BLOOD AND MARROW TRANSPLANT CLINICAL TRIALS NETWORK

# Initial Systemic Treatment of Acute GVHD: A Phase II Randomized Trial Evaluating Etanercept, Mycophenolate Mofetil (MMF), Denileukin Diftitox (ONTAK), and Pentostatin in Combination with Corticosteroids

# BMT CTN PROTOCOL 0302
# VERSION 7.0


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- University Hospitals of Cleveland
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# PROTOCOL SYNOPSIS – BMT CTN PROTOCOL 0302

## Initial Systemic Treatment of Acute GVHD: A Phase II Randomized Trial Evaluating Etanercept, Mycophenolate Mofetil (MMF), Denileukin Diftitox (ONTAK) and Pentostatin in Addition to Corticosteroids

**Principal Investigator:** Daniel Weisdorf, M.D.

**Study Design:** The study is a randomized Phase II, four arm treatment trial. The primary purpose of the study is to define new agents with promising activity against acute GVHD suitable for testing against corticosteroids alone in a subsequent Phase III trial.

Corticosteroids have been used as primary therapy for acute GVHD for many years. Historical published and unpublished data from the University of Minnesota, Dana Farber Cancer Institute and the Fred Hutchinson Cancer Center define an expected 35% complete response (CR) at Day +28 of corticosteroid therapy for previously untreated patients with acute GVHD.

In this trial, patients with newly diagnosed acute GVHD will receive corticosteroids plus one of four new agents. A control arm of only corticosteroids will not be employed. Each agent will be assessed for safety (stopping rules defined) and efficacy (≥ 35% CR rate at Day 28 of therapy).

**Primary Objective:** The primary objective is to estimate the proportion of CR at Day 28 of therapy for newly diagnosed acute GVHD for each of these agents given in combination with corticosteroids.

**Secondary Objectives:** Secondary objectives are to determine: proportion of partial response (PR), mixed response and progression at Day 28; proportion of treatment failure (no response, progression, administration of additional therapy for GVHD, or mortality) by Day 14; the incidence of GVHD flares requiring increasing therapy before Day 90. In addition, the following endpoints will be examined: incidence of discontinuation of immune suppression without flare by Days 90, 180 and 270 post-therapy, incidence of chronic GVHD by 9 months, overall survival at 6 and 9 months post initiation of therapy, incidence of systemic infections within 3 months of initiation of therapy, incidence of EBV-associated lymphoma**.**

**Eligibility:** Patients must be greater than or equal to 6 years of age and have acute GVHD requiring systemic therapy. No previous systemic immune suppressive therapy for acute GVHD is allowed except for a maximum 48 hours of prior corticosteroid therapy (≥ 1 mg/kg/day methylprednisolone). Patients receiving ONTAK, pentostatin, or etanercept within 7 days of screening will be excluded. Patients receiving MMF for GVHD prophylaxis will be randomized into one of the other three treatment arms. Patients must have an absolute neutrophil

count (ANC) greater than 500/μL and an estimated creatinine clearance greater than 30 mL/minute.

**Treatment Description:** The treatment schedules are as follows: **Etanercept** [25 mg subcutaneously twice weekly for up to 4 weeks; discontinue if in CR by 4 weeks]. Mycophenolate mofetil (**MMF)** [20 mg/kg (maximum 1 gm) PO or IV BID; continue through prednisone taper, then taper MMF over 4 weeks]. Denileukin Diftitox (**ONTAK®)** [9 mcg/kg IV Days 1, 3, 5, 15, 17, 19]. **Pentostatin** [1.5 mg/m$^2$ daily for 3 days; Days 1-3 and repeat Days 15-17].

All patients will receive methylprednisolone 2 mg/kg/day IV (or prednisone 2.5 mg/kg/day PO) divided in 2-3 daily doses for at least 7 days. Prednisone may be tapered as tolerated to no less than 0.75 mg/kg/day (methylprednisolone 0.6 mg/kg/day) at Day 28 of therapy. If not in CR at Day 28, patients will be followed for study endpoints, but assigned therapy can be continued or changed per institutional guidelines.

In addition to prescribed study drug plus corticosteroids, all patients should receive transfusion support per institutional practice; anti-infective prophylaxis directed towards CMV, gram positive (encapsulated) bacteria, pneumocystis carinii and fungal infections

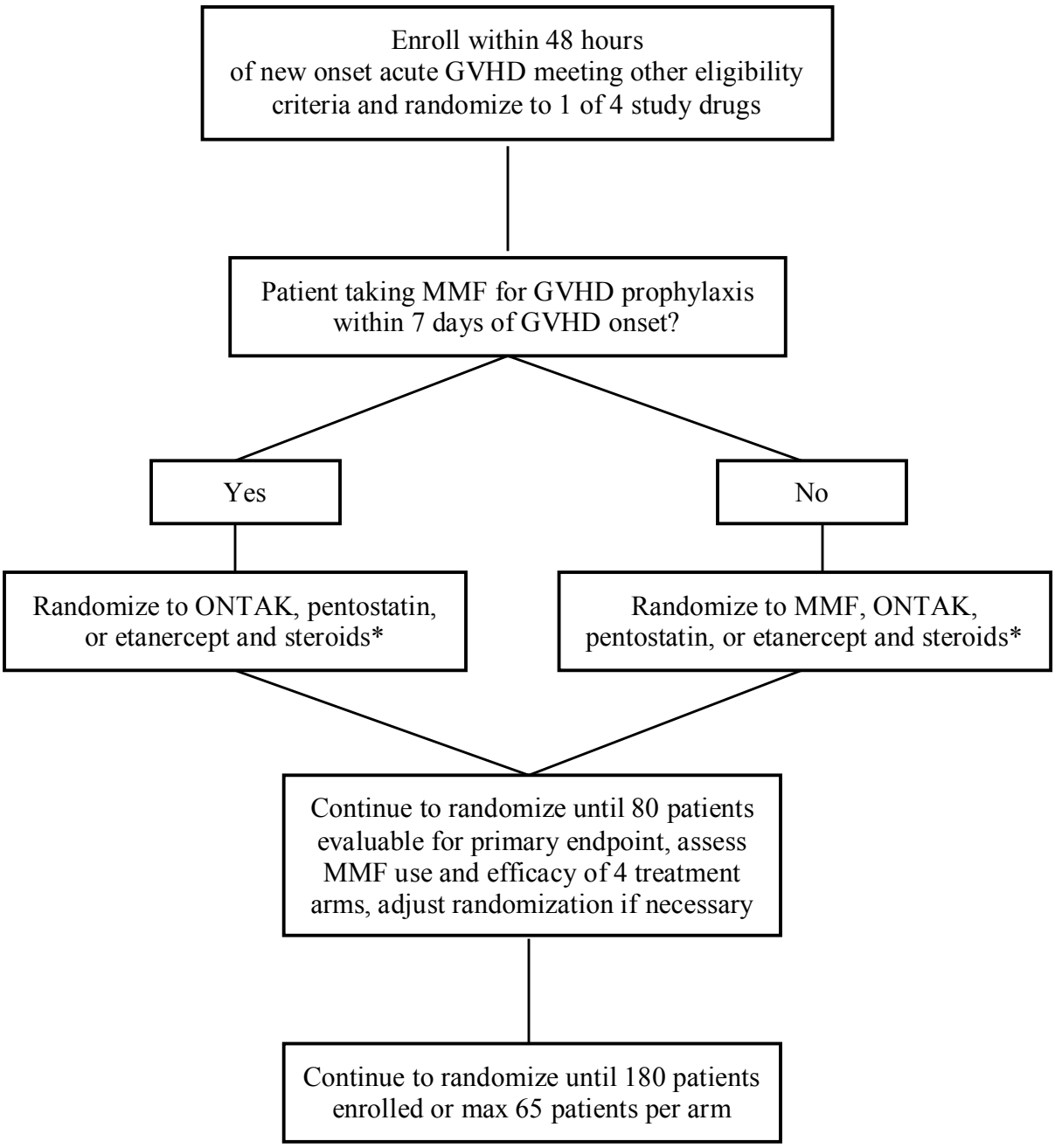
Weekly GVHD organ stage scores, overall clinical grade, biopsy information for GVHD and relevant differential diagnosis will be recorded and reported to the BMT CTN Data Coordinating Center (DCC).

**Accrual Objective:** A maximum of 180 patients, but at most 65 per arm, will be randomized to one of the four treatment arms. After 80 patients are evaluable for the primary endpoint, if in any experimental arm, observed responses suggest a posterior probability > 85% that an arm will have a true CR < 35%, enrollment to the arm will cease and the arm will be excluded from further consideration due to lack of evidence for efficacy.

**Accrual Period:** The estimated accrual period is 12 months.

**Study Duration:** Patients will be followed for 9 months following initiation of therapy.

# STUDY DESIGN SCHEMATIC




* Steroids = 2.5 mg/kg prednisone/day PO or IV equivalent in methylprednisolone (2 mg/kg) divided 2-3 times daily

Primary Endpoint:
Proportion of CR at Day 28 of therapy.

# STUDY SCHEMA

**Aim:** To determine if any of four new agents, given in addition to corticosteroids as initial therapy for acute GVHD improves response rate and overall clinical outcome.

| **Inclusion Criteria** | **Exclusion Criteria** |
|---|---|
| 1. Prior allogeneic hematopoietic stem cell transplant using either bone marrow, PBSC or cord blood.<br>2. De novo acute GVHD diagnosed **within 48 hours** prior to enrollment. Biopsy confirmation of GVHD is strongly recommended but not required. Enrollment should not be delayed awaiting biopsy or pathology results. The patient must have had no previous systemic immune suppressive therapy given for treatment of acute GVHD except for a maximum 48 hours of prior corticosteroid therapy (≥ 1 mg/kg/day methylprednisolone).<br>3. GVHD developing following DLI for prophylaxis or planned DLI.<br>4. ≥ 6 years of age at enrollment.<br>5. Clinical status at enrollment to allow tapering of steroids to not less than 0.6 mg/kg/day methylprednisolone (0.75 mg/kg/day prednisone) at Day 28 of therapy (e.g. persisting malignant disease suggesting the need for accelerated taper of immunosuppression).<br>6. ANC greater than 500/μL.<br>7. Estimated creatinine clearance greater than 30 mL/minute<br>8. Signed informed consent and/or assent.<br>9. Assent and educational materials provided to, and reviewed with, patients under the age of 18. | 1. ONTAK, pentostatin or etanercept given within 7 days of enrollment.<br>2. Isolated limited skin GVHD as the sole manifestation of acute GVHD.<br>3. Active uncontrolled infection.<br>4. Patients that have undergone an unscheduled (or not part of original transplant therapy plan) DLI.<br>5. Patients unlikely to be available at the transplant center on Day 28 and 56 of therapy.<br>6. A clinical syndrome resembling de novo chronic GVHD developing at any time after allotransplantation.<br>7. Other investigational therapeutics for GVHD within 30 days, including agents used for GVHD prophylaxis.<br>8. If any prior steroid therapy (for indication other than GVHD), treatment at doses > 0.5 mg/kg/d methyl-prednisolone within 7 days prior to onset of GVHD.<br>9. Patients who are pregnant, breast feeding, or if sexually active, unwilling to use effective birth control for the duration of the study.<br>10. Adults unable to provide informed consent<br>11. Patients with a history of intolerance to any of the study drugs. |

**If any improvement after 7 days of full dose corticosteroids + study drug**: Taper steroids as tolerated to no less than 0.75 mg/kg/day prednisone (or 0.6 mg/kg/day methylprednisolone) on Day 28. Improvement is defined as any clinically recognizable lessening of skin rash, redness, or extent; lessening of diarrhea or lowered bilirubin (though it does not have to be greater than or equal to one stage improvement in any involved organ), without worsening in any organ.

**Suggested prednisone taper for responders (round to nearest 5 mg of prednisone):**
2.5 mg/kg/day divided in 2-3 doses Day 0-6
2.5 mg/kg/day once daily Day 7-13
2 mg/kg/day Day 14-21
1.4 mg/kg/day Day 21-28
0.75 mg/kg/day Day 29-35
0.6 mg/kg/day Day 36-42
0.4 mg/kg/day Day 43-49
0.25 mg/kg/day Day 50-56
0.1 mg/kg/day Day 57-63
0.1 mg/kg every other day, Day 63-69;
Discontinue on Day 70

**Primary endpoint:**
Proportion of CR at Day 28 of therapy

**Secondary endpoints:**
- Proportion of PR, mixed response and progression at Day 28
- Proportion of NR, progression, administration of additional therapy for GVHD, or mortality by Day 14
- Incidence of GVHD flares requiring increasing therapy before Day 90
- Incidence of discontinuing immune suppression without flare by Day 90, 180 and 270 post therapy
- Incidence of Chronic GVHD by 9 months
- Survival at 6 and 9 months after initiation of treatment
- Incidence of systemic infections within 3 months of therapy
- Incidence of EBV lymphoma within 9 months of therapy

If acute GVHD progresses within 7 days or no response within 14 days, then treat with alternative secondary GVHD therapy off study, but follow for study endpoints.

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# CHAPTER 1

## 1 BACKGROUND AND RATIONALE

Acute graft-versus-host disease (GVHD) is the major complication of allogeneic hematopoietic stem cell (HSC) transplantation. Acute GVHD produces significant morbidity and complicates patient management resulting in organ toxicity, frequent infections, malnutrition and substantial delay in recovery from transplantation.

Acute GVHD usually occurs within the first 30-60 days of allogeneic HSCT and may involve the skin, liver and intestinal tract. T lymphocytes contained in the donor graft respond in vivo to disparate major (HLA) or minor (non-HLA) histocompatibility antigens on host tissues and a cascade of events leads to the signs and symptoms of acute GVHD. Greater HLA disparity between the donor and the recipient results in a higher incidence of acute GVHD.

The syndrome of acute GVHD includes the following signs and symptoms. Skin involvement (maculopapular exanthem) is usually the first sign of acute GVHD. Lesions may be pruritic or painful, red to violaceous in color, and often involve the palms and soles. Acute GVHD of the intestines may involve the stomach, small bowel and colon producing persistent nausea and vomiting or profuse diarrhea, intestinal bleeding, crampy abdominal pain and ileus. Liver GVHD produces cholestatic liver injury with hyperbilirubinemia and some hepatocellular enzyme elevations.

The use of cyclosporine (CSA) or tacrolimus (FK) plus short course methotrexate (MTX) has lowered the risk for acute GVHD compared to single agent or other combination therapy, but still 35-50% of patients develop Grade 2-4 acute GVHD [2-4, 5]. Older recipients or those transplanted from alternative (unrelated or partially matched) donors may develop more frequent or possibly more therapy-resistant acute GVHD.

**GVHD Therapy**
Corticosteroids have been the primary therapy for acute GVHD for over three decades. Various additional immunosuppressive strategies have been tested as GVHD therapy but neither anti-thymocyte globulin (ATG), CD5-immunotoxins, IL-1 antagonists nor other agents have been demonstrably helpful in either control of GVHD symptoms or improvement in survival [6-11]. Published response rates of complete response (CR) to acute GVHD therapy with corticosteroids range from 25-41% [12-14]. These rates will be used as benchmarks for assessing efficacy of promising new agents. New immunosuppressive agents and strategies are required to improve the management of GVHD and decrease the toxicities of the immunosuppressive regimens.

### 1.1 Study Goal

This study will test four new agents (etanercept, MMF, denileukin diftitox (ONTAK) and pentostatin), each in combination with corticosteroids as initial therapy of acute GVHD in a prospective, randomized Phase II trial. The primary purpose of the study is to identify agents

with sufficient promise for definitive testing in a subsequent Phase III trial in comparison to corticosteroids alone.

## 1.2 Background Data: Therapy of Acute GVHD

Recently, several new agents have been tested for GVHD therapy. Relevant data (peer-reviewed manuscripts, abstracts and/or personal communication) about the most promising new agents are briefly summarized below.

### 1.2.1 Daclizumab

A report from Germany [15] summarized GVHD treatment in 16 patients; 12 had steroid resistant acute GVHD. Patients (n=7) received daclizumab in a dosage of 1 mg/kg body weight (BW) on Day 1-5 and once every week thereafter until Day 28. Eight patients received 1 mg/kg on Day 1 and 2, followed by one dose per week through Day 28. Of the 12 evaluable patients, 6 responded to therapy. However, the rate of infections was high with 14 of 16 patients experiencing infections, 2 of them fatal. Four of 16 (25%) patients survived.

Przepiorka et al., [16] reported on 43 patients with steroid refractory acute GVHD. Daclizumab was administered at 1 mg/kg IV on study Day 1, 8, 15, 22 and 29 (n=24) or at 1 mg/kg IV on study Day 1, 4, 8, 15 and 22 (n=19) given either weekly (n=29) or 3 times per week (n=19). CR at Day 42 was 29% using the weekly regimen and 47% using the 3 times per week regimen. Improved 120 day survival (29% versus 53%) was also seen with the second regimen.

Wolff [17] reported a study of 12 patients treated with daclizumab and steroids. Six patients achieved CR, 4 partial responses, 1 non-response and 1 early death. A randomized trial of daclizumab plus corticosteroids versus corticosteroids alone (Dana Farber/ Minnesota/other centers) showed no benefit and some added toxicity with daclizumab [42].

### 1.2.2 Infliximab

Couriel et al., [18] reported a preliminary analysis of a randomized trial (n=60) showing no benefit of infliximab over steroids alone. The trial was stopped early. However, Sleight et al. [19] reported a series of 24 pediatric patients with 16 of the patients refractory to steroids. In this series, the patients received infliximab plus various other therapies. Improvement was seen in 15/16 evaluable patients with skin GVHD, 11/14 with gut GVHD, and 1/3 with liver GVHD; only 2 of 16 are surviving. Kobbe et al. [20] reported PR in 3 of 4 patients receiving 10 mg/kg/week.

### 1.2.3 Etanercept

In a report of 10 patients with chronic GVHD, Chiang et al. [21] found improvement of GVHD in 7 of the 8 patients completing an 8-week course. In another series, 13 patients received 25 mg subcutaneously twice weekly for up to 8 weeks [22]. Ten of 13 had a CR, most within 2 weeks. CR was more frequent for Grade 2 than Grade 3 acute GVHD.

### 1.2.4 Sirolimus

Benito et al. [23] reported 21 cases of steroid refractory disease treated with sirolimus at a median of 37 days post-transplant. Ten patients stopped treatment early (5 NR, 2 myelosuppression, 2 seizures). Ten patients had thrombotic microangiopathy (TMA). However, in 5 of 10, TMA did not appear correlated with sirolimus therapy. Of the 18 patients who received at least 6 doses, 12 responded (5 CR and 7 PR). Six of the 21 patients are surviving. [J. Antin, personal communication]. It is recognized that sirolimus has complex pharmacology and drug interactions. The formulation is currently not suitable for concurrent use with voriconazole. Information on dose modifications required for combined use of sirolimus and voriconazole may be available during 2004. It is promising but additional clinical pharmacology is needed.

### 1.2.5 Mycophenolate Mofetil (MMF)

Basara et al. [24] reported that treatment with MMF improved GVHD in 65% of 17 patients with acute GVHD (N=24; 17 acute GVHD; 65% improved). A report from Seattle (R Nash personal communication) showed similar results with 6 CR and 2 PR in 19 patients.

### 1.2.6 Pentostatin

Bolanos-Meade et al. [25] reported a dose escalation trial for treatment of steroid resistant acute GVHD using 1-4 mg/$m^2$ daily x 3 days. The best dose was 1.5mg/$m^2$ x 3 days, then repeated q 2 weeks. Of 20 treated patients, 11 achieved CR, 3 achieved PR, 3 had mixed responses and 3 progressed while on therapy. A recent update (personal communication, G. Vogelsang) totaling 23 treated patients showed 14 CR and 3 PR among 22 evaluable patients. At higher doses, late infections were considered dose limiting. The drug was well tolerated with only modest elevations of liver function tests and thrombocytopenia, each observed in only a single patient.

### 1.2.7 Denileukin Diftitox (ONTAK)

Shaughnessy et al. [26] described 11 patients treated with ONTAK 4.5 µg/kg/d x 5 days followed by the same dose weekly x 4. Of the 11, there were 5 responders, including 2 CR and 1 PR with 2 lesser responses.

Ho et al. reported 10 patients receiving 9 mcg/kg IV Days 1, 15 or Days 1, 3, 5, 15, 17 and 19. CR was seen in 3 patients, PR in 4 and mixed response in 2 (78% CR+PR). A subsequent update [27] included 28 patients with steroid refractory GVHD [grade II (n = 10), grade III (n = 13), grade IV (n = 5)]. Seven received ONTAK at dose level I (9 µg/kg IV on Days 1 and 15), 16 at dose level II (9 µg/kg IV Days 1, 3, 5, 15, 17 and 19), and 5 at dose level III (9µg/kg IV Days 1-5, 15-19), which yielded dose-limiting toxicity (1 acute renal failure, 3 elevated hepatic transaminases). At Day 28, 7 of 22 evaluable (32%) had CR and 9 (41%) PR, for an overall response rate of 73%. Among the 9 with PR, 3 additional patients subsequently entered CR after Day 29 without additional GVHD therapy, resulting in an overall CR rate of 45%. Response rate was highest at dose level II (CR = 45% and PR = 27%). Of the 22 evaluable for GVHD response, 9 (41%) are alive.

### 1.2.8 Study Agents

Based on the available data presented above, the four agents considered most promising for Phase II multi-center testing were etanercept, MMF, ONTAK and pentostatin. Each study agent has promising preliminary data reported from single or several center studies, suitable safety experience and tolerable applicability for multi-center Phase II testing as initial systemic therapy for acute GVHD.

### 1.2.9 Staging of Acute GVHD

Several schemas for staging and grading of acute GVHD have been published [28, 41]. In this study we will classify patients by the CIBMTR grading schema (Grades A-D), which has been shown to be more predictive of survival than earlier versions [7, 28].

# CHAPTER 2

## 2 STUDY DESIGN

### 2.1 Study Design

This trial is designed to evaluate the safety and efficacy of four new agents for acute GVHD in a prospective, multi-center, two-stage, randomized, four-arm Phase II trial. Each study arm will include corticosteroids plus a new agent. After 80 patients are evaluable for the primary endpoint, each arm will be assessed for safety using Bayesian posterior probability rules.

*Study Design without Steroid Only Control*
In this Phase II trial design, a corticosteroid only control arm will not be used since such an arm would not aid in judging which experimental arm was suitable for consideration in a subsequent Phase III randomized trial given the limited statistical power for comparison between arms. Each agent will be tested for potential efficacy against well established historical data published from several centers ($\geq$ 35% CR rate at Day 28 of therapy). A control cohort would not assist this comparison and would delay completion of this prospective Phase II test of individual agents given as an adjunct to corticosteroids.

### 2.2 Primary Objective and Rationale for Study Design

The primary purpose of the study is to define new agents with promising activity against acute GVHD suitable for testing against corticosteroids alone in a subsequent comparative, Phase III trial. Careful review of published and unpublished data on therapy of acute GVHD documents an expected complete response (CR) rate for steroids alone of 35%. Each of the four study agents selected are reported to have promising activity as therapy of acute GVHD in preliminary testing but have limited Phase II data available. This trial will more formally test their safety and efficacy as supplemental to corticosteroids alone.

The choice of agents for study in the subsequent Phase III trial against steroids alone will include consideration of the initial CR rate (the primary endpoint) as well as several important assessments of other safety and efficacy parameters (the secondary endpoints). Those considered suitable for subsequent testing will have demonstrable primary efficacy as well as satisfactory estimates (developed within this randomized Phase II trial) of GVHD control without excessive rates of the anticipated complications of infection, GVHD flare, chronic GVHD or early mortality. Thus, in this Phase II trial, a control arm will be omitted and deferred until the formal, prospective Phase III trial, which will test the best agent(s) against the control of steroids alone.

## 2.3 Eligibility

### 2.3.1 Inclusion Criteria

Biopsy of involved tissue is strongly encouraged, but not required for study entry. The clinical diagnosis of acute GVHD requiring systemic therapy with corticosteroids is necessary for enrollment on the trial. Enrollment and randomization includes commitment to continue both steroids and study drug as indicated by the protocol as well as the required follow-up observations. If the intention to treat a patient is dependent on histologic results, the patient should not be enrolled on the BMT 0302 study until the results are known. Patients should only be enrolled with a clinically established diagnosis. Histologic confirmation of acute GVHD is not required, but can be used as supportive evidence for the diagnosis. If an unexpected biopsy result occurs, the center should contact the protocol chair.

1. Prior allogeneic hematopoietic stem cell transplant using either bone marrow, peripheral blood stem cells or cord blood. Recipients of non-myeloablative transplants are also eligible.

2. De novo acute GVHD requiring systemic therapy within 48 hours of diagnosis [28]. GVHD is defined as the presence of skin rash and/or persistent nausea, vomiting, and/or diarrhea and/or cholestasis as the clinical criteria compatible with the diagnosis of acute GVHD when present in the right clinical setting and not directly attributable to other etiologies such as drug rash, enteric infection, or hepatotoxic syndromes (e.g., veno occlusive disease of the liver). The patient must have had no previous systemic immune suppressive therapy given for treatment of acute GVHD except for a maximum 48 hours of prior corticosteroid therapy ≥ 1 mg/kg/day methylprednisolone.

   Note that patients with stage II skin or isolated upper gastrointestinal (GI) only are eligible. However, the protocol team <u>does not recommend</u> initiating systemic therapy with steroids for limited skin or upper GI only GVHD, but if the institutional treatment plan and treating physician initiates corticosteroid therapy at ≥ 2.0 mg/kg/day methylprednisolone (2.5 mg/kg/day prednisone), the patients are eligible for enrollment.

3. Patients that have undergone a donor lymphocyte infusion (DLI) as part of their original transplant therapy plan.

4. Greater than or equal to 6 years of age at the time of enrollment.

5. Clinical status at enrollment to allow tapering of steroids to not less than 0.6 mg/kg/day methylprednisolone (0.75 mg/kg/day prednisone) at Day 28 of therapy.

6. Absolute neutrophil count (ANC) greater than 500/μL.

7. Estimated creatinine clearance greater than 30 mL/minute (see Table 2.3.1).

8. Written informed consent and/or assent from patient, parent or guardian.

9. Documentation that the assent document and education materials have been provided to, and reviewed with, patients under the age of 18.

**Table 2.3.1**
**ESTIMATED CREATININE CLEARANCE (mL/min)**

| **Serum Creatinine** | **Ideal Body Weight 20 kg 10 years/15** | **40 kg 20years/ 30/40** | **60 kg 20years/ 30/40** | **80 kg 20years/ 30/40** | **100 kg 20years/ 30/40** |
|---|---|---|---|---|---|
| 0.8 | Male 45/43<br>Female 38/37 | Male 83/76/69<br>Female 71/65/59 | Male 125/115/104<br>Female 106/97/89 | | |
| 1.0 | Male 36/35<br>Female 31/30 | Male 67/61/56<br>Female 57/52/47 | Male 100/92/83<br>Female 85/78/71 | | |
| 1.2 | Male 30/29<br>Female 26/25 | Male 56/51/46<br>Female 47/43/39 | Male 83/76/83<br>Female 59/65/71 | Male 111/102/93<br>Female 94/87/79 | |
| 1.4 | Male 26/25<br>Female 22/21 | Male 48/44/40<br>Female 41/37/34 | Male 71/66/60<br>Female 51/56/51 | Male 95/87/79<br>Female 81/74/68 | Male 119/109/99<br>Female 101/93/84 |
| 1.6 | Male 23/22<br>Female 19/18 | Male 42/38/35<br>Female 35/33/30 | Male 63/57/52<br>Female 53/49/44 | Male 83/76/69<br>Female 71/65/59 | Male 104/96/87<br>Female 89/81/74 |
| 1.8 | Male 20/19<br>Female 17/16 | Male 37/34/31<br>Female 32/29/26 | Male 56/51/46<br>Female 47/43/39 | Male 74/68/62<br>Female 63/58/53 | Male 93/85/77<br>Female 79/72/66 |
| 2.0 | Male 18/17<br>Female 15/15 | Male 33/31/28<br>Female 28/26/24 | Male 50/46/42<br>Female 43/39/35 | Male 67/61/56<br>Female 57/52/47 | Male 83/76/69<br>Female 71/65/59 |
| 2.2 | | Male 30/28/25<br>Female 26/24/22 | Male 46/42/38<br>Female 39/35/32 | Male 61/56/51<br>Female 52/47/43 | Male 76/69/63<br>Female 64/59/54 |

Note: Modified from Cockcroft Gault equation; (140 – age)*wt/([Cr]*72= clearance Females: 0.85 x the calculated result.

Calculated or measured creatinine clearance for patients can also be used.

### 2.3.2 Exclusion Criteria

1. Patients receiving ONTAK, pentostatin or etanercept within seven days of screening for enrollment.

2. Patients with isolated limited skin GVHD (stage I) as the sole manifestation of acute GVHD.

3. Patients with uncontrolled infections will be excluded. For bacterial or viral infections, patients must be receiving definitive therapy and have no signs of progressing infection for 72 hours prior to enrollment. For fungal infections patients must be receiving definitive systemic anti-fungal therapy and have no signs of progressing infection for 1 week prior to enrollment.

   Progressing infection is defined as hemodynamic instability attributable to sepsis or new symptoms, worsening physical signs or radiographic findings attributable to infection. Persisting fever without other signs or symptoms will not be interpreted as progressing infection.

4. Patients that have undergone an unscheduled DLI or a DLI that was not part of their original transplant therapy plan or GVHD developing following DLI given for treatment of persistent or recurrent malignancy after transplantation would be excluded.

5. Patients unlikely to be available at the transplantation center on Day 28 and 56 of therapy.

6. A clinical syndrome resembling de novo chronic GVHD developing at any time after allotransplantation. See Chapter 2 of the BMT CTN Manual of Procedures for details of de novo chronic GVHD.

7. Patients receiving other investigational agents for GVHD, including GVHD prophylaxis agents within 30 days of screening for eligibility. The following agents are not considered experimental and therefore are not excluded: cyclosporine, tacrolimus, sirolimus, corticosteroids (prednisone, methylprednisolone, prednisolone, dexamethasone, or other), antithymocyte globulin (atgam, thymoglobulin or other), and methotrexate.

8. Patients receiving methyprednisolone > 0.5 mg/kg/day (or 0.6 mg/kg/day predisone) within 7 days of onset of acute GVHD. If steroid therapy has been administered for a non-GVHD related condition and tapered to ≤ 0.5 mg/kg/day methylprednisolone (0.6 mg/kg/day prednisone) for seven or more days prior to the onset of acute GVHD, the patient is eligible.

9. Patients who are pregnant, breast feeding, or, if sexually active, unwilling to use effective birth control for the duration of the study.

10. Adults unable to provide informed consent.

11. Patients with a history of intolerance to any of the study drugs.

2.3.3 Informed Consent

The informed consent process will begin at recognition of subject eligibility and consent will be obtained per institutional practices before study therapy is initiated.

## 2.4 Treatment Plan

Treatment should be initiated as soon as possible after randomization. A maximum of 48 hours is allowable.

2.4.1 Risks of All Study Drugs

2.4.1.1 Risks of infection

All four study drugs are potent immunosuppressives. All have been associated with increased risks of opportunistic infection and in this setting, where corticosteroids and the intrinsic immunosuppression of GVHD are apparent, particular caution must be paid to the risks of additional infection. Anti-infective prophylaxis (see Section 2.4.11) is required for all patients enrolled in this trial.

Each of these study drugs can add to risks of infection due to their immunosuppressive potency. Patients with sustained bacteremia (≥ 2 days of bacteremia), persistent (≥ 5 days) culture negative fever or hemodynamic instability (sepsis syndrome with hypotension requiring > 30 ml/kg fluid resuscitation or pressor support) should have their study drug held at the attending physician's discretion. Study drug can be reinstituted when control of the infection episode and hemodynamic stability is achieved. Replacement doses or extension of study drug therapy is not recommended.

2.4.1.2 Drug associated toxicities

Study drug should be held for any Grade 3-4 toxicities that are considered probably related to study drug.

2.4.2 Etanercept (ENBREL®, Amgen, Inc.)

2.4.2.1 Mechanism of action

ENBREL is a recombinant DNA derived protein composed of tumor necrosis factor (TNF) receptor linked to the FC portion of human IgGl. Etanercept binds TNF and blocks its

interaction with target cell surface receptors [29-31]. It has anti-inflammatory activity that has been demonstrated in rheumatoid arthritis and tested in other inflammatory states including Wegener's granulomatosis, inflammatory bowel disease, and other autoimmune and inflammatory states.

2.4.2.2 Etanercept drug supply

Etanercept will be provided by the BMT CTN for 4 weeks or approximately 8 doses. The unreconstituted drug is supplied in vials containing 25 mg etanercept and must be stored between 2ºC and 8ºC (without freezing) in the refrigerator. The reconstituted vial (using 1 mL of bacteriostatic water, added slowly without shaking to minimize foaming) creates a solution of 25 mg/mL after approximately 10 minutes of dissolution. Reconstituted solutions should be administered as soon as possible after reconstitution. If not administered immediately following reconstitution, reconstituted etanercept can be stored under refrigeration (2ºC - 8ºC) for up to 28 days. No other diluents except for bacteriostatic water for injection can be used and the drug should not be filtered when infused.

2.4.2.3 Dose and administration

Etanercept is to be administered subcutaneously within 28 days of reconstitution and stored in the refrigerator before use.

It is to be administered no more often than once every 72 hours. It is administered subcutaneously in rotating sites and should not be reinjected into a site with residual injection site reaction (new sites must be at least 1 inch from previous injection site). The thigh, abdomen, and upper arm are preferred sites of injection.

Patients will receive 25 mg subcutaneously twice weekly for up to 4 weeks. Dosing for patients less than 17 years of age or with body surface area (BSA) < 0.6 $m^2$ should be 0.4 mg/kg subcutaneously twice weekly with a maximum of 25 mg within 24 hours (the adult dose). No premedications are required.

2.4.2.4 Drug interactions

No specific drug interactions have been recognized and no dose modifications will be required for metabolic or excretory dysfunction (liver or renal impairment).

2.4.2.5 Risks and toxicities

Administration of etanercept may cause injection site reactions with redness, tenderness, or warmth in up to one-third of patients. Headache, dizziness, and rash may occur in a fraction of patients and upper respiratory tract symptoms with cough, sinusitis, rhinitis, and pharyngitis can occur. It is uncertain whether there are infectious or inflammatory symptoms accompanying long-term use of etanercept.

Serious opportunistic infections, including tuberculosis, have been identified. Rarely, pancytopenia has been observed.

2.4.2.6 Discontinuation

If patient is in CR (no signs of acute GVHD symptoms) by 4 weeks after therapy was initiated, etanercept is discontinued.

For additional information on this study drug please see "Prescribing Information", at http://www.enbrel.com/

2.4.3 Mycophenolate Mofetil (CellCept®, Roche)

2.4.3.1 Mechanism of action

Mycophenolate mofetil (MMF) is a morpholinoethyl ester of mycophenolic acid (MPA). A product of several penicillium species, MPA possesses antibacterial, antifungal, antiviral, antitumor and immunosuppressive properties. MMF is a pro-drug since the immunosuppressive activity is evident only after hydrolysis to MPA [32-34]. MMF was developed to enhance the bioavailability of MPA. MPA mediates its effect by inhibiting inosine monophosphate dehydrogenase (IMPDH), an enzyme that catalyzes the oxidation of inosine monophosphate to xanthine monophosphate, an intermediate metabolite in the synthesis of guanosine triphosphate (GTP). Lymphocytes rely on the de novo purine synthesis pathway for the nucleotides necessary for DNA synthesis; other cells can also rely on the salvage pathway. The action of MPA results in the depletion of the nucleotide pool in cell synthesis. An additional mode of action of MPA may be that, by depletion of GTP, it inhibits recruitment of leukocytes to sites of inflammation by inhibiting the glycosylation of lymphocyte glycoproteins involved in intercellular adhesion. Pharmacokinetics will be studied twice in each subject receiving MMF (Appendix C-5).

2.4.3.2 MMF drug supply

MMF will be provided by the BMT CTN for up to 8 weeks. Oral MMF is available as 500 mg tablets and 250 mg hard gelatin capsules in bottles of 100 each. Capsules should be stored at room temperature. A liquid suspension is also available (200 mg/mL, 225 mL bottle) and should be stored at 25ºC. Once reconstituted the solution may be stored at room temperature or under refrigeration (do not freeze) for 60 days. Enteric coated MMF (Myfortic) should not be used.

Intravenous: MMF for intravenous use will be supplied as a lyophilized powder. It will be reconstituted by injecting 5% Dextrose in Water (D5W) into each vial.

Intravenous MMF does not contain an antibacterial preservative; therefore, it should be prepared under aseptic conditions. Reconstitute each vial with 14 mL D5W. Gently shake vial to dissolve the drug. Further dilute into D5W for a final concentration of 6 mg/mL. Dilute 1-g doses in 140 mL D5W and 1.5-g doses into 210 mL D5W. Intravenous mycophenolate is incompatible

with normal saline and other saline containing diluents. Only D5W solution should be used for reconstitution and infusion solution. Infusion lines should be flushed with D5W only.

The Pharmacist at each center should prepare each dose of drug to avoid errors. Because of the potential teratogenic effects of MMF, pharmacists should follow the guidelines established at their centers for the preparation of similar types of solutions.

2.4.3.3 Dose and administration

Patients with body surface area (BSA) > 1.5 $m^2$ will receive 20 mg/kg (actual body weight) PO or IV twice daily (maximum 1 g per dose). Intravenous doses are infused over a two-hour period.

For patients from 1.25 to 1.5 $m^2$, administer 750 mg IV or as capsules PO twice daily.

For patients < 1.5 $m^2$ receiving suspension (not capsules), MMF as oral suspension can be given at a dose of 600 mg/$m^2$ PO twice daily (maximum 1 g twice daily).

If patients with GI GVHD are assigned to MMF, and they cannot tolerate oral fluids greater than 500 mL per day (200 mL/$m^2$), then MMF should be administered intravenously for the first 3-7 days of therapy and converted to oral MMF as soon as possible after the initial 3 days of therapy if oral medications and this limited fluid intake are tolerated.

A study of MMF absorption and pharmacokinetics is part of the trial (Appendix C-5) but levels will not be used for dose modification.

2.4.3.4 Risks and toxicities

Administration of MMF may produce diarrhea, leukopenia, sepsis, vomiting and possibly a higher incidence of certain viral infections (CMV, VZV, Herpes Simplex). Gastrointestinal bleeding and perforation have also been described infrequently.

2.4.3.5 Guidelines for MMF dose adjustment

If any toxicity is judged on clinical grounds related to MMF administration, dose adjustment can occur as follows:

**Gastrointestinal (GI) Toxicity:** If GI toxicity requires medication for control of persistent vomiting or diarrhea (not related to GVHD), a dose reduction to 50% can be instituted or, if symptoms persist and are severe (Grade 3 or 4), the drug can be stopped. If GI toxicity does not resolve within 48-72 hours of stopping MMF, then this toxicity is not likely related to MMF and the drug should be restarted. The study site principal investigator should be consulted before discontinuing MMF for GI toxicity.

**Myelosuppression:** If the ANC is less than 1000/μL, MMF dosing should be reduced by 50%. If the ANC is less than 500/μL, the drug should be discontinued until neutrophil recovery occurs. Use of filgrastim and/or discontinuation of other myelosuppressive agents is allowed.

**Guidelines for Renal Insufficiency:** As free MPA exposure has not been directly correlated with renal dysfunction, MMF dosing should not be modified unless renal failure requiring dialysis is required. If dialysis is needed, MMF can be reduced to 25% - 50% of the starting dose.

**Hepatic Dysfunction:** No adjustments of MMF are required for liver dysfunction.

2.4.3.6 Drug interactions

MMF activity is decreased with oral administration of antacids and cholestyramine. Acyclovir or ganciclovir blood levels may increase during co-administration with MMF due to competition for tubular secretion of these drugs, especially if renal impairment is present. Probenecid may also augment MPA levels due to inhibition of tubular secretion. High doses of salicylates (or other highly protein bound drugs) may increase the free fraction of MPA and exaggerate the potential for myelosuppression.

2.4.3.7 Discontinuation

If in CR at Day 28, continue MMF at the same dose through the completion of the prednisone taper, then taper MMF and discontinue over four more weeks.

2.4.4 Denileukin Diftitox (ONTAK®, Ligand Pharmaceuticals)

2.4.4.1 Mechanism of action

ONTAK is a fusion protein combining sequences from IL-2 with sequences from diphtheria, DAB-389. It binds with the CD25 and delivers the toxin that inhibits intracellular protein synthesis, rapidly leading to death in any cells that are bound. It has activity against cutaneous T cell lymphoma, but its anti T-lymphocyte lytic effects have been used for immunosuppressive therapy in treatment of GVHD and other conditions [35, 36].

ONTAK is administered intravenously and is distributed within 2-5 minutes and is metabolized in the liver by proteolytic degradation with a terminal half-life 70-80 minutes before inactivation.

2.4.4.2 ONTAK drug supply

ONTAK will be provided by the BMT CTN for 4 weeks or 6 doses. It is supplied as a frozen solution (≤ -10°C) containing 150 micrograms/mL (2 mL). It is diluted for administration using preservative free normal saline. It is recommended that 9 mL of saline is added for each mL of ONTAK maintaining a concentration of at least 15 micrograms/mL during preparation. It is swirled gently with vigorous shaking avoided and should be administered within 6 hours of thawing and dilution. It should not be put into glass containers or syringes.

2.4.4.3 Dose and administration

ONTAK will be administered at 9 micrograms/kg (actual body weight) intravenously on Days 1, 3, 5, 15, 17, and 19 of study. If ONTAK is unavailable on Day 1, it should be administered on Days 2, 4, 6, 15, 17 and 19 of the study. The rate of infusion for initial ONTAK administration should be over approximately 1 hour, with subsequent infusions shortened to no less than 15 minutes as tolerated by the patient. No dose modifications are permitted.

2.4.4.4 Drug interactions

Specific drug interactions have not been reported.

2.4.4.5 Risks and toxicities

Administration of ONTAK may cause infusion reactions with fever, chills, or hypotension sometimes resembling acute hypersensitivity events during or within 24 hours of infusion. Dyspnea and chest tightness, flushing, and pruritis may occur.

Additionally, vascular leak with hypoalbuminemia, edema and, if severe, hypotension may occur in patients receiving ONTAK. Lymphopenia with risk of infection and anemia may develop. Gastrointestinal side effects of diarrhea, anorexia, nausea, and vomiting are possible but are seen more frequently in doses considerably higher than used in this trial. Reversible hepatic transaminase elevations can also be seen. Myelosuppression with leukopenia or thrombocytopenia can occur, but is uncommon.

2.4.4.6 Guidelines for dose adjustment

ONTAK should be stopped for Grade 4 drug associated toxicity and drug held for 4 days for Grade 3 toxicity that improves to less than Grade 3 within the 4 days.

Flulike symptoms with headache, fever, chills, arthralgia and myalgias may occur, but tend to decrease in incidence and severity with repeat treatments. Slowing the infusion to 30 minutes has reduced the severity of these symptoms.

Infusion-associated reactions can be managed by temporarily discontinuing or interrupting the infusion and then restarting at a slower rate. Administering the dose of corticosteroids due for GVHD therapy as a pre-medication along with acetaminophen and/or diphenhydramine may minimize infusion-associated reactions.

2.4.5 Pentostatin (Nipent®, Supergen Pharmaceuticals)

2.4.5.1 Mechanism of action

Pentostatin is a purine anti-metabolite that inhibits adenosine deaminase preventing the deamination of adenosine to inosine. Accumulation of deoxyadenosine and dATP leads to

reduction in DNA synthesis and subsequent cell death. The drug distributes rapidly to body tissues and has a terminal half-life of 5-15 hours with urinary excretion, mostly as unchanged drug [37, 38, 39].

2.4.5.2 Pentostatin drug supply

Pentostatin will be provided by the BMT CTN for 4 weeks or 6 doses. Pentostatin is supplied as a powder for reconstitution (10 mg/vial) in preparation for injection. Vials are stored in the refrigerator between 2°C and 8°C. It is diluted for infusion with 5% dextrose injection USP or 0.9% saline. Because it contains no preservatives, the diluted prepared solution must be used within 24 hours.

2.4.5.3 Dose and administration guidelines

Patients will receive 1.5 mg/m$^2$ daily (BSA calculated using actual body weight) for 3 days on Days 1-3 and Days 15-17 of study for a total of 6 doses. If pentostatin is unavailable on Day 1, it should be administered on Days 2-4 and 15-17 of the study. It will be infused intravenously over 15-30 minutes.

2.4.5.4 Drug interactions

Fludarabine and cyclophosphamide should not be co-administered as pentostatin toxicity is exaggerated.

2.4.5.5 Risks and toxicities

Mild to moderate nausea, vomiting, anorexia, diarrhea and skin rash are the most common adverse reactions, occurring in 30-50% of patients. Thrombocytopenia, anemia, and to a lesser extent, leukopenia may occur, though of modest severity, even with higher doses than used in this trial. Modest elevation of hepatic enzymes and increased risk of infection may occur.

2.4.5.6 Guidelines for dose adjustment

**Myelosuppression:** If the ANC is less than 1000/μL, pentostatin dosing should be reduced by 50%. If the ANC is less than 500/μL, the drug should be discontinued until neutrophil recovery occurs. Use of filgrastim and/or discontinuation of other myelosuppressive agents is allowed.

**Renal Insufficiency:** Pentostatin dose reductions for renal insufficiency are made based on estimated creatinine clearance (Table 2.3.1). For clearance of 30-50 mL per minute, the dose should be reduced to 50% or 0.75 mg/m$^2$ IV in the same schedule. The drug should not be used if clearance is less than 30 mL per minute.

**Hepatic Insufficiency:** Dose reduction is not required for liver dysfunction.

2.4.6 Corticosteroids (prednisone or methylprednisolone)

- All patients will receive methylprednisolone 2 mg/kg/day IV (or prednisone 2.5 mg/kg/day PO) divided in 2-3 daily doses for 7 days.

- Patients unable to tolerate oral fluids of at least 500 mL/day (200 mL/$m^2$) must be treated with IV steroids for at least the first 3 days to ensure bioavailability.

- For responding patients between Day 8 and Day 28, the dose of methylprednisolone must be **at least** 0.6 mg/kg/day (0.75 mg/kg/day prednisone).

- Clinical judgment should guide the pace of the steroid taper between Day 8 and Day 28 and a suggested taper schedule for responding patients (whose GVHD improves in at least one organ by at least one stage and without worsening in any other organ) is shown

- For patients that have not showed improvement in GVHD within the first week, the taper can be slower than the recommended rate, but should not be faster.

- After Day 28, the steroid dose is determined by the clinical judgment of the treating physician.

2.4.6.1 Steroid taper

**Suggested taper for responders (methylprednisolone IV)**

| | |
|---|---|
| 2 mg/kg/day divided in 2-3 doses Days 0-6<br>2 mg/kg/day once daily Days 7-13<br>1.5 mg/kg/day Days 14-21<br>1.0 mg/kg/day Days 22-28<br>0.5 mg/kg/day Days 29-35 | 0.4 mg/kg/day Days 36-42<br>0.3 mg/kg/day Days 43-49<br>0.2 mg/kg/day Days 50-56<br>0.1 mg/kg/day Days 57-63<br>0.1 mg/kg every other day Days 64-69<br>Discontinue on Day 70 |

**Suggested taper for responders (prednisone orally)**

| | |
|---|---|
| 2.5 mg/kg/day divided 2-3 doses Days 0-6<br>2.5 mg/kg/day once daily Days 7-13<br>2 mg/kg/day Days 14-21<br>1.4 mg/kg/day Days 22-28<br>0.75 mg/kg/day Days 29-35 | 0.6 mg/kg/day Days 36-42<br>0.4 mg/kg/day Days 43-49<br>0.25 mg/kg/day Days 50-56<br>0.1 mg/kg/day Days 57-63<br>0.1 mg/kg every other day Days 64-69<br>Discontinue on Day 70 |

### 2.4.7 Cyclosporine or Tacrolimus

Cyclosporine or tacrolimus should be continued at therapeutic doses adjusted as necessary for renal, central nervous system (CNS) or other toxicity using conventional management guidelines. The recommended therapeutic trough level of cyclosporine is 200-400 ng/mL measured by whole blood HPLC; institutional levels for other assays. The recommended therapeutic trough level for tacrolimus is 5-15 ng/mL.

### 2.4.8 GVHD Progression or Non-response

If acute GVHD **progresses** (new organ involvement or increased organ specific symptoms sufficient to increase the organ stage by one or more) within 7 days or there is **no response** (no reduction in any GVHD organ staging) within 14 days, the patient may be treated with alternative secondary GVHD therapy and/or taken off assigned study therapy. Patient must still be followed for all study endpoints.

### 2.4.9 GVHD Complete Response or Partial Response

Complete response at Day 28 is the primary endpoint evaluation for the trial. Patients with complete response will continue corticosteroids according to the taper above. Etanercept, ONTAK, or pentostatin will not be further administered. MMF (as per Section 2.4.3 above) will be continued through the duration of prednisone taper and then tapered over 4 weeks and stopped.

Patients not in CR at Day 28 will not have further study drug supplied (with the exception of MMF), but may continue assigned study drug or alternative therapy at the investigator or institutional discretion. Patients must still be followed for all study endpoints.

### 2.4.10 Acute GVHD Flare

Patients who have achieved CR at Day 28 and who have subsequent flare of acute GVHD (recurrence of GVHD signs or symptoms) can be treated with the same or alternative agents at the investigator/institutional discretion. Alternatively they can have a modest increment of corticosteroid dosing (recommended increase is prednisone 0.25 mg/kg/day x 7 days) and resume a taper if a response is observed.

### 2.4.11 Supportive Care

In addition to prescribed study drug plus corticosteroids, all patients should receive the following:

- Transfusion support per institutional practice
- Anti-infective prophylaxis or preemptive therapy directed towards: CMV, gram positive (encapsulated) bacteria, pneumocