blood trough levels within relatively narrow therapeutic ranges with the goal of preventing combined toxicities of sirolimus and calcineurin inhibitors.

The suggested frequency for monitoring sirolimus and CNI blood levels varies from monthly to twice weekly based upon the guidance provided in **Table 2.5b.** Monitoring frequency takes into consideration changes in renal function and changes in concomitant medications which are known to affect levels by altering the metabolism of either sirolimus or CNIs. It is important to recognize that drug-related toxicities occur with greater frequency when sirolimus and CNIs are given concomitantly (also see Sections 2.5.1.3-2.5.1.5).

Whole blood levels of CNIs will be measured by high performance liquid chromatography (HPLC) or HPLC with tandem mass spectrometric detection (preferred methods) or equivalent method with correlation coefficient ≥ 0.90 .

TABLE 2.5b. RECOMMENDED MONITORING AND DOSE MODIFICATION OF THERAPY WITH SIROLIMUS, CYCLOSPORINE OR TACROLIMUS

Parameter	Target Range	Result	Action	Test Frequency on Therapy < 1 month	Test Frequency on Therapy ≥ 1 month
Neutrophils	≥3000/µL	<1300/µL	Stop sirolimus ⁴	weekly	weekly
		1300-1999/µL	none	weekly	weekly
		2000-3000/µL	none	weekly	every 2 weeks
		>3000/µL	none	weekly	Monthly ²
Platelets	≥ 100,000/µL	<30,000/µL	Stop sirolimus ⁴	weekly	weekly
		30,000-49,999/µL	none	weekly	weekly
		50,000-100,000/µL	none	weekly	every 2 weeks
		>100,000/µL	none	weekly	monthly
Hyperlipidemia ¹	age normal	≤ 2.5 x upper N	Consider dietary manipulations	weekly	monthly
		> 2.5 x upper N	Consider appropriate drug therapy	weekly	weekly
		>800 mg/dL	Start appropriate lipid lowering agent	weekly	weekly
Sirolimus <i>with CNI</i> (trough levels)	3-12 ng/mL	> 15 ng/mL ³	Stop sirolimus; monitor for HUS	every 3-4 days	every 3-4 days
		12-15 ng/mL	Reduce sirolimus 20-25%	every 3-4 days	every 3-4 days
		10 – 12 ng/mL & serum Cr ≥ 2 x N	Reduce sirolimus 20-25%	every 3-4 days	every 3-4 days
		3-12 ng/mL on > 3 successive tests	Less frequent monitoring	weekly	monthly
		< 3 ng/mL & serum Cr < 2 x N	Increase sirolimus 20-25%	weekly	weekly
Sirolimus <i>without CNI</i> (trough levels)	3-12 ng/mL	> 12-18 ng/mL	Reduce sirolimus by 20-25%	weekly	weekly
		3 – 12 ng/mL on > 3 successive tests	Less frequent monitoring	weekly	monthly
		< 3 ng/mL	Increase sirolimus 20-25%	weekly	weekly
Cyclosporine (trough level)	120-200 ng/mL	> 360 ng/mL	Stop/reduce CSP, recheck level & serum Cr	every 1-3 days	every 3-4 days
		>200 & serum Cr normal	reduce dose of cyclosporine	every 3-4 days	every 3-4 days
		120-200 ng/mL & serum Cr ≤ 2.5 x N	continue same dose	every 1-2 wks	monthly
		80-200 ng/mL & serum Cr ≥ 2.5 x N	monitor for HUS	every 3-4 days	every 3-4 days
Tacrolimus (trough level)	5-10 ng/mL	>20 ng/mL	Stop/reduce TAC, recheck level & serum Cr	every 1-3 days	every 3-4 days
		>10 ng/mL and serum Cr normal	reduce dose of tacrolimus	every 3-4 days	every 3-4 days
		5-10 ng/mL & serum Cr ≤ 2.5 x N	continue same dose	every 1-2 wks	monthly
		5-10 ng/mL & serum Cr ≥ 2.5 x N	monitor for HUS	every 3-4 days	every 3-4 days

Notes: ¹ Fasting serum triglycerides. ² Every 2 weeks if on ganciclovir or valganciclovir. ³ Hold dose and recheck level before readministering sirolimus at lower dose. ⁴ If patients develop neutropenia, thrombocytopenia or hyperlipidemia while taking sirolimus please consult Section 2.8 of protocol for further details on management. HUS; Hemolytic Uremic Syndrome. [See also Section 2.5.1.4 (TMA/HUS/TTP) and “Sirolimus & CNI Therapy Physician Guide”]

Other Important Considerations:

- **Children** generally require a larger total daily dose of CNI by weight than adults. If aged < 6 years, every 8 hour administration may be required to maintain desirable serum trough levels. For those patients in whom adequate serum trough levels cannot be maintained using intermittent oral or IV dosing, continuous IV infusion may be warranted.
- **Review of concomitant medications for potential interactions that may significantly alter serum CNIs levels (see Section 2.5.2.7-2.5.2.8) is essential** because CNIs undergo extensive metabolism by the hepatic and intestinal cytochrome P-450 system which may impact toxicity and efficacy of CNIs.
- Although elevated CNI blood levels are more frequently associated with toxicity (especially renal or hepatic), **“therapeutic” CNI blood levels may also be associated with toxicity** (e.g. significant tremors). Therefore, dose decreases are recommended for significant organ toxicities that manifest despite a “therapeutic” CNI level.
- **Emesis:** A repeat dose of CNI may be given if emesis occurs within 15 minutes of a dose.
- CNIs should be administered at a consistent time each day and in relation to meals. Neoral oral solution should be administered in orange or apple juice at room temperature.
- Subjects should **avoid beverages containing the enzyme bergamottin** (grapefruit juice, Sunny Delight, Fresca, and Squirt) when taking CNIs.
- **Distal paraesthetic pain or burning during the infusion** may be alleviated by extending the IV infusion time for Sandimmune from the standard of 1 hour to as long as 6 hours. Alternatively, cyclosporine may be administered as a continuous infusion over 24 hours for patients intolerant of intermittent administration.
- Monitor closely for an acute allergic reaction for the first 30 minutes after starting tacrolimus IV infusion and at frequent intervals thereafter.
- **Intravenous route of administration is preferred for patients with progressive GVHD of the liver or gastro-intestinal tract** until GVHD is clinically improved and the oral route reliable.
- If the oral route becomes temporarily unfeasible and conversion to the intravenous formulation is required the following recommendations are made:

Starting Oral Formulation	Conversion to IV formulation
Prograf®	IV Prograf = 0.25 x total daily dose of oral Prograf but should be given as continuous daily infusion
Neoral®	IV Sandimmune = 0.4 x total daily dose of Neoral
Gengraf®	IV Sandimmune = 0.4 x total daily dose of Gengraf
Sandimmune®	IV Sandimmune = 0.25 x total daily dose of oral Sandimmune

2.5.2.4. Tacrolimus adverse reactions

The primary toxicities in large controlled trials were reversible renal insufficiency, hypertension, hyperglycemia, hypomagnesemia, hyperkalemia, and neurological toxicity (tremor and headache). With the IV formulation, there is also an infusional toxicity of the cremaphor diluent. There is increased risk of opportunistic infections and secondary malignancies. Some of these toxicities are recognized complications of transplantation.

TABLE 2.5c. TACROLIMUS TOXICITIES

	Common >20%	Occasional 5-20%	Rare <5%
Immediate Within 1-2 days of receiving drug	<i>Headache</i> (L), <i>hypertension</i> (L), nausea, vomiting, anorexia, immunosuppression (L), diarrhea, constipation, fever	Chest pain	Anaphylaxis with the injection, allergic reaction, hypotension, asthma, dyspnea, increased cough, flu like syndrome, pleural effusion, seizure (L), tachycardia, angina
Prompt Within 2-3 weeks, prior to the next course	<i>Tremor</i> (L), decrease in GFR, <i>elevated creatinine/BUN, anemia</i> , insomnia, asthenia, pain (abdominal, back, pain), <i>hyperglycemia</i> , <i>hypomagnesemia</i> (L), <i>hyper-/hypokalemia</i> (L), <i>hypophosphatemia</i> , <i>paresthesiae</i> , opportunistic infections	Alopecia, dizziness, elevated LFTs, UTI, peripheral edema, rash, pruritis, hyperlipidemia, hypercholesterolemia, leukocytosis, thrombocytopenia	Dyspepsia, dysphagia, gastritis, esophagitis, flatulence, CNS abnormalities (confusion (L), somnolence (L), depression (L), anxiety, anxiousness, abnormal dreams, emotional lability, hallucinations, psychosis, hypertonia, incoordination, neuropathy, nervousness encephalopathy, abnormal vision, tinnitus, coagulation disorder, leukopenia (L), polycythemia, anemia, leukocytosis, thrombosis, phlebitis, <i>arthralgia, myalgia, electrolyte abnormalities</i>
Delayed Any time later during therapy, excluding the above conditions			Acne, exfoliative dermatitis, skin discoloration, photosensitivity reaction, skin ulcer, delayed wound healing, hirsutism (hypertrichosis) (L), gingival hyperplasia, abnormal vision, amblyopia, ear pain, otitis, tinnitus, GI hemorrhage, GI perforation, cholelithiasis, cholestatic jaundice, chronic renal dysfunction, renal failure, post transplant diabetes mellitus (L), Reversible, concentration dependent <i>myocardial hypertrophy</i> (L), elevated liver function tests, liver damage, ascites
Late Any time after completion of treatment			Lymphoproliferative disorders, skin malignancies
Unknown Frequency and Timing	Fetal toxic effects of tacrolimus have been noted in animals. Tacrolimus is transported across the placenta and its use during pregnancy has been associated with neonatal hyperkalemia and renal dysfunction. Tacrolimus is excreted in human milk, nursing should be avoided.		

(L) Toxicity may also occur later.

2.5.2.5. Cyclosporine adverse reactions

The toxicities of cyclosporine are similar to tacrolimus but not overlapping (see Section 2.5.2). Common toxicities include: hypertension, tremor, hirsuitism, hyperlipidemia, hyperkalemia, hypomagnesemia, elevated creatinine/BUN, hyperglycemia, paraesthesiae, gingival hyperplasia, abnormal LFTs, acne, reduced bicarbonate, abdominal discomfort, anorexia, nausea, vomiting, CNS disturbances and potential for TTP/HUS/TMA similar to tacrolimus and exacerbated by sirolimus (see Section 2.5.2.4), gynecomastia, and with the IV form: flushing, erythema, skin bullae.

2.5.2.6. Management of tacrolimus and cyclosporine toxicities

Please see Section 2.5.1.4 and Table 2.5b for management of nephrotoxicity, Thrombotic Microangiopathy and hyperlipidemia.

2.5.2.7. Tacrolimus drug interactions

Tacrolimus is a substrate for cytochrome CYP 3A4 enzyme systems.

Drugs that may increase tacrolimus blood concentrations include CYP 3A4 inhibitors:

- Calcium Channel Blockers: diltiazem, nicardipine, nifedipine, verapamil. Tacrolimus should be monitored and a dose adjustment may be necessary.
- Triazole antifungal agents: fluconazole, itraconazole, clotrimazole, posaconazole, voriconazole, ketoconazole. See Section 2.7.1 for **recommendations for preemptive dose reduction of CNI doses when initiating triazole antifungal therapy** (Table 2.7a.) and anticipated need to increase CNI doses after triazole antifungal therapy is discontinued (Table 2.7b.).
- Macrolide antibiotics: clarithromycin, troleandomycin.
- Gastrointestinal prokinetics: cisapride, metoclopramide.
- Other: cyclosporine, ethinyl estradiol, omeprazole, lansoprazole, methylprednisolone.

Drugs that may decrease tacrolimus blood concentrations include CYP 3A4 inducers:

- Anticonvulsants: carbamazepine, Phenobarbital, phenytoin.
- Antimicrobials: Rifampin, rifabutin, caspofungin.
- Herbs: St. John's Wort (*Hypericum perforatum*).

Due to potential for additive or synergistic impairment of renal function, care should be taken when administering tacrolimus with drugs that may be associated with renal dysfunction. These include but are not limited to amphotericin B products, aminoglycosides, vancomycin, trimethoprim with sulfamethoxazole, fenofibrate, gemfibrozil, statins, angiotensin II receptor antagonists, and diuretics.

2.5.2.8. Cyclosporine drug interactions

The drug interactions for cyclosporine are similar or identical to tacrolimus (see Section 2.5.2.7). Postmarketing cases of myositis, myopathy and rhabdomyolysis have been reported with concomitant administration of cyclosporine with HMG-CoA reductase inhibitors (statins) which should be used with caution and at doses which are reduced doses according to label recommendations.

2.5.3. Prednisone

2.5.3.1. Initiating prednisone therapy

Prednisone is administered initially as a single early morning dose of 1 mg/kg/day [or equivalent (adults: maximum dose 100 mg, age < 17 years: adjusted body weight as per institutional guidelines for patients who weigh > 110% of ideal body weight)].

If prednisone (or equivalent) at a dose of 1 mg/kg/day is contraindicated (e.g. poorly controlled diabetes, hypertension, osteoporosis, avascular bone necrosis, major mood disturbance) patients may begin prednisone between 0.5 - 1 mg/kg/day.

Prednisone therapy continues at the initial dose until there is objective evidence of improvement in manifestations of chronic GVHD.

2.5.3.2. Monitoring

Prednisone therapy will be monitored according to institutional guidelines.

2.5.3.3. Prednisone adverse reactions

The side effects of prednisone are broad and well recognized. They include but are not limited to: fluid and sodium retention, hypokalemia, hypertension, myopathy, muscle wasting, altered body habitus with cushingoid appearance, osteoporosis, tendon rupture, pancreatitis, peptic ulcer, impaired wound healing, thin fragile skin, petechiae and ecchymoses, ruddy facies, negative nitrogen balance, pseudotumor cerebri, headache, secondary adrenal insufficiency, suppression of growth in children, hyperglycemia, diabetes mellitus, posterior subcapsular cataracts, glaucoma, euphoria, insomnia, mood swings, personality changes, severe depression, emotional instability, and frank psychosis.

2.6. Approach to Tapering Immunosuppressive Therapy

The recommended sequence is to taper prednisone, then CNI (for study subjects on Arm 2) and, finally sirolimus. The suggestions for taper schedules are explained in Sections 2.1 (schematic overviews) and 2.6.1 - 2.6.2 below. Minor variations in these tapers are expected based on the physician discretion which considers individual clinical circumstances.

2.6.1. Prednisone Taper

The initial taper of prednisone (or equivalent) from the starting dose of 0.5-1 mg/kg/**every day** is attempted within 2 weeks after the first evidence of improvement in GVHD and takes place over 4-8 weeks to achieve a dose of 0.5-1 mg/kg/**every-other-day**.

Every-other-day dosing, in which twice the usual daily dose of prednisone is administered every other morning provides the patient requiring long-term pharmacologic dosing with the beneficial effects of glucocorticoids while minimizing certain undesirable effects, including pituitary-adrenal suppression, the cushingoid state, glucocorticoid withdrawal symptoms, and growth suppression in children. Note that this approach may not be appropriate for every patient because of complications associated with large swings in prednisone dose.

Once an alternating-day prednisone regimen is achieved, it is recommended that the dose of prednisone be held constant for 10-12 weeks until all reversible manifestations of chronic GVHD have resolved, after which a second taper may be attempted. The tempo of this second taper may follow individual institutional guidelines but it is recommended that the extent of the taper be approximately calibrated to the magnitude of an individual patient's alternating-day prednisone dose. For example, a patient whose prednisone dose has been stable at 0.5 mg/kg/every-other-day may attempt to taper prednisone completely. However, a patient whose prednisone dose has been stable at 1.0 mg/kg/every-other-day is recommended first to taper over 4-8 weeks to 0.5 mg/kg/every-other-day, followed by 2-3 months of further observation before attempting a complete taper.

2.6.2. Sirolimus and Calcineurin Inhibitor Tapers

Arm 1 (Prednisone + Sirolimus):

Sirolimus may be tapered at the discretion of the managing physician over 1-3 months after prednisone has been discontinued. The recommended approach is to monitor and confirm the stability of GVHD response for 1-3 months before tapering sirolimus. Please see Section 2.6.1 regarding the taper of prednisone.

Arm 2 (Prednisone + Sirolimus + Calcineurin Inhibitor):

After prednisone is discontinued, the order and approach for tapering sirolimus or the CNI occurs at the discretion of managing physician. The recommended approach is to monitor and confirm the stability of GVHD response for 1-3 months before tapering the CNI over 3-6 months. A similar 1-3 month period of monitoring to confirm the stability of GVHD response to stopping the CNI is recommended prior to attempting taper or discontinuation of sirolimus. Please see Section 2.6.1 regarding the taper of prednisone.

2.7. Supportive Care

All supportive care will be in keeping with BMT CTN Manual of Procedures and local institutional guidelines that reflect reasonable standard practices appropriate to the patient with chronic GVHD as outlined by the Ancillary Therapy and Supportive Care Working Group Report of the NIH Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD.² Supportive care should be administered in a similar fashion to subjects randomized to both arms of the study.

2.7.1. Concomitant Azole Therapy

Antifungal prophylaxis: Triazole antifungal medications are expected to increase serum CNI and sirolimus levels, therefore, dosages of CNIs and sirolimus, should be adjusted accordingly using the guidelines recommended in Table 2.7a. and 2.7b. **Due to extreme interactions with sirolimus, voriconazole is contraindicated during sirolimus therapy.** In the event of suspected or documented fungal infection, alternative antifungal therapy should be used wherever possible.

TABLE 2.7a. - PRE-EMPTIVE DOSE REDUCTION OF SIROLIMUS OR CNIs WHEN AZOLES ARE INITIATED AT STEADY STATE LEVELS OF SIROLIMUS OR CNIs

Antifungal	Cyclosporine		Tacrolimus		Sirolimus	
	Dose ↓	Comment	Dose ↓	Comment	Dose ↓	Comment
Voriconazole	50%	Strongly advised	67%	Strongly advised	90%	Essential*
Posaconazole	25%	Consider	67%	Advised	67%	Advised
Itraconazole	50%	Advised	50%	Advised	ND	ND
Fluconazole	25%	Consider	25%	Consider	50%	If > 400 mg QD

***Notes:**

1. **Voriconazole should not be used unless deemed absolutely clinically necessary.**
2. If an voriconazole must be added to control fungal infection then following the 90% reduction in sirolimus dosing⁵⁵ and/or 50%-67% reduction in CNI dosing SRL and/or CNI serum levels should be measured 24-48 hours later and then every 3-4 days until levels are stable and in the desired range. If voriconazole is given intravenously or if voriconazole and sirolimus are not given together, these guidelines may not apply because the effect on bioavailability of sirolimus will be weaker.
3. Note that sirolimus tablets should not be split or crushed. Fractional dose of sirolimus may be achieved by drawing an appropriate volume of the 1 mg/mL oral liquid formulation into a 1 mL syringe and swallowed directly (or mixed with water or orange juice; no other liquids, including grapefruit juice, should be used for dilution).
4. If posaconazole or itraconazole or high-dose fluconazole are added then SRL and/or CNI serum levels should be followed 48-72 hours later and then every 3-5 days until levels are stable and in the desired range.

TABLE 2.7b. - ANTICIPATE DOSE INCREASE OF SIROLIMUS OR CNIs WHEN AZOLES ARE STOPPED DURING CONCOMITANT SIROLIMUS OR CNI THERAPY

Antifungal	Cyclosporine		Tacrolimus		Sirolimus	
	Dose ↑	Comment	Dose ↑	Comment	Dose ↑	Comment
Voriconazole	2-fold	Dose increase may not be necessary for 5-10 days	3-fold	Dose increase may not be necessary for 5-10 days	10-fold	Dose increase may not be necessary for 5-10 days
Posaconazole	1.3-fold		3-fold		ND	
Itraconazole	2-fold		2-fold		ND	
Fluconazole	1.3-fold		1.3-fold		2-fold	

Note: Although sirolimus and CNI doses may need to be substantially increased when azole therapy is stopped, the azole mediated inhibition of cytochrome CYP 3A4 (and other) and P-glycoprotein may take 5-10 days to abate and therefore immediate dose increases are not advised. Rather, sirolimus and CNI dose increases should be cautious and based on more frequent monitoring of the sirolimus and/or CNI levels as appropriate.

2.8. Drug Information

2.8.1. Description, Administration and Storage

2.8.1.1. Sirolimus (rapamycin, Rapamune®)

Sirolimus is a naturally occurring compound produced by *Streptomyces hygroscopicus*. In addition to its immunosuppressive properties, sirolimus has antifungal, antiviral and antineoplastic properties (see also Section 1.3).

1) Oral solution:

Sirolimus oral solution is supplied in cartons of 2 oz (60 mL fill) or 5 oz (150 mL fill) amber glass bottles, or foil pouches. The oral solution contains sirolimus at a concentration of 1 mg/mL and the following inactive ingredients: Phosal 50 PG® (phosphatidylcholine, propylene glycol, monodiglycerides, ethanol, soy fatty acids, and ascorbyl palmitate) and polysorbate 80. The oral solution also contains 1.5% - 2.5% ethanol. The appropriate dose of sirolimus oral solution should be measured using the provided amber colored oral syringe and is diluted in at least 2 oz (1/4 cup) of water or orange juice to improve palatability. **No other liquids, including grapefruit juice, should be used for dilution.** After vigorous mixing, the diluted dose should be taken immediately. Refill the container with an additional volume (recommended minimum of 4 oz (1/2 cup) of water or orange juice, stir vigorously, and drink or administer at once to assure delivery of all of the medication. Small children may not be able to consume the recommended volumes of water or orange juice suggested for dilution and may need lesser volumes.

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. Rapamune® Oral Solution bottles and pouches should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). The syringe should be discarded after one use. After dilution, the preparation should be used immediately.

2) Tablets:

Sirolimus tablets are available as white, tan or yellow-to-beige triangular-shaped tablets marked “RAPAMUNE 1 mg,” “RAPAMUNE 0.5 mg” or “RAPAMUNE 2 mg” respectively, in bottles containing 100 tablets or cartons containing 10 blister cards each with 10 tablets (0.5 mg and 1 mg formulations only). Each tablet contains sirolimus and the following inactive ingredients: sucrose, lactose, polyethylene glycol 8000, calcium sulfate, microcrystalline cellulose, pharmaceutical glaze, talc, titanium dioxide, magnesium stearate, povidone, poloxamer 188, polyethylene glycol 20,000, glyceryl monooleate, carnauba wax, and other ingredients. The 0.5 mg and 2 mg tablets also contain yellow iron (ferric) oxide and brown iron (ferric) oxide. Sirolimus tablets should be stored at 20° to 25°C (68° - 77°F). Cartons should be used to protect blister cards and strips from light. Sirolimus tablets should be dispensed in a tight, light-resistant container. Sirolimus tablets should not be split or crushed.

3) Pharmacology:

The absorption of sirolimus is rapid after administration of Rapamune® Oral Solution, with a mean T_{max} of 1-2 hours in different study populations. Oral bioavailability is only 14% in stable renal transplant patients due to first pass metabolism in the liver and the intestinal wall, plus countertransport in the gut lumen by P-glycoprotein. Mean bioavailability of sirolimus after administration of the tablet is about 27% higher relative to the oral solution. However, clinical equivalence has been demonstrated for the 2-mg dose. Coadministration with high fat meals leads to reduced C_{max} , prolonged T_{max} and increased AUC, meaning that sirolimus should be taken consistently with or without food. The distribution of sirolimus is notable for extensive partitioning into blood cells and approximately 92% is bound to human plasma proteins. Sirolimus is a substrate for CYP3A4 and P-glycoprotein, and is extensively metabolized by O-demethylation and/or hydroxylation to at least 7 major metabolites. The parent compound contributes to more than 90% of the immunosuppressive activity. The excretion of Sirolimus is 91% fecal and only 2.2% via the urine.

Whole blood sirolimus trough levels in renal transplant recipients who were administered daily doses of 2 mg or 5 mg at 4 hours after Neoral®) were 8.59 ± 4.01 ng/mL and 17.3 ± 7.4 ng/mL, respectively, as measured by LC/MS/MS. Whole blood trough levels significantly correlated with steady state AUC ($r^2=0.96$). Six days of multiple dosing were required to achieve steady state. Alternatively, a loading dose of three times the maintenance dose will provide near steady state concentrations within one day in most patients.

The mean \pm SD terminal elimination half life ($T_{1/2}$) of sirolimus after multiple dosing in stable renal transplant patients was 62 ± 16 hours. The mean $T_{1/2}$ increased from 79 ± 12

hours in subjects with normal hepatic function to 113 ± 41 hours in patients with impaired hepatic function. Dosage reduction is recommended for patients with mild to moderate hepatic impairment. Limited pharmacokinetic data are available in pediatric patients with chronically impaired renal function but indicates similar T_{max} (0.62-1.6 h) and $T_{1/2}$ (31-111h). Clearance is slower in males compared to females but dose adjustments based on gender are not recommended. There were no differences between African Americans and non-African Americans.

2.8.1.2. Tacrolimus (FK-506, Prograf®)

Tacrolimus is a macrolide immunosuppressant produced by *Streptomyces tsukubaensis* that inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines like interleukin-2 and gamma interferon. The net result is the inhibition of T-lymphocyte activation. Tacrolimus may also inhibit cellular activities such as nitric oxide synthetase activation and apoptosis, and may potentiate the action of corticosteroids in these processes.

1) Oral capsules:

Tacrolimus (Prograf®) is available as oblong shaped, light yellow (0.5 mg), white (1 mg), or grayish-red (5 mg) capsules supplied in bottles containing 100 capsules or cartons containing 10 blister cards each with 10 capsules. Capsules should be stored at 25°C (77°F).

2) IV solution:

Tacrolimus IV solution is supplied in 1 mL ampoules that contain 5 mg of anhydrous tacrolimus (concentration of 5 mg/mL) and should be stored between 5°C to 25°C (41°F – 77°F).

Tacrolimus Injection must be diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection before use to a concentration between 0.004 mg/mL and 0.02 mg/mL. Diluted infusion solution should be stored in glass or polyethylene containers and should be discarded after 24 hours. The polyoxyethylated castor oil contained in the concentrate for intravenous infusion can cause phthalate stripping from PVC. **It is strongly recommended that glass bottles and non-PVC tubing be used to minimize patient exposure to phthalates.** 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).

3) Pharmacology:

The absorption of tacrolimus after oral administration is incomplete and variable and

absolute bioavailability was 17-23% and the T_{max} was 1.5-3 hours in different study populations. The rate and extent was greatest under fasted conditions and least with high fat or high carbohydrate meals. Tacrolimus binding to plasma proteins is 99% which is independent of concentrations ranging from 5-50 ng/mL. Tacrolimus is also highly associated with erythrocytes. Tacrolimus is a substrate for mixed-function oxidase system, primarily CYP3A4, and is extensively metabolized by demethylation and hydroxylation to a possible 8 major metabolites. The parent compound is primarily responsible for the immunosuppressive activity. The excretion of orally administered tacrolimus is 92% fecal and 2.3% via the urine. Typical whole blood tacrolimus trough levels after the recommended oral doses of 0.1-0.2 mg/kg/day were 5-20 ng/mL as measured by LC/MS/MS. The mean terminal elimination half life ($T_{1/2}$) of tacrolimus after multiple dosing in different study populations was 11-34 hours. The $T_{1/2}$ in children was generally at the lower range and showed that children needed higher doses than adults to achieve similar trough concentrations. The mean $T_{1/2}$ was reduced by 2-3-fold in patients with impaired hepatic function. Dosage reduction is recommended for patients with mild to moderate hepatic impairment. A retrospective comparison of African American and Caucasian kidney transplant patients indicated that African American patients required higher tacrolimus doses to attain similar trough concentrations; there were no gender-based differences.

2.8.1.3. Cyclosporine

Cyclosporine is a cyclic polypeptide immunosuppressant agent produced by the fungus species *Beauveria nivea*. While the molecular structure of cyclosporine is distinct from tacrolimus, their mechanism of action is almost indistinguishable and involves inhibition of calcineurin and the resulting activation cascade leads to inhibition of T cell signaling (see Section 2.8.1.2). Cyclophilin is the cytosolic binding protein for cyclosporine that is distinct from FK-binding protein.

1) Oral formulations:

Cyclosporine is most commonly prescribed as soft gelatin capsules (Neoral®) or modified oral solution (Neoral® Oral Solution) that have increased bioavailability in comparison to the less commonly used Sandimmune formulations. Neoral® and Sandimmune® cannot be used interchangeably and care must be exercised when converting from high doses of Sandimmune® to Neoral®. Neoral® capsules are available in two strengths: 25 mg (oval, blue-gray) or 100 mg (oblong, blue-gray) imprinted in red. Neoral® Oral solution is available in 50 mL bottles of yellow liquid containing 100 mg/mL of cyclosporine. Both Neoral® formulations contain 11.9% v/v alcohol. Neoral® capsules and oral solution should be stored at 20°-25°C (68°F -77°F) and not in the refrigerator. Sandimmune® capsules are available in two strengths: 25 mg (oblong, pink) or 100 mg (oblong, dusty rose), in unit dose packages of 30 capsules that should be stored at 25°C (77°F). Sandimmune® Oral solution is available in 50 mL bottles of yellow liquid containing 100 mg/mL of cyclosporine. Both Sandimmune® formulations contain ~12.5% v/v alcohol.

2) IV solution:

Sandimmune® IV solution is supplied in 5 mL ampoules that contain 50 mg of cyclosporine per mL, Cremaphor and 32.9% alcohol v/v. It should be stored at temperatures below 30°C (86°F) but not in the refrigerator and should be protected from light.

3) Pharmacology:

The absorption of cyclosporine after oral administration is incompletely dependent on the individual patient, patient population and the formulation. Cyclosporine is excreted in human milk. The absolute oral bioavailability of Sandimmune® was 10%-89% in adults and had not been determined for Neoral®. Mean cyclosporine AUC is generally 20%-50% greater, and C_{max} 40%-106% greater, with Neoral® compared to Sandimmune®, respectively. The T_{max} following oral administration of Neoral® is 1.5-2 hours. The rate and extent of absorption were lower with low or high fat meals. Cyclosporine binding to plasma proteins is 90%, primarily lipoproteins. Cyclosporine is 33%-47% in the plasma, 4%-9% in lymphocytes, and 41%-58% in erythrocytes. Cyclosporine is extensively metabolized CYP3A4 and to a lesser extent in the gastrointestinal tract and kidney. At least 25 metabolites have been identified in bile, feces, blood and urine but the parent compound is primarily responsible for the immunosuppressive activity. Elimination is primarily biliary with only 6% of the dose excreted in urine. The mean terminal elimination half life ($T_{1/2}$) of cyclosporine is 8.4 hours (range 5-18 hours). The $T_{1/2}$ in children was generally at the lower range and showed that children needed higher doses than adults to achieve similar trough concentrations. The mean $T_{1/2}$ was reduced by 2-3-fold in patients with impaired hepatic function. Dosage reduction is recommended for patients with mild to moderate hepatic impairment. A retrospective comparison of African American and Caucasian kidney transplant patients indicated that African American patients required higher tacrolimus doses to attain similar trough concentrations; there were no gender-based differences.

2.8.1.4. Glucocorticoids

Naturally occurring glucocorticoids cause profound and varied metabolic effects which include modification of the body's immune system.

The most commonly used glucocorticoid formulations for the treatment of chronic GVHD are prednisone tablets which are available in a variety of strengths ranging from 1 mg up to 50 mg. The most commonly used intravenous formulation is methylprednisolone.

CHAPTER 3

3. STUDY ENDPOINTS

3.1. Definition of High-Risk vs. Standard-Risk

The definition of high-risk and standard-risk patients is as follows:

- 1) High-risk defined as meeting one or more of the following criteria:
 - a) Patients with platelets < 100,000
 - b) Patients > 50% skin involvement
 - c) Patients with bronchiolitis obliterans
 - d) Patients receiving prednisone ≥ 0.5 mg/kg/day (or equivalent) at the time of cGVHD diagnosis
- 2) Standard-risk not meeting the high-risk criteria above.

3.2. Primary Endpoint

Treatment success will be defined according to the local physician's response.

Phase II Endpoint

The primary endpoint is a comparison of the proportion of treatment successes in patients at 6 months after randomization in both study arms. *Treatment success is defined as a study subject who is alive and who achieved a complete or partial response. The subject did not receive secondary systemic immunosuppressive therapy and had no subsequent progression or additional secondary immunosuppressive therapy added through 6 months after randomization.* Relapse is considered a competing risk and is not included in the primary endpoint definition.

Phase III Endpoint

The primary endpoint is a comparison of the proportion of treatment success in patients at 24 months after randomization in both study arms. *Treatment success is defined as a study subject who is alive and who achieved complete resolution of all reversible manifestations at 24 months after randomization and did not receive secondary systemic immunosuppressive therapy.*

3.3. Secondary Endpoints

The secondary endpoints will explore clinically relevant presumed associations with the primary endpoint of response. These will be evaluated at baseline, and between 2 months and 3 years after the start of therapy depending on the specific endpoint.

3.3.1. Prednisone Sparing

The percent reduction in the average daily dose of prednisone (or equivalent) will be calculated at 6 and 12 months (Phase II/III) and, additionally at 24 and 36 months (Phase III) after study entry based upon the prednisone doses that will be captured on case report forms up to 36 months from randomization.

3.3.2. Use of Secondary Therapy

Treatment failure is defined at the time any secondary systemic therapy is added to control manifestations of chronic GVHD. Secondary systemic therapy includes any intervention intended to control chronic GVHD through an immunosuppressive effect from oral or parenteral administration of any systemic medication not originally given under the auspices of this protocol for treatment of chronic GVHD. Examples include, but are not limited to the following: cyclosporine or tacrolimus(Study Arm 1 only), and extracorporeal photopheresis, mycophenolate mofetil, azathioprine, rituximab, infliximab, daclizumab, etanercept, antithymocyte globulin, pentostatin, thalidomide, and hydroxychloroquine (Arms 1 and 2).

Any increase in the dose of prednisone (or equivalent) and any resumption of treatment with prednisone, after previous discontinuation following the diagnosis of chronic GVHD for any reason is not considered as secondary systemic therapy. However, prednisone pulses exceeding 2 mg/kg/day are considered secondary therapy.

Any increase in the dose of CNI or sirolimus, or resumption of treatment with CNI or sirolimus after previous discontinuation for any reason is not considered as secondary systemic therapy, if the drug in question was included in the immunosuppressive regimen when treatment for chronic GVHD was started.

A change in treatment from cyclosporine to tacrolimus or vice versa because of drug toxicity is not considered as secondary treatment, but any such change made because of uncontrolled chronic GVHD is considered as secondary treatment. Sirolimus may be discontinued for toxicities without counting as treatment failure unless a new systemic therapy is added for control of GVHD.

Topical therapy, including glucocorticoid creams, topical tacrolimus, oral beclomethasone or budesonide, topical azathioprine and ophthalmic glucocorticoids, is not considered as secondary systemic therapy.

3.3.3. Symptomatic Patient-Reported Chronic GVHD

Symptomatic chronic GVHD will be defined as severity of chronic GVHD symptoms as reported by patients on the Patient Surveys. Prevalence rates will be calculated and compared between groups at 1 and 2 years (Phase II/III) and, additionally at 3 years for Phase III component.

3.3.4. Permanent Discontinuation of all Systemic Immunosuppressive Therapy

Cumulative incidence curves of discontinuation of all systemic IST at 1 and 2 years (Phase II/III) and, additionally at 3 years for the Phase III component, will be calculated based upon the time that all systemic IST has been discontinued after resolution of all reversible manifestations of chronic GVHD. Death or drop out with lack of follow-up information after resolution of all reversible manifestations of chronic GVHD and discontinuation of all systemic IST but before the final analysis is expected to occur infrequently and will be categorized as successful discontinuation of IST for the purposes of this study since the treatment had been successful in controlling chronic GVHD. Discontinuation of immunosuppressive medications for the purpose of inducing an anti-tumor response after the development of recurrent or secondary malignancy will not be counted as successful discontinuation of IST. If a patient requires only physiological doses of glucocorticoid to treat adrenal insufficiency when the final immunosuppressive agent is discontinued then it is acceptable to assign the designation: “permanent discontinuation of all systemic immunosuppressive therapy”.

3.3.5. Overall Survival

Overall survival probabilities will be compared between treatment arms adjusting for significantly imbalanced covariates. Patients are considered a failure of this endpoint if they die from any cause. The time to this event is the time from randomization to death, loss to follow up or end of study whichever comes first. Overall survival will be assessed 1 and 2 years from the time of randomization (Phase II/III) and, additionally at 3 years for the Phase III component.

3.3.6. Progression-Free Survival

Progression-free survival will be assessed 1 and 2 years from the time of randomization (Phase II/III) and, additionally at 3 years for the Phase III component. Progression of a cancer is defined as any clinical evidence of progression or relapsed disease, or any therapy used to treat persistent, progressive, or relapsed disease including withdrawal of immunosuppressive therapy or DLI.

3.3.7. Biomarkers

T-reg, B-cell and plasma BAFF levels will be enumerated at baseline and months two and six after randomization as long as patients do not start secondary therapy. If secondary systemic immunosuppressive therapy is prescribed, samples should be drawn for T reg, B cell, and plasma BAFF levels prior to initiation of secondary therapy.

3.3.8. NIH and Other New Response Instruments

An important secondary endpoint of this study will be to test more objective, clinically relevant CR and PR criteria using instruments that have been recommended along the lines of the NIH Consensus guidelines⁵². A comprehensive assessment of organ involvement using the NIH Consensus response assessment tools and other study instruments will be performed at baseline,

2 months, 6 months, 1 year and 2 years (Phase II/III) and additionally at 3 years (Phase III), and in the event that a subject prematurely discontinues the study.

3.3.9. Quality of Life

Health Related Quality of Life will be described prior to the initiation of therapy for patients utilizing the FACT-BMT self report, transplant specific questionnaire and the generic quality of life tool, the SF-36. The questionnaires will be scored according to standard procedures. They will be due at baseline and months 2, 6, 12, 24, and 36.

3.4. Safety Endpoints

Monitoring of several safety endpoints will be conducted monthly and be used to form the basis of the stopping guidelines detailed in Section 5.4. The key safety endpoint is mortality at 56 days after randomization. Two additional non-hematological toxicities will also be monitored: Thrombotic Microangiopathy (TMA) and Pneumonitis (NIP).

3.4.1. Mortality

The rate of mortality will be monitored up to 56 days post-randomization. Monitoring will be performed monthly beginning after the third month of enrollment until enrollment is closed. At least three events must be observed in order to trigger review.

3.4.2. Thrombotic Microangiopathy (TMA)

This complication might be expected to occur more frequently in the sirolimus and calcineurin-inhibitor containing arm than in the other arm (see Section 2.5.1.4).

3.4.3. Pneumonitis (NIP)

Sirolimus associated NIP is poorly understood but has been reported after solid organ transplantation. NIP appears to be uncommon although the exact incidence is unknown. NIP should be suspected if a patient develops fatigue, fever and/or dyspnea within weeks to months after starting sirolimus; especially in patients > age 40 years, when patients have been converted from CNI-based IST to sirolimus-based IST, and when sirolimus levels have exceeded 12 ng/mL. Radiological pulmonary infiltrates are invariably observed and often resemble bronchiolitis obliterans-organizing pneumonia (BOOP), more currently referred to as cryptogenic organizing pneumonia (COP). Sirolimus associated NIP symptoms are generally readily reversible within 14-28 days when sirolimus is stopped; although, pulmonary infiltrates may take 3-6 months to resolve. Sirolimus should not be restarted if sirolimus-associated NIP is diagnosed.

CHAPTER 4

4. PATIENT REGISTRATION, RANDOMIZATION AND ENROLLMENT

4.1. Approaching Patients, Eligibility Screening and Obtaining Consent

Subjects will be approached for this protocol after they have developed chronic GVHD. Transplant physicians will evaluate patient eligibility for randomization on to this protocol (see Section 2.3). If the patient has already been discharged from the transplant center, the patient's current managing physician may contact the transplant center to determine patient eligibility for randomization. Eligibility criteria will be verified and ineligible patients will proceed off study and no further follow-up will be obtained. Eligible patients willing to potentially participate in the trial will have a thorough discussion about the protocol with a transplant center physician who is an investigator or sub-investigator. If necessary, this discussion may take place by telephone. The patient (or guardian) will be given a copy of the IRB-approved consent document to review before this discussion. During the discussion, the purposes of the study, procedures and alternative forms of therapy will be presented as objectively as possible, and the potential benefits and risks of participation will be explained. The patient (or guardian) will be given a copy of the entire signed consent document to keep. The last page of the consent document must be signed by the patient (or guardian) and by the physician who discussed the protocol. Transplant center personnel will record the documentation of patient consent in EMMES AdvantageEDCSM (Electronic Data Capture, an Internet-based data entry system) and patients will be enrolled through AdvantageEDC.

4.2. Randomization

Once the subject is deemed eligible and has given written informed consent, and the transplant center has confirmed patient eligibility in AdvantageEDC, randomization occurs. See Section 5.1.2 for further details on the randomization process.

4.3. Treatment Scheduling

Treatment scheduling begins as soon as possible after a patient has undergone baseline screening evaluations and randomization in AdvantageEDC (see Section 4.4, and in particular, baseline screening Section 4.4.1.1).

4.4. Study Monitoring

4.4.1. Patient Assessments

A) **Study Endpoint Assessments** are study specific and include comprehensive NIH style evaluations of chronic GVHD and blood for biomarkers as shown in **Table 4.4a**. Results of these assessments will be recorded on Case Report Forms. A recommended target day range for these visits (**Table 4.4b.**) is computed based on date of signed written consent and is displayed for printing in AdvantageEDC.

Patients who discontinue therapy early will still be followed through the duration of the study for the scheduled Study Endpoint Assessments (Table 4.4a.) and Clinical Assessments (Table 4.4b) relevant to analysis of secondary endpoints.

B) Clinical Assessment Visits that are considered standard care for the average patient being treated with CNI and/or sirolimus based therapy for chronic GVHD. Assessments include history, physical exam, height, weight, directed evaluation of GVHD/toxicity, CBC and platelets, blood chemistry panel, serum lipids, pulmonary function tests, drug levels, and prednisone dose as shown in **Table 4.4a**. A recommended target day range for these visits (**Table 4.4c**) is computed based on date of signed written consent and is displayed for printing in AdvantageEDC. *However, the visit frequency and target day range should be dictated mainly by good clinical practice with a focus on safe delivery of immunosuppressive therapy as recommended by the monitoring approach in Table 2.5b. and/or the form entitled: “Sirolimus & CNI Therapy Physician Guide.”*

TABLE 4.4a. STUDY ASSESSMENT SCHEDULE

BASELINE through END OF STUDY	Baseline	Months Post-Study Entry						
		1	2	3	6	12	24	36
Study Endpoint Assessments/Testing								
Comprehensive Chronic GVHD Assessment ¹	X		X		X	X	X	X
Required Peripheral Blood Sample (Biomarkers: BAFF, T-cell and B-cell)	X		X		X			
	And at initiation of secondary immunosuppressive therapy							
Optional Peripheral Blood Sample (Future Testing of Biomarkers)	X		X		X			
Optional Buccal Swab (Future Testing of Biomarkers)	X							
Clinical Assessments/Testing								
History, Physical Exam, Weight, Height ²	X		X	X	X	X	X	X
Pregnancy Test (if applicable)	X							
Toxicity Assessment			X	X	X	X		
Prednisone Dose at end of Month ³	X	X	X	X	X	X	X	X
Pulmonary Function Tests	X			X	X	X		
CBC, Differential, Platelet Count ⁴	X	X	X	X	X	X		
Serum Cholesterol/Triglycerides ⁴	X	X ⁵	X	X	X	X		
Blood Chemistry Panel ⁴	X	X	X	X	X	X		
Sirolimus Level ⁴		X	X	X	X	X		
Cyclosporine or Tacrolimus Level ⁴		X	X	X	X	X		

Notes:

¹ Comprehensive cGVHD assessment includes Provider Survey, Patient Survey, 2 minute walk (optional), grip test (optional), and Schirmer's eye exam (optional). Per institutional standards for other times between visits.

² Height is only required at baseline.

³ Q monthly (4-12 mo) then every other mo. (12-36 mo).

⁴ Frequency of labs not recorded on CRF at times indicated follows the recommendations outlined in "Sirolimus & CNI Therapy Physician Guide."

⁵ Cholesterol at 1 month.

TABLE 4.4b. TARGET DAY RANGE FOR STUDY ENDPOINT ASSESSMENT

Study Visit	Target Day post Study Entry
Baseline	See Section 4.4.1.1
Month 2	60 ± 10 days
Month 6	180 ± 30 days
Month 12	365 ± 45 days
Month 24	730 ± 60 days
Month 36	1095 ± 90 days

TABLE 4.4c. TARGET DAY RANGE FOR CLINICAL ASSESSMENT

Study Visit	Target Day post Study Entry
Baseline	See Section 4.4.1.1
Month 1	30 ± 5 days
Month 2	60 ± 10 days
Month 3	90 ± 14 days
Month 4	120 ± 14 days
Month 5	150 ± 14 days
Month 6	180 ± 14 days
Month 7	210 ± 14 days
Month 8	240 ± 14 days
Month 9	270 ± 14 days
Month 10	300 ± 14 days
Month 11	330 ± 14 days
Month 12	365 ± 30 days
Month 14	425 ± 30 days
Month 16	485 ± 30 days
Month 18	545 ± 45 days
Month 20	605 ± 45 days
Month 22	665 ± 45 days
Month 24	730 ± 60 days
Month 26	795 ± 60 days
Month 28	855 ± 60 days
Month 30	915 ± 90 days
Month 32	975 ± 90 days
Month 34	1035 ± 90 days
Month 36	1095 ± 90 days

4.4.1.1. Pre-study evaluations

The following observations should be made at enrollment/randomization ± 14 days:

1. Pregnancy test (if applicable).
2. History, physical examination, height and weight.
3. Comprehensive Chronic GVHD assessment [to include provider survey, patient survey, 2 minute walk (optional), grip test (optional) and Schirmer's eye exam (optional)].
4. Complete blood count (CBC) with differential and platelet count, blood chemistry panel to include serum creatinine, bilirubin (total), alkaline phosphatase, AST, ALT, LDH.
5. Peripheral blood (18 mL) for plasma BAFF levels and regulatory T-cell and B-cell immunophenotyping. This should be collected and processed according to instructions summarized in Appendix C and detailed in the BMT CTN 0801 Laboratory Sample Guide.
6. (OPTIONAL) If consent for optional future research sample was obtained, patient buccal swabs, and an additional peripheral blood sample (10 mL, or 6 mL for patients < 40 kg) should be collected and stored for the future undefined testing of biomarkers related to cGVHD. These samples should be collected and processed according to instructions summarized in Appendix C and detailed in the BMT CTN 0801 Laboratory Sample Guide.
7. Fasting serum cholesterol and triglycerides.
8. Pulmonary function tests including full spirometry are required (DLCO is recommended although not required).

4.4.1.2. Study evaluations

The following observations should be made according to Tables 4.4a or 4.4c:

1. History, physical examination, and weight at months 2, 3, 6, 12, 24, and 36.
2. Comprehensive Chronic GVHD assessment [to include provider survey, patient survey, 2 minute walk (optional), grip test (optional) and Schirmer's eye exam (optional)] for assessment of response at months 2, 6, 12, 24, and 36.
3. Toxicity assessments at months 2, 3, 6, and 12.
4. CBC with differential and platelet count, blood chemistry panel to include serum creatinine, bilirubin (total), alkaline phosphatase, AST, ALT, LDH, at least weekly for the first 4 weeks. CBCs are also indicated at other times during the course of monitoring sirolimus therapy based on the frequencies recommended in **Table 2.5b** or **Sirolimus & CNI Therapy Physician Guide**. Data will be collected on Case Report Forms at months 1, 2, 3, 6, 12, but not necessarily at other times except in the event of Adverse Event Reporting.
5. Fasting serum cholesterol and triglycerides at months 2, 3, 6, and 12.

6. Pulmonary function tests including full spirometry are required (DLCO is recommended although not required) at months 3, 6, and 12.
7. Sirolimus levels are done at the frequencies recommended in **Table 2.5b** or **Sirolimus & CNI Therapy Physician Guide**. Sirolimus levels are recorded on the Case Report Forms at months 1, 2, 3, 6, and 12. Levels are unnecessary during taper.
8. Tacrolimus or cyclosporine levels at the frequencies recommended in **Table 2.5b** or **Sirolimus & CNI Therapy Physician Guide**. Tacrolimus or cyclosporine levels are recorded on the Case Report Forms at months 1, 2, 3, 6, and 12. Levels are unnecessary during taper.
9. Peripheral blood (18 mL) for plasma BAFF levels and regulatory T-cell and B-cell immunophenotyping at 2 months and 6 months and initiation of secondary systemic immunosuppressive therapy, if applicable. This should be collected and processed according to the instructions summarized in Appendix C and detailed in the BMT CTN 0801 Laboratory Sample Guide.
10. (OPTIONAL) If consent for optional future research sample was obtained, an additional peripheral blood sample (10 mL, or 6 mL for patients < 40 kg) should be collected at 2 months and 6 months and stored for the future undefined testing of biomarkers related to cGVHD (see instructions summarized in Appendix C and detailed in the BMT CTN 0801 Laboratory Sample Guide).

4.4.2. 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.

4.4.3. Reporting Patient Deaths

Deaths must be reported to the BMT CTN Data and Coordinating Center (DCC) 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 the initial reporting. However, once the cause of death is known, the form must be updated.

4.4.4. Reporting Serious Adverse Events

4.4.4.1. 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 4-5 AEs must be reported within 24 hours of the event. Unexpected, grade 3 AEs must be reported within three business days of knowledge of the event. Unexpected, grade 3-5 AEs will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. 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.

4.4.5. CIBMTR Data Reporting

Centers participating in BMT CTN trials must register pre and post-transplant outcomes on all consecutive hematopoietic stem cell transplants done at their institution during their time of participation to the Center for International Blood and Marrow Transplant Research (CIBMTR). Registration is done using procedures and forms of the Stem Cell Transplant Outcomes Database (SCTOD). (Note: Federal legislation requires submission of these forms for all US allograft recipients.) Additionally, CIBMTR pre- and post- transplant Report Forms must also be submitted for all patients enrolled on this trial according to the randomization assigned to the patient at the time of initial registration with the CIBMTR according to the randomization assigned to the patient at the time of initial registration with the CIBMTR.

CHAPTER 5

5. STATISTICAL CONSIDERATIONS

5.1. Study Design

The protocol is designed as a combined Phase II/III, randomized, open label, multicenter, prospective comparative study of sirolimus plus prednisone (SP) versus sirolimus/calcineurin-inhibitor plus prednisone (SPC) for the treatment of chronic GVHD. The target enrollment is 300 patients across both phases of the study.

After 50 patients per study arm in the Phase II study have been accrued (100 total) and followed for the 6 month CR+PR rate, a decision will be made about whether to continue in the Phase III study. At this point an additional 200 patients will be randomized on the Phase III study, so that the final sample size for the Phase III comparison will be 300 patients. A schema illustrating this study design is given in the Protocol Synopsis.

5.1.1. Accrual

It is estimated that 7 years of accrual (3 years for phase II study) will be necessary to enroll the targeted sample size. Both Core and Affiliate Centers will enroll patients on this study. Accrual will be reported by race, ethnicity, gender, age (children defined as < 17 years). Accrual will not be halted while waiting for the 6-month response assessment on the last patient enrolled in the Phase II study; over-run of the Phase II accrual target is considered acceptable as long as there are no safety concerns.

5.1.2. Randomization

All patients will be randomized 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

Treatment success will be defined according to the local physician's response.

Phase II Endpoint: The proportion of subjects with complete or partial responses after 6 months of therapy.

Phase III Endpoint: The proportion of subjects with complete resolution of all reversible manifestations at 24 months after starting therapy in both study arms.

The primary analysis will be performed using the intent-to-treat principle so that all randomized patients will be included in the analysis.

5.1.4. Primary Hypotheses

The primary hypothesis is that avoidance of a calcineurin inhibitor in the treatment of chronic GVHD might facilitate the development of tolerance and improve the CR/PR rates at 6 months and ultimately the 24 month CR rates compared to immunosuppressive therapy regimens that contain a calcineurin inhibitor (the comparator arm, denoted SPC). We are testing the null hypothesis.

$$H_0: p_{SP} = p_{SPC}$$

Against the alternatives

$$H_1: p_{SP} > p_{SPC}$$

Where p refers to the CR/PR rate at 6 months in the initial Phase II study and to the CR rate at 24 months for the subsequent Phase III study.

5.2. Details of Study Design and Interim Monitoring Plan for Efficacy and Futility

The primary objective of the Phase II component is to estimate the CR/PR rate for each treatment arm, and determine whether the SP arm is sufficiently promising to expand the trial into a Phase III study. In the Phase II trial, the Z statistic for comparing the CR/PR rates at 6 months between the two treatments will be computed. The SP treatment will not be considered further if the Z statistic comparing its CR/PR rate to the SPC arm is ≤ 0.9 . This is approximately equivalent to rejecting the SP treatment for further consideration if its CR/PR rate is not at least 9% better than the SPC arm, assuming an approximately 40% baseline CR+PR rate. If the Z statistic comparing the CR/PR rates at 6 months is at least 3.53, then we will consider this sufficient evidence of efficacy that we will not continue randomizing patients in a Phase III trial, but will just follow the patients already enrolled for the 2-year CR rate. This corresponds approximately to a 35% observed improvement in CR+PR rates compared to the SPC arm.

If the Phase II study progresses to Phase III, the primary endpoint of the study will be switched to the proportion of patients in CR at 24 months. Additional interim analyses based on this new primary endpoint are planned, after approximately 50 and 100 patients per arm have been followed for two years, and the target final sample size is 150 patients per arm. Although these interim analyses will likely not have an effect in terms of stopping accrual to the study, they are put into place to monitor whether the study results are sufficiently strong or futile to indicate early presentation of study results. This is especially important if accrual is lagging. The stopping boundaries to be used and the decision algorithm for this study are given in Table 5.2.

TABLE 5.2: BOUNDARY VALUES FOR INTERIM MONITORING

Interim Analysis	Number of patients evaluable per treatment arm	Boundary	Decision
1	50 evaluable at 6 months	$Z_6 \leq 0.9$	SP not sufficiently promising in terms of 6-month CR+PR rate; stop study
		$Z_6 \geq 3.53$	Stop for efficacy based on a large observed difference in 6-month CR+ PR rates; stop study but continue to follow-up enrolled patients for two-year outcomes
		$0.9 < Z_6 < 3.53$	Treatment is promising based on 6 month outcomes, and continue enrolling to the Phase III part of the study
2	50 evaluable at 2 years	$Z_{24} \geq 3.12$	Stop for efficacy: study has demonstrated sufficient evidence of an improvement in two year outcomes
		$Z_{24} < 3.12$	Continue
3	100 evaluable at 2 years	$Z_{24} \leq 0.88$	Stop for futility; study is not likely to demonstrate an improvement in two year outcomes
		$Z_{24} \geq 2.46$	Stop for efficacy; study has demonstrated sufficient evidence of an improvement in two year outcomes
		$0.88 < Z_{24} < 2.46$	Continue
4	150	$Z_{24} < 2.01$	No evidence of an improvement in two year outcome
		$Z_{24} \geq 2.01$	Statistically significant improvement in two year outcome

5.2.1. Details of Stopping Boundary Calculations

Stopping boundaries for the comparison of 24 month CR rates are based on an error spending approach, using the error spending function $\alpha(t) = \min(\alpha t^3, \alpha)$, which is similar to an O'Brien-Fleming boundary. These will be conservative since they ignore the impact of the aggressive futility boundary in the phase II trial on the overall type I error rate. However, they provide control of the type I error rate even if the futility boundary is not followed exactly. We also add an option for stopping the study early for efficacy at the end of the phase II study. This is meant to account for unexpectedly large differences in the 6 month CR+PR rate, which are unlikely to disappear when looking at the 24 month CR rate. This additional stopping rule is designed very conservatively, and therefore has negligible impact on the type I error rate under the overall null hypothesis that there is no difference between the treatments on either outcome.

For the futility boundary after the initial Phase II look, we use a stopping rule based on conditional power of less than 5% at the estimated effect size.

These boundary values are shown in Table 5.2 above for the proposed monitoring schedule using Monte Carlo integration, but they may be recalculated using the error spending function if the timing of the analyses do not match up with the planned schedule.

5.3. Operating Characteristics and Power Considerations

Simulations were performed to study the operating characteristics of the study design and monitoring algorithm. Binary observations modeling the 6-month CR+PR rate for the first 100 patients were generated according to the vector of probabilities in each group $p(6)=(pspc(6),psp(6))$. Binary observations for all 300 patients analyzed in the Phase III trial were generated according to 24-month CR probabilities $p(24)=(pspc(24),psp(24))$. Note that 100 patients will have both the 6-month and the 24-month binary outcomes, so that a model for the association between these must be postulated. Odds ratios (OR) between these outcomes ranging from 1 (independent) to 5 to 100 (strongly positively associated) were used in simulations. Baseline 6-month CR+PR rates were expected to be approximately 40%, while baseline 24-month CR rates were expected to be around 28%, although we also considered rates as high as 40%. Several type I error rate scenarios were investigated to demonstrate control at a 2.5% one-sided level, in which $p(6)$, $p(24)$, and the odds ratio were modified. For each scenario in Table 5.3a. the stopping probabilities for futility or efficacy at each interim analysis, as well as the overall type I error rate, are given. Note that the type I error rate is maintained conservatively in all situations as expected. Also worth noting is that if there is no difference in 6-month CR+PR rates, there is an 82% chance that the study will stop at the end of the Phase II component and an 18% chance that the study will proceed to Phase III.

TABLE 5.3a.: SIMULATION RESULTS FOR TYPE I ERROR RATE

p(6)	p(24)	OR	P(stop for futility) at look		P(stop for efficacy) at look				Overall Power
			Phase II	3	Phase II	2	3	4	
(0.4,0.4)	(0.4,0.4)	1	0.821	0.146	0.000	0.000	0.001	0.003	0.005
		5	0.822	0.126	0.000	0.001	0.003	0.005	0.009
		100	0.818	0.096	0.001	0.001	0.006	0.009	0.016
(0.4,0.4)	(0.28,0.28)	1	0.820	0.145	0.000	0.000	0.001	0.003	0.005
		5	0.819	0.126	0.000	0.001	0.003	0.005	0.009
		100	0.821	0.100	0.000	0.001	0.005	0.008	0.015

Next we simulated the power to identify a true difference. Note that for a difference in outcomes to be detectable, it needs to be present in both the 6-month CR+PR rate and the 24-month CR rate. The power to detect a 20% improvement in both outcomes is given in Table 5.3b. below

for several configurations of outcome proportions and odds ratios measuring association between outcomes. This study design has at least 80% power to identify a 20% improvement in both 6-month CR+PR rates and 24-month CR rates between the SP arm and the SPC arm. Also worth noting, if the SP arm is 20% better than the SPC arm on the 6-month CR+PR rate, there is only a 13% chance of stopping the study for futility at the first Phase II analysis.

TABLE 5.3b.: SIMULATION RESULTS FOR POWER UNDER THE ALTERNATIVE HYPOTHESIS

p(6)	p(24)	OR	P(stop for futility) at look		P(stop for efficacy) at look				Overall Power
			Phase 2	3	Phase 2	2	3	4	
(0.6,0.4)	(0.6,0.4)	1	0.131	0.021	0.093	0.137	0.369	0.212	0.810
		5	0.131	0.018	0.090	0.137	0.380	0.208	0.815
		100	0.130	0.010	0.090	0.105	0.429	0.203	0.828
(0.6,0.4)	(0.48,0.28)	1	0.130	0.015	0.090	0.141	0.409	0.186	0.826
		5	0.131	0.013	0.091	0.136	0.420	0.183	0.829
		100	0.132	0.008	0.092	0.117	0.453	0.174	0.836

5.4. Safety Stopping Guidelines

Monitoring of several 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. The stopping guidelines serve as a trigger for consultation with the DSMB for additional review. The key safety endpoint to be monitored is mortality. Two additional non-hematological toxicities also will be monitored: Thrombotic Microangiopathy (TMA) and Pneumonitis (NIP). Each outcome will be monitored using a Sequential Probability Ratio Test (SPRT) as described below. A version for censored exponential data will be used for monitoring mortality up to 56 days, while a version for binary data will be used for monitoring the incidence of the other outcomes by 56 days. The SPRT conserves type I error at 5% across all of the monthly examinations. No additional control of the type I error across multiple toxicity outcomes or across multiple treatment groups will be used.

5.4.1. Mortality

The rate of mortality will be monitored up to 56 days post-randomization. Monitoring will be performed monthly beginning after the third month of enrollment until enrollment is closed. At least three events must be observed in order to trigger review. Each month, the null hypothesis that the 56-day mortality rate is less than or equal to 20% is tested, separately for each of the treatment arms. This is based on a cohort of 76 high-risk patients (thrombocytopenia and progressive onset) out of 170 patients with cGVHD at the University of Minnesota. An

extension of the SPRT for censored exponential data will be used for monitoring, as described in greater detail below and in Appendix D.

This sequential testing procedure conserves type I error at 5% across all of the monthly examinations. The SPRT can be represented graphically. At each monthly interim analysis, the total time on study is plotted against the total number of endpoints (e.g., patients experiencing death). The continuation region of the SPRT is defined by two parallel lines. Only the lower boundary will be used for monitoring to protect against excessive 56-day mortality. If the graph falls below the lower boundary, the SPRT rejects the null hypothesis, and concludes that there are more events than predicted by the observed time on study. Otherwise, the SPRT continues until enrollment reaches the maximum of 150 patients for a treatment continuing in Phase III.

This procedure assumes a censored exponential distribution for the time until death during the first 56 days, and censors follow-up time after 56 days. Only deaths that occur on or before the patient has been followed for 56 days are counted. Total time on study is computed as time from registration to death, or to 56 days, whichever comes first, summed for all patients on study.

The usual measures of performance of an SPRT are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$ and of accepting H_1 when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta_i)$. The test for overall mortality was developed from the following SPRT:

- A SPRT contrasting 20% versus 35% 56-day rate of mortality results in decision boundaries with a common slope of 6.336 and a lower intercept of -24.049, with nominal type I and II errors of 7% and 15%, respectively.

The actual operating characteristics of the truncated test, shown in Table 5.4.a, were determined in a simulation study that assumed uniform accrual of 150 individuals over a seven-year time period, and exponential time to failure after randomization.

TABLE 5.4.a: OPERATING CHARACTERISTICS OF SEQUENTIAL TESTING PROCEDURE FROM A SIMULATION STUDY WITH 10,000 REPLICATIONS

Day 56 Mortality

True 56-Day Rate	20%	30%	35%	40%
Probability Reject Null after 50 patients	0.034	0.379	0.660	0.868
Probability Reject Null after 150 patients	0.052	0.756	0.971	0.999
Mean Month Stopped	82.7	45.5	26.4	17.0
Mean # Endpoints in 56 Days	28.9	23.9	16.0	11.7
Mean # Patients Enrolled	144.6	80.7	47.4	30.6

For example, the testing procedure for a treatment which continues into Phase III testing rejects the null hypothesis in favor of the alternative 5% of the time when the true 56-day mortality rate is 20%, and 76% of the time when the rate is 30%. This corresponds to a type I error rate of $\alpha = 0.05$ and a type II error rate of $\beta = 0.24$. When the true 56-day mortality rate is 30%, on average, the DSMB will be consulted 45 months after opening, when 24 events have been observed in 81 patients. For a treatment arm which only enrolls up to 50 patients, the stopping rule will have 87% power to reject the null hypothesis when the Day 56 mortality rate is 40%.

5.4.2. Non-hematological Toxicities

The cumulative incidences (C.I.) of Thrombotic Microangiopathy (TMA) and Pneumonitis (NIP) at Day 56 will be monitored separately for each arm. **Definitions of TMA and NIP** are detailed in Section 3.3 (Safety Endpoints). Each month, the null hypothesis that the cumulative incidences of TMA or NIP is $\leq 10\%$ will be tested against the alternative that it is $> 10\%$. This will be done using a SPRT for binary outcomes. As above, the SPRT can be represented graphically. At each interim analysis, the total number of patients enrolled is plotted against the total number of patients who have experienced toxicity. The continuation region of the SPRT is defined by two decision boundaries. Only the upper boundary will be used for monitoring the study to protect against high incidences of toxicity. If the graph falls above the upper boundary, the SPRT rejects the null hypothesis, and concludes that the toxicity rate is higher than predicted by the observed number of patients enrolled on study. Otherwise, the SPRT continues until enrollment reaches the target goal.

The tests for TMA and NIP are identical and were developed from the following SPRT:

- A SPRT contrasting 10% versus 25% 56-day incidence, which results in decision boundaries with a common slope of 0.166 and an upper intercept of 2.413, with nominal type I and II errors of 6% and 15%, respectively.

The actual operating characteristics of this truncated test, shown in Table 5.4.b, were determined in a simulation study that assumed uniform accrual of 150 individuals over a seven-year time period.

TABLE 5.4.b: OPERATING CHARACTERISTICS OF SEQUENTIAL TESTING PROCEDURE FROM A SIMULATION STUDY WITH 10,000 REPLICATIONS**Day 56 Incidence of TMA**

True 56-Day Rate	10%	15%	20%	25%
Probability Reject Null after 50 patients	0.038	0.226	0.546	0.818
Probability Reject Null after 150 patients	0.049	0.379	0.856	0.991
Mean Month Stopped	82.8	64.6	36.3	19.9
Mean # Endpoints in 56 Days	14.4	16.8	12.4	8.2
Mean # Patients Enrolled	144.3	112.0	61.8	32.8

For example, the testing procedure for a treatment which continues into Phase III testing rejects the null hypothesis in favor of the alternative 5% of the time when the true 56-day TMA rate is 10%, and 86% of the time when the rate is 20%. This corresponds to a type I error rate of $\alpha = 0.05$ and a type II error rate of $\beta = 0.14$. When the true 56-day TMA rate is 20%, on average, the DSMB will be consulted 36 months after opening, when 12 events have been observed in 62 patients. For a treatment arm which only enrolls up to 50 patients, the stopping guidelines will have 82% power to reject the null hypothesis when the Day 56 TMA rate is 25%. Operating characteristics for NIP are identical, since the monitoring guidelines are the same.

5.5. Demographics and Baseline Characteristics

Demographic 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, and donor gender, conditioning intensity, donor type, peripheral blood versus marrow, time from transplant to enrollment, time from start of immunosuppressive therapy to enrollment, and percent of patients with high risk cGVHD. Between group comparisons will be performed for continuous variables via a Kruskal-Wallis Test and for categorical variables, via the Chi-Square Test.

5.6. Analysis of Secondary Endpoints

- Prednisone Sparing:** The median percent reduction in average daily dose among survivors will be estimated for each treatment with a 95% confidence interval by 6 and 12 months after transplant for the Phase II component and additionally by 24 and 36 months for the Phase III component. These will be compared between treatments using the Kruskal-Wallis Test.
- Use of Secondary Therapy:** The proportion of patients who received secondary therapy at months 6, 12, 24, and 36 will be computed and compared using a Chi-Square test.

3. Patient-Reported Symptomatic Chronic GVHD Symptoms: The proportion of patients with symptomatic chronic GVHD will be estimated for each treatment with a 95% confidence interval at 1 and 2 years for the Phase II component and additionally at 3 years for the Phase III component. These proportions will be compared between groups using the Chi-Square Test.

We will also estimate the correlation between patient-reported symptoms on the Patient Survey and physician-reported symptoms on the Provider Survey.

4. Discontinuation of all Systemic Immunosuppressive Therapy: The cumulative incidence of discontinuation of all systemic IS therapy will be estimated by treatment with a 95% confidence interval at 1 and 2 years for the Phase II component and additionally at 3 years for the Phase III component. Death will be considered the competing risk. Cumulative incidence curves will be compared between groups using Gray's Test.

5. Survival and Progression-free Survival: Survival and progression-free survival from time of randomization will be computed using the Kaplan-Meier estimates along with a 95% confidence interval for each group at 1 and 2 years for the Phase II component and additionally at 3 years for the Phase III component. Survival curves will be compared across treatment using the Logrank Test.

6. Biomarkers: T regulatory cell numbers, B cell numbers, and plasma BAFF levels will be enumerated. BAFF to B cell ratios will also be computed. T reg numbers and BAFF to B cell ratios will be compared to respective values within groups using the Paired T-Test, or between groups using the Two Sample T-Test. Biomarker data will also be correlated with CR+PR status at 6 months using logistic regression.

7. NIH Consensus Clinically Relevant Adjudicated CR and PR Rates: The proportion of patients experiencing a CR or PR according to the NIH consensus definition will be estimated for each treatment with a 95% confidence interval. They will be compared between treatment arms similar to the comparison of the primary endpoint. Agreement between the NIH consensus guidelines CR/PR evaluation and the CR/PR outcome based on the primary endpoint will be computed using the Kappa statistic. As feasibility of this outcome is also of interest, the proportion of patients with complete data for this evaluation will also be estimated.

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

8. Health Quality of Life: Health quality of life will be compared between both treatment groups utilizing the FACT-BMT self report, transplant specific questionnaire and the generic quality of life tool, the SF-36. Only English speaking patients are eligible to participate in the HQL component of this trial. The HQL assessments will be performed at baseline, months two and six, then at one, two and three years after randomization. Pairwise t-tests at each time point will be used as a descriptive analysis. Reasons for missing data will be explored, and linear mixed models will be used to model QOL after accounting for data missing at random. We will also consider missing data models for QOL conditional on survival at each time point, using methods of Kurland and Heagerty.⁵⁶

5.7. Safety Analysis

The reporting of serious adverse events will be consistent with standard BMT CTN procedures. The type and severity of adverse events will be described.

APPENDIX A
HUMAN SUBJECTS

APPENDIX A

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 B
INFORMED CONSENTS

PATIENT CONSENT
PATIENT ASSENT

Informed Consent to Participate in Research



Your Name: _____

Study Title: A Phase II/III Randomized, Multi-center Trial Comparing Sirolimus plus Prednisone and Sirolimus/Calcineurin Inhibitor plus Prednisone for the Treatment of Chronic Graft-versus-Host-Disease.

Protocol: 0801

Co-Investigator: Paul Carpenter, M.B., B.S.
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Transplant Center

Investigator: _____

Sponsor: The National Institutes of Health (NIH) gave financial support for this research study through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN).

Therakos, a company that manufactures the CELLEX™ extracorporeal photopheresis device, has provided some financial support to help pay the costs of this study. This company did not plan or design this study. Therakos will not have a part in analyzing the results of this study.

Introduction

You are invited to join a research study. The primary goals of this study are to compare two treatments for chronic graft-versus-host disease (GVHD), and evaluate how well your chronic GVHD responds to treatment.

This study will be done over three years. One hundred people will participate at up to 50 clinics. Each clinic in this study will offer two study treatments. We will explain the two study treatments for your clinic in this Consent Form. Every clinic will report their results, so we can compare the two treatments at the end of the study.

The standard treatment for chronic GVHD is a corticosteroid (such as prednisone) and a calcineurin inhibitor (cyclosporine or tacrolimus). Patients who have chronic GVHD that is not controlled by the standard treatment usually have other drugs added to their treatment. Or less often, they may receive other drugs instead of standard treatment.

In this study chronic GVHD will be treated with another drug (sirolimus) in addition to either:

- A corticosteroid, or
- A corticosteroid and a calcineurin inhibitor, such as cyclosporine or tacrolimus

Sirolimus will be added to both treatments because research suggests that it may have positive effects on immune cells and improve GVHD treatment.

You will be randomly assigned to receive one of the two following treatments:

2-Drug Treatment	3-Drug Treatment
<ul style="list-style-type: none">▪ A corticosteroid (such as prednisone)▪ Sirolimus	<ul style="list-style-type: none">▪ A corticosteroid▪ A calcineurin inhibitor (cyclosporine or tacrolimus)▪ Sirolimus

This study will try to learn if it is better to add sirolimus to standard treatment or to replace a calcineurin inhibitor with sirolimus.

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

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

- Being in any research study is voluntary.
- You may or may not benefit from being in the study. Knowledge we gain from this study may benefit others.
- If you join the study, you can quit the study at any time.

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

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

1. Background

This research study is sponsored by The National Institutes of Health (NIH) through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). This study is being done to compare two treatments for chronic GVHD in people who have had an allogeneic transplant.

Chronic GVHD

Chronic GVHD is a medical condition that can become very serious. Chronic GVHD is a common development after allogeneic transplant. It happens when the donor cells attack and damage your tissues.

Chronic GVHD may cause:

- Skin rashes
- Mouth pain or dryness
- Eye dryness
- Nausea (feeling sick to your stomach) or diarrhea
- Liver inflammation
- Damage to other organs, such as the lungs, throat, and vagina

Treatments for chronic GVHD

Corticosteroid (or steroid) medications are the most basic treatment for chronic GVHD. However, less than half of patients will become free of chronic GVHD when steroid treatment is used alone. Several other drugs are often added to steroids to help treat chronic GVHD, including a calcineurin inhibitor (cyclosporine or tacrolimus) and/or sirolimus.

Information from earlier research suggests that different combinations of sirolimus, cyclosporine or tacrolimus, and steroids may help to reduce chronic GVHD.

Sirolimus, cyclosporine and tacrolimus all work by blocking the growth of new immune cells that can cause chronic GVHD. Research suggests that sirolimus also supports the growth of other immune cells called “T-reg” which limit the immune response that causes GVHD.

Cyclosporine and tacrolimus may slow the growth of these immune cells. Sirolimus, cyclosporine and tacrolimus are approved by the U.S. Food and Drug Administration (FDA) to prevent rejection after organ transplantation. They have been used for years as treatments for GVHD that do not respond to standard steroid therapy, or to prevent GVHD after allogeneic transplant.

2. Purpose

You are invited to join this research study because you have chronic GVHD that needs treatment.

The primary goals of this study are to compare two treatments for chronic GVHD and evaluate how well your chronic GVHD responds to your treatment. We will also collect extra blood samples for future research on GVHD.

3. Right to Ask Questions and/or Withdraw

You have the right to ask questions about the study at any time. If you have questions about your rights as a participant or you want to leave the study, please contact:
[Insert contact info]

Being in this study is voluntary. You can choose to not be in this study, or leave this study at any time.

If you choose to not take part or to leave this study, your regular medical care will not be affected in any way. This includes standard care for your chronic GVHD. If you decide to leave this study after taking the study treatment, or are asked to leave by your doctor for medical reasons, you will be asked to come back to the doctor's office for tests for your safety.

Even if you withdraw from the study, the information collected from your participation will be included in the study evaluation, unless you specifically ask that it not be included.

Your study doctor and study staff will be available to answer any questions that you may have about your participation in, or your withdrawal from this study.

4. Procedures

Your study participation will last for 3 years, and includes your treatment and follow-up. One hundred patients will participate at up to 50 clinics around the country.

The number of required clinic visits during this time will depend on your treatment and the combination of drugs you receive. You will need to have health evaluations before you start treatment and for several years after you finish your treatment. These tests and examinations are

standard care for patients with chronic GVHD and would be done even if you were not part of this study.

One of the objectives of this study is to evaluate how the treatment affects the quality of the patient's life and whether there is a difference between the treatment arms. Therefore, all patients will be asked to complete questionnaires asking about their chronic GVHD symptoms and quality of life at study entry, 2, 6, 12, 24 and 36 months. It will take you approximately half an hour to complete the questionnaires.

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

Before You Start Your Treatment

Before you join the study, we will evaluate your general health, medical history, and your current medications. It is important that you do not participate in the study if you suffer from an allergy to any of the drugs used in this study; or if you are pregnant, breastfeeding or are likely to become pregnant during the study.

You will have at least one clinic visit before you start your treatment for this study. This visit will collect information about your:

- Physical health (including history, height, weight and temperature)
- Comprehensive Chronic GVHD assessment [to include provider survey, patient survey, 2 minute walk (optional), grip test (optional) and Schirmer's eye exam (optional)]
- Lung function
- Cancer re-staging (if appropriate)
- Organs affected by GVHD
- Routine blood tests, including cell counts, cholesterol, liver and kidney function, and levels of GVHD treatment medications in your blood (if it applies to you)
- Blood samples (4-5 teaspoons, or 3-4 teaspoons for patients under 90 pounds) for protocol-defined GVHD studies
- Pregnancy test (if applicable)

Study Participation

You will receive either the 2-drug treatment or the 3-drug treatment.

If you are assigned to the 2-drug treatment, you will receive:

2-Drug Treatment
<ul style="list-style-type: none">▪ <u>Prednisone</u>: once each day until 2 weeks improvement in GVHD symptoms is observed, and then slowly lowered and then stopped▪ <u>Sirolimus</u>: once each day until all prednisone has stopped. After up to 3 months of stable improvement is observed, the amount of sirolimus will be slowly lowered and then stopped

If you are assigned to the 3-drug treatment, you will receive:

3-Drug Treatment
<ul style="list-style-type: none">▪ <u>Prednisone</u>: once each day until 2 weeks improvement in GVHD symptoms is observed, and then slowly lowered and then stopped.▪ <u>Sirolimus</u>: once each day.▪ <u>CNI</u>: two times each day.

Depending on how well you respond to the treatment, you may need to visit the clinic every week for another 24 weeks. Your visits may drop to once each month, if your doctor feels it is appropriate. You may need to take your assigned treatment for up to two years. Your doctor will lower the amount of drugs you will need to take, as your chronic GVHD symptoms get better.

Once you stop taking your assigned treatment, the study team will follow your health for up to 2 years. Your total length of study participation may last up to 3 years, including follow-up once you finish your treatment.

Study Evaluations

We will evaluate your health at specific points during your study participation:

1. History, physical exam, and weight at 2, 3, 6, 12, 24 and 36 months.
2. Comprehensive Chronic GVHD assessment [to include provider survey, patient survey, 2 minute walk (optional), grip test (optional) and Schirmer's eye exam(optional)] to measure your response to treatment at 2, 6, 12, 24 and 36 months.
3. Routine blood tests, including: cell counts, and liver and kidney function, at least weekly for the first 4 weeks.
4. Cholesterol tests at 1, 2, 3, 6, and 12 months.
5. Lung (pulmonary) function tests at 3, 6 and 12 months.
6. Blood tests to check sirolimus levels will be done at least every week for the first 4 weeks and potentially twice a week if you are taking cyclosporine or tacrolimus.

Sirolimus levels from Week 5 to the end of your treatment are then done weekly to monthly, depending on your treatment.

7. Blood tests to check cyclosporine or tacrolimus levels are done at least weekly for the first 4 weeks and then at least monthly after Week 5 based on your treatment.
8. Blood samples (4-5 teaspoons, or 3-4 teaspoons for patients under 90 pounds) for protocol-defined GVHD studies at 2 months and 6 months or when any new oral or injected medication is added to treat your GVHD.

Randomization

A computer will randomly assign you to receive either the standard treatment or the study treatment. This means that you will be put into a group by chance. It is like flipping a coin or drawing names out of a hat. You will have an equal chance of being chosen for either treatment.

Additional Health Evaluations and Procedures

Before and after photographs may be taken to document your chronic GVHD.

5. Alternative Treatments

Current available treatments which may be used to treat graft-versus-host disease (GVHD) include:

- Corticosteroids (prednisone) with or without cyclosporine or tacrolimus (standard treatment for GVHD)
- Other medications
- Participation in another clinical trial (if available, check with your doctor)

Every treatment option has benefits and risks. Your study doctor will discuss these options with you. If you decide not to participate in this research study, your medical care will not be affected in any way.

6. Risks and Discomforts

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

a.) Side Effects of Study Drugs and Treatments

All drugs can have side effects, including the standard therapy for GVHD (steroids) and the other drugs being tested in this study. Your doctor will watch you carefully and will change your treatment if side effects develop.

Please see Table: Risks and Side Effects of Study Drugs and Treatments at the end of this section for more information on the drugs used in the study treatments.

b.) Infections

Because chronic GVHD is caused by an immune attack on your tissues from the transplanted donor cells, all treatments for chronic GVHD include drugs to control (suppress) that immune attack. There is a higher risk of infection in patients with chronic GVHD and in people who take steroids like prednisone or methylprednisolone, the standard therapy for chronic GVHD.

You will need to take several antibiotics to prevent infection. You will also be watched carefully for any infections while you are being treated for chronic GVHD. Tell your doctors promptly if you get a fever, chills, cough or any other symptoms that might be a sign of an infection.

c.) Risks of Other Procedures Including Blood Draws

There are no major risks associated with drawing blood. Having your blood drawn can be uncomfortable and can sometimes cause a bruise. In rare cases, a blood draw can cause fainting. Only trained people will draw your blood.

d.) Reproductive Risks

The drugs used in this research study, including sirolimus and CNIs may cause injury or birth defects if you take them during pregnancy. Because of this, it is important that you are not pregnant or breast-feeding and do not become pregnant during the course of the study.

If you are a woman and pregnancy is a possibility, you will need to take a pregnancy test before you start the study. You should discuss ways to not become pregnant while you are participating on the study.

e.) Unforeseen Risks

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

f.) Other Treatments or Medications

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

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

Table: Risks and Side Effects of Study Drugs and Treatments

	Prednisone	Sirolimus	Calcineurin Inhibitor (Cyclosporine or Tacrolimus)
Common (more than 20% of patients)	<ul style="list-style-type: none"> ▪ Fluid retention and bloating ▪ Problems with the liver, pancreas or adrenal glands ▪ High blood pressure ▪ Enlarged heart ▪ Muscle wasting ▪ Thinning of bones ▪ Torn tendons ▪ Stomach ulcers ▪ Slow wound healing ▪ Thin fragile skin ▪ Broken blood vessels in the skin, esp. face ▪ Diabetes ▪ Slowed growth in children ▪ Cataracts ▪ Mood swings or emotional changes ▪ Insomnia 	<ul style="list-style-type: none"> ▪ High blood pressure ▪ Nausea (feeling sick to your stomach) ▪ Diarrhea and/or constipation ▪ Infection ▪ Fever ▪ Liver or kidney problems ▪ Weight gain ▪ Muscle pain ▪ High cholesterol ▪ Acne 	<ul style="list-style-type: none"> ▪ Headache and/or dizziness ▪ Uncontrollable shaking of a part of the body ▪ Diarrhea and/or constipation ▪ Nausea and/or vomiting ▪ Heartburn ▪ Stomach pain ▪ Loss of appetite ▪ Difficulty falling asleep and/or staying asleep ▪ Weakness ▪ Back and/or joint pain ▪ Burning, numbness, pain, or tingling in the hands or feet ▪ Rash ▪ Itching

Table: Risks and Side Effects of Study Drugs and Treatments, continued

	Prednisone	Sirolimus	Calcineurin Inhibitor (tacrolimus or cyclosporine)
Less Common (less than 20% of patients)		<ul style="list-style-type: none"> ▪ Chest pain ▪ Insomnia (unable to sleep) ▪ Upset stomach or vomiting ▪ Shortness of breath ▪ Low blood counts ▪ Skin rashes or hives ▪ Slow wound healing 	
Rare but Serious (less than 2-3% of patients)		<ul style="list-style-type: none"> ▪ Low blood pressure ▪ Lung problems, including asthma ▪ Loss of appetite ▪ Serious infections ▪ Blood clots ▪ Skin problems ▪ Kidney failure ▪ Secondary cancers ▪ Bone degeneration (necrosis) 	<ul style="list-style-type: none"> ▪ Decreased urination ▪ Pain or burning on urination ▪ Swelling of the arms, hands, feet, ankles, or lower legs ▪ Weight gain ▪ Unusual bleeding or bruising ▪ Seizures ▪ Coma (loss of consciousness for a period of time)

7. Possible Benefits

Taking part in this study may or may not make your health better.

We do know that the information from this study will help doctors learn more about possible therapies for the treatment of chronic GVHD. This information could help future allogeneic transplant patients.

New Information Available During the Study

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

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

8. Privacy, Confidentiality and Use of Information

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

Your personal information may be given out if required by law. If information from this study is published or presented at scientific meetings, your name and other personal information will not be used. Your study number is not related to your name, social security number or medical record number at [insert facility name].

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

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

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

9. Ending Your Participation

The study doctor or the study sponsor may stop the study at any time. We may ask you to leave the study if you do not follow directions or if you suffer from side-effects of the treatment. If you are asked to leave the study, the reasons will be discussed with you.

Possible reasons to end your participation in this study include:

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

- The study is stopped for any reason.

10. Physical Injury as a Result of Participation

It is important that you tell your study doctor or study staff if you feel that you have been hurt or injured because of taking part in this study.

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

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

11. Compensation or Payment

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

12. Costs & Reimbursements

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

13. Ethical Review

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

14. Further Information

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

[Insert name and contact details]

15. Independent Contact

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

[Insert appropriate contact details]

16. Web Information

A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>, as required by U.S. Law. This web site will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site at any time.

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

A. Purpose

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

A Phase II/III Randomized, Multi-center Trial Comparing Sirolimus plus Prednisone and Sirolimus/Calcineurin Inhibitor plus Prednisone for the Treatment of Chronic Graft-versus-Host-Disease.

B. Individual Health Information to be Used or Disclosed

My individual health information that may be used or disclosed to do this research includes:

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

C. Parties Who May Disclose My Individual Health Information

The researcher and the researcher's staff may collect my individual health information from: (List hospitals, clinics or providers from which health care information can be requested).

D. Parties Who May Receive or Use My Individual Health Information

¹ HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of 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 Investigator and the researcher's staff
Paul Carpenter, M.B., B.S., Co-Investigator
Mutka Arora, M.D., Co-Investigator
- Study Sponsors
National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI), both of the National Institutes of Health (NIH); Blood and Marrow Transplant Clinical Trials Network (BMT CTN)
- U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)
- U.S. government agencies that are responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments.

E. Right to Refuse to Sign this Authorization

I do not have to sign this authorization. If I decide not to sign the authorization, I will not be allowed to participate in this study or receive any treatment related to research that is provided through the study.

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

F. Right to Revoke

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

If I withdraw this authorization, 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. This authorization does not have an expiration date.

Blood and DNA Samples for Research

Please note: This section of the Informed Consent Form is about an additional research study that will be done with people who are taking part in the main study. You may take

part in this additional study if you want to. You can still be a part of the main study even if you say ‘no’ to take part in this additional study.

Your blood and cells have a material known as DNA. This is a molecule that holds a person's genetic information.

We ask for your permission to collect and store extra blood and DNA. These samples will be used for future research.

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

Genome-wide association studies are a way for scientists to identify genes involved in human disease. This method searches the genome for small genetic changes that are more common in people with a particular disease than in people without the disease. Each study can look at hundreds of thousands of genetic changes at the same time. Researchers use data from this type of study to find genes that may add to a person's risk of developing a certain disease.

If your coded genetic and clinical information is used in such a study, the researcher is required to add the DNA test results and non-identifying information into a public research database. This public database is called the NIH Genotype and Phenotype Database and it is managed by the National Center for Biotechnology Information (NCBI). The NCBI will never have any information that would identify you, or link you to your information or research samples.

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

Procedures

- We will collect extra blood samples at the same time you have routine blood tests done on 3 study visits; before you start treatment (baseline), 2 months and 6 months.
- The amount of blood collected from you is small – about 2 teaspoons (10mL) each time.
- We will also collect samples of your DNA by swabbing four sections of the inside of your cheeks with cotton swabs.
- Your blood and DNA samples will be collected confidentially and your name will not be on the containers.
- These samples may be stored indefinitely for future research.

Withdrawal

If you agree to allow your blood and DNA samples to be used for research, you can change your mind at any time. If you change your mind, please contact [the Principal Investigator at your transplant center] in writing to state that you are withdrawing permission for your blood to be used for research. His/her mailing address is on the first page of this Consent Form. Any unused samples will be destroyed if you withdraw your permission. **If you choose not to participate in this additional research there will be no change in your care.**

Benefits

You will not benefit directly from providing blood and DNA samples for this study.

Risks

There are no major risks associated with drawing blood. Having your blood drawn can be uncomfortable and can sometimes cause a bruise. In rare cases, it can cause fainting. Only trained people will draw your blood. There are no major risks with swabbing the inside of your cheeks.

Confidentiality and Your Medical Information

The results of GVHD research done with your blood and DNA will not be part of your medical record and will not be shared with you.

If you agree to allow your blood and DNA samples to be used for research, they will be collected confidentially and your name will not be on the tubes. Only the study doctors or staff working with them will study the results from your blood and DNA samples.

Information gained from research on your blood and DNA may be used to develop diagnostic procedures or new treatments for GVHD in the future. Your blood will not be sold to any person, institution, or company for financial gain or profit.

Making Your Choice

Please read each sentence below and think about your choice. After reading each sentence, make your selection by checking one of the boxes. If you have any questions, please talk to your doctor or nurse, or call our research review board at [IRB's phone number].

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

I agree to allow my blood and DNA to be used for future research.

I do not agree to allow my blood and DNA to be used for future research.

Signature

Date

TITLE: A Phase II/III Randomized, Multi-center Trial Comparing Sirolimus plus Prednisone and Sirolimus/Calcineurin Inhibitor plus Prednisone for the Treatment of Chronic Graft-versus-Host-Disease.

Participant Name

Date

Participant Signature

Date

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

Counseling Physician

Date

Signature of Counseling Physician

Date

Assent to Participate in Research



Study Title: A Phase II/III Randomized, Multi-center Trial Comparing Sirolimus plus Prednisone and Sirolimus/Calcineurin Inhibitor plus Prednisone for the Treatment of Chronic Graft-versus-Host-Disease.

Protocol: 0801

A. Why am I here?

We are inviting you to join our study because you have chronic graft-versus-host disease (GVHD).

Chronic GVHD happens when donor cells from your transplant attack parts of the body like your skin, stomach or liver. Both children and adults can get chronic GVHD. It can be a very serious problem for some people.

B. Why are you doing this study?

We want to compare two ways to treat chronic GVHD. This will help us learn which may be a better treatment.

C. What will happen to me?

If you say you want to be in the study, we will ask you for several things:

- Permission to let us read your medical records and x-rays.
- Check-ups with the study doctors.
- Some blood from you (about 3-5 teaspoons). A very small needle will be used to get blood.
- Answer some questions about how you feel.

You will be assigned to have either Treatment #1 or Treatment #2. The doctors will use a computer to decide who goes into each group. Each treatment uses a different mix of drugs.

Treatment #1

- A steroid
- Cyclosporine or tacrolimus
- Sirolimus

Treatment #2

- A steroid
- Sirolimus

We will watch you carefully for fevers, any sign of infection or other problems.

D. Will it hurt?

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

E. Will the study help me?

We don't know if the study will help you or not. Your GVHD may stay the same, it may get better, or it may get worse.

F. What if I have questions?

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

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

G. Do I have to be in this study?

If you don't want to be in the study, you need to tell us and your parent or guardian. Your doctor will not be angry or upset if you don't want to join.

Whether you are in the study or not, you will still need to have treatment for your GVHD.

You can say yes now and change your mind at any time.

Please talk this over with your parent or guardian before you decide if you want to be in the study. We will also ask your parents to give their permission for you to join this study.

Use of Blood and DNA Samples

Some of your blood will be used to test for GVHD. Additional blood (1-2 teaspoons) and a sample of cells from inside your cheek (DNA) will be collected and saved for future studies. Your blood has a material known as DNA. DNA is a molecule that holds your genetic information. We may study your blood and your DNA in future GVHD research.

To be in the study, you must agree to have your blood used for GVHD and other regular tests, but you do not have to agree to have your blood or cheek cells (DNA) used in future research.

Please check one of the boxes below to show how you want your blood and cheek cells (DNA) to be used.

Yes, you may use my blood and cheek cell (DNA) samples for future research.

No, you may not use my blood and cheek cell (DNA) samples for future research.

Writing your name on this page means that you agree to be in the study, and know what will happen to you. If you decide to quit the study, all you have to do is tell the person in charge.

You and your parent or guardian will get a copy of this form after you sign it.

Signature of Participant's Guardian

Date

Printed Name of Participant's Guardian

Signature of Participant (if 18 years old or older)

Date

Printed Name of Participant

Signature of Researcher

Date

Printed Name of Researcher

APPENDIX C
LABORATORY SAMPLE INFORMATION

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LABORATORY SAMPLE INFORMATION

BIOMARKER BLOOD SAMPLES

Peripheral blood samples (18 mL) will be collected from the patient for T-cell and B-cell immunophenotyping and plasma BAFF levels, at baseline prior to initiation of treatment for cGVHD, at the 2 months and 6 months post-treatment time points, and at the initiation of secondary systemic immunosuppressive therapy. Additional biomarkers of interest may also be evaluated that might be identified via other research reported during the period that this study is conducted. Optional patient buccal swabs and peripheral blood samples (10 mL, or 6 mL for patients < 40 kg) may be collected and stored for the future undefined testing of biomarkers related to cGVHD. These samples should be collected and shipped according to the detailed instruction in the BMT CTN 0801 Laboratory Sample Guide.

SCHEDULE OF LABORATORY EVALUATIONS
Protocol-Defined Research Testing and for Future, Undefined Research

Protocol-Defined cGVHD Assessments				
Research Sample	Type of Sample	Type of Processing and Storage	Dates Samples Obtained	Shipping Specifications
BAFF Levels by ELISA	6 mL peripheral blood collected in a 6 mL fill EDTA lavender-top blood tube.	Centrifuge the EDTA containing whole blood samples at 1000-1300 x g or 2100 rpm for 10 minutes within 60 minutes of collection. Remove the separated plasma. Transfer 0.5 mL aliquots to 4-6 cryovials (depending on volume of plasma recovered) and freeze at -70°C in a scientific grade freezer.	At baseline prior to initiation of treatment for cGVHD, and on Day 60 and Day 180 [or upon initiation of secondary systemic immunosuppressive therapy (whichever is sooner)].	Batch ship yearly, frozen on dry ice in a provided shipping container, FedEx priority overnight to the BMT CTN Research Sample Repository.
Regulatory T-cell and B-cell Immunophenotyping	12 mL peripheral blood samples will be collected in 6 mL fill EDTA lavender top blood tubes.	No processing or storage required at transplant center.	At baseline prior to initiation of treatment for cGVHD, and on Day 60 and Day 180 or upon initiation of secondary systemic immunosuppressive therapy (whichever is sooner)].	Ship blood tubes directly to designated project lab (DFCI) on day of collection by priority overnight FedEx.

Optional Research Samples for Future Undefined Research				
Research Sample	Type of Sample	Type of Processing and Storage	Dates Samples Obtained	Shipping Specifications
Baseline, Pre-Treatment Patient Plasma & Patient Whole Blood (Source of Donor DN A)	10 mL peripheral blood (6 mL for patients < 40 kg) collected in an EDTA lavender-top tube.	Within 60 minutes of collection, gently mix the 10 mL blood tube by gently inverting the tube for 60 seconds. Pipette a 1.0 mL aliquot of the whole blood into each of 3 cryovials and freeze at -70°C in a scientific grade freezer (make three 0.5 mL whole blood aliquots if the alternate 6 mL tube was used). Centrifuge the remaining whole blood (7 mL) in the EDTA tube (4.5 mL if 6 mL fill blood tube used) at 1000-1300 x g or 2100 rpm for 10 minutes. Remove the separated plasma. Transfer 0.5 mL aliquots to 3-7 cryovials (depending on volume of plasma recovered) and freeze at -70°C in a scientific grade freezer.	At baseline prior to initiation of treatment for cGVHD.	Batch ship yearly, frozen on dry ice in a provided shipping container, FedEx priority overnight to the BMT CTN Research Sample Repository.
Baseline, Pre-Treatment Patient Buccal Swab (Patient DNA)	4 buccal swabs	Transplant centers will have the patient collect buccal cell samples per instructions. The 4 swabs should be dried for a minimum of 2 hours before they are processed for long-term frozen storage. Each dried swab is placed into a separate cryovial (4 cryovials), the stick cut so that the cotton swab tip fits well within the cryovial. Secure the cryovial cap. Cryovials will need to be promptly stored at -70°C in a scientific grade freezer until batch-shipped to the BMT CTN Research Sample Repository.	At baseline prior to initiation of treatment for cGVHD.	Batch ship yearly, frozen on dry ice in a provided shipping container, FedEx priority overnight to the BMT CTN Research Sample Repository.
Post-Treatment Patient Plasma Samples	10 mL peripheral blood (6 mL for patients < 40 kg) collected in an EDTA lavender-top tube.	Within 60 minutes of collection, centrifuge the EDTA containing whole blood tube at 1000-1300 x g or 2100 rpm for 10 minutes. Remove the separated plasma. Transfer 0.5 mL aliquots to 4-10 cryovials (depending on volume of blood processed and plasma recovered) and freeze at -70°C in a scientific grade freezer.	Day 60 and Day 180	Batch ship yearly, frozen on dry ice in a provided shipping container, FedEx priority overnight to the BMT CTN Research Sample Repository.

APPENDIX D

DERIVATION OF A SEQUENTIAL TEST STATISTIC FOR CENSORED EXPONENTIAL DATA

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Background – The Sequential Probability Ratio Test

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

The usual measures of performance of such a procedure are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$, and of accepting H_0 when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta_j) \equiv E_j(N)$. Wald and Wolfowitz showed that among all tests, sequential or not, for which $\Pr_0(\text{reject } H_0) \leq \alpha$ and $\Pr_1(\text{reject } H_0) \leq \beta$, and for which $E_j(N)$ are finite, $j=0,1$, the SPRT with error probabilities α and β minimizes $E_0(N)$ and $E_1(N)$. If, in addition, the x_1, x_2, \dots are independent and identically distributed (i.i.d.) with density function $f(x, \theta)$, with monotone likelihood ratio in $\tau(x)$, then any SPRT for testing θ_0 against $\theta_1 (> \theta_0)$ has non-decreasing power function.

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

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

Derivation of the SPRT for Uncensored Exponential Survival Times

For example, we wish to construct a sequential test for the composite null hypothesis that the rate of treatment-related mortality (TRM) at 100 days is less than or equal to 30% versus the alternative hypothesis that it is greater than or equal to 50%. For the derivation of the uncensored SPRT, we will require that the type I error of the test be less than 5%, and that the test provide 80% power to reject the null hypothesis under a specified alternative that the true rate is 50%. A maximum sample size of 50 patients will be permitted.

Let us assume that the survival times, T_1, T_2, \dots, T_n , are completely observed (uncensored) and are i.i.d. with exponential density function $f(T, \theta) = \theta e^{-\theta T}$. These assumptions will be relaxed to incompletely observed data subsequently. In the exponential parameterization, a 100-day survival rate of 70% translates into a mean survival of 0.768 years ($\theta_0 = 1.303$), and 50% translates into a mean survival of 0.395 years ($\theta_1 = 2.532$).

The SPRT is derived with reference to a simple null and alternative hypothesis, in this case, $H_0 : \theta = \theta_0 = 1.303$ versus $H_1 : \theta = \theta_1 = 2.532$. However, since the log-likelihood ratio for the exponential, $\log \prod_i^n f(x_i; \theta_1) - \log \prod_i^n f(x_i, \theta_0) = n(\log(\theta_1) - \log(\theta_0)) - (\theta_1 - \theta_0) \sum_i^n T_i$, is a monotone function of $\sum_i^n T_i$, the power of the test is non-decreasing in θ . Thus the SPRT is a one-sided level .05 test of a composite null ($H_0 : \theta \leq \theta_0 = 1.303$) versus a composite alternative ($H_1 : \theta \geq \theta_1 = 2.532$), with power of $1 - \beta = .80$ at the selected alternative $\theta = \theta_1 = 2.532$.

The SPRT can be represented graphically. The continuation region is bounded by two parallel lines with common slope $(\log \theta_0 - \log \theta_1) / (\theta_0 - \theta_1) = 0.541$, and intercepts $\log A / (\theta_0 - \theta_1) = -2.256$ and $\log B / (\theta_0 - \theta_1) = 1.270$, for the lower and upper bounds, respectively. As each individual unit is put on trial and observed to fail, the cumulative sum of failure times, $\sum_i^n T_i$, is recomputed, and plotted against the current sample size, n . When this graph crosses the lower boundary, the null hypothesis is rejected.

The maximum sample size of 50 patients requires that the SPRT be truncated. We choose to truncate the SPRT by declaring that if the test has failed to terminate after 50 patients, that the null hypothesis will be accepted. Since the probability that the untruncated SPRT would reject the null at a sample size of 50 is negligible, it makes little difference how the final boundary value is selected, and this rule is chosen for simplicity.

Derivation of a Modified SPRT for Censored Exponential Data

The assumption of uncensored exponential survival times is flawed. However, we consider it reasonable to assume the hazard for TRM is constant over the first 100 days post-transplant, and

we will restrict our attention to this time interval. Furthermore, it is not practical to conduct a clinical study by putting each individual on trial, and waiting until that individual is observed to fail. We relax our assumptions as follows. Firstly, each individual's time on study will be computed as time from transplant to failure, or to the 100 day time point, whichever comes first. Secondly, we will put individuals on trial as soon as they become available, without waiting for the previous individual to fail.

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

Now we relax the second assumption, and put individuals on trial as soon as they become available, without waiting for the previous individual to fail. Assume that three years are required for accrual of 50 patients to the study, and that the final analysis takes place 100 days after the last patient is entered. Putting all of this together, we propose a modified truncated SPRT, where at any interim time point, s , ranging from 0 to 3 years 100 days, the sum of observed time on study, $\sum_i^n X_i(s)$ is plotted against the number of observed failures, $d(s)$. In practice, monitoring will be scheduled monthly after the start of enrollment to the study. A further modification to the SPRT was to only use the lower boundary for stopping since the primary focus of the monitoring is to protect against unacceptable 100-day TRM rates.

Operating Characteristics of the Modified SPRT Test for Censored Exponential Data

Recall that the uncensored SPRT targeted a drop in survival at Day 100 from 70% to 50%, with type I and II errors of 5% and 20%. Since only the lower boundary is used for monitoring, the continuation region of the test was bounded below by a line with a slope of 0.541 and intercept of -2.256. The effect of truncation is to reduce the power of the test. In order to compensate for this, we raise the lower boundary to make it easier to cross. Under the further assumption of uniform accrual over a three year period, and monthly interim analyses over the course of the study, the operating characteristics of the modified SPRT were obtained from a simulation study. These simulation show that an intercept of -1.741, corresponding to setting parameters α and β to 10% and 15%, result in empirical type I and II error rates of about 5% and 20%.

Table D-1 Operating Characteristics of Sequential Testing Procedures from a Simulation Study with 100,000 Replications**Treatment-Related Mortality (TRM)**

True 100-Day Rate	30%	35%	40%	45%	50%
Probability Reject Null	0.07	0.20	0.41	0.66	0.86
Mean Month Stopped	34.5	32.3	28.5	23.5	18.5
Mean # Endpoints in 100 Days	13.8	15.0	15.1	14.0	12.1
Mean # Patients Enrolled	48	45	40	33	26

While the motivation for this testing procedure is largely heuristic rather than theoretical, the simulation results validate the approach. When the true rate of TRM on or before Day 100 was 30%, the test crossed the lower boundary in 7119 of 100,000 replications, for an estimated type I error rate of 7%. When the true rate of TRM on or before Day 100 was 50%, the test failed to cross the boundary in 14226 of 100,000 replications, for an estimated type II error rate of 14%. And on average, the boundary will be crossed at 18.5 months, when 26 patients will be enrolled to the study.

It is interesting to note that the SPRT derived above for exponential failure times with censoring at 100 days, has operating characteristics which are similar to those of a more traditional SPRT, derived for binomial variates with success probability equal to the 100 day failure rate. Using time to failure rather than a simple binary indicator of failure, leads to little improvement in power when failure times are censored relatively soon after entry on study. We speculate that if the constant hazard rate over the first 100 days were high, the exponential test would reject faster than the binomial test, but have not conducted simulation studies to demonstrate this.

APPENDIX E

END OF PHASE II DECISION MAKING CONSIDERATIONS

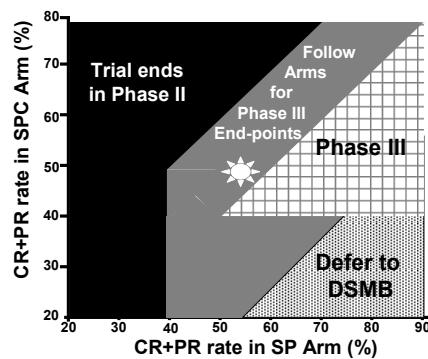
APPENDIX E

END OF PHASE II DECISION MAKING CONSIDERATIONS

The adaptive Phase II/III design of Protocol 0801 allows efficient transition from Phase II to Phase III, given certain outcomes in the Phase II stage of the trial. The Phase II stage may result in a variety of outcomes, not all of which would favor proceeding directly to the Phase III as written. This Appendix outlines the principles that will be used to determine how to proceed at the end of Phase II. Overall response rates (CR+PR) at 6 months for the experimental arm (SP) will be plotted (see examples plotted as * below) relative to the respective comparator arm (SPC) as shown in the figures below. Assumptions include: 1) the overall response rate for SPC is about 40%; and, 2) overall response rates in the experimental arm (SP) < 40% are not of interest to pursue further.

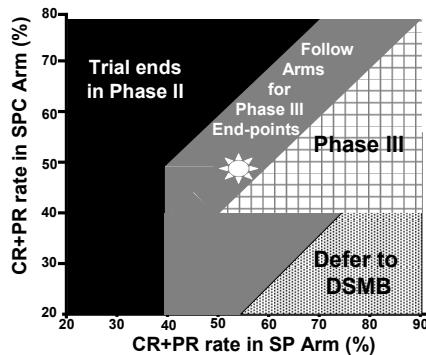
Scenario 1: The Phase III trial as written is not worth pursuing

SP is inferior to comparator (SPC) and the trial ends at Phase II.



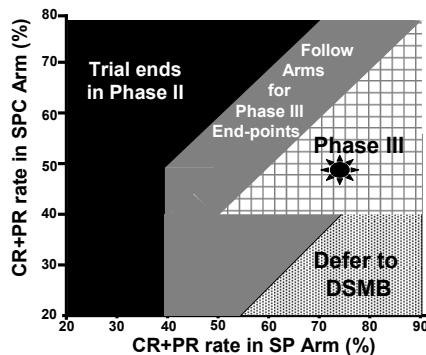
Scenario 2: The Phase II study is possibly worth pursuing after further follow-up

The SP arm might be of sufficient interest to continue into a future Phase III trial but Phase II short term outcome data does not warrant proceeding directly to Phase III. In this scenario, both arms would have similar CR+PR rates higher than the estimated 40% benchmark for the SPC arm. In this situation, how to proceed may require more information on longer term endpoints. Considerations would also include, in rank order, toxicity, convenience and cost of both study arms, as well as BMT CTN priorities and other available therapies at the time.



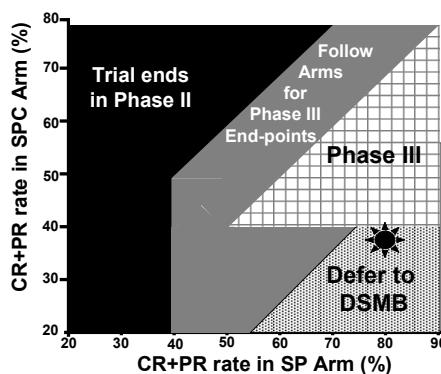
Scenario 3: Proceed to Phase III as written

This is a scenario in which the experimental arm (SP) is superior to the comparator (SPC) and has a CR+PR rate of at least 40%.



Scenario 4: Experimental Arm (SP) is substantially superior ($\geq 35\%$) to SPC

In this example, while the comparator arm (SPC) exceeds the 40% benchmark, the experimental arm (SP) is considered sufficiently more superior over SPC that proceeding directly to Phase III (SP versus SPC) may not be justified. The DSMB would advise whether SP and SPC should be tested head-to-head in Phase III or if SP should be tested against a novel therapy of interest. Considerations would also include, in rank order, toxicity, convenience and cost of all study arms, as well as BMT CTN priorities and other available therapies at the time.



APPENDIX F

REFERENCES

APPENDIX F

REFERENCES

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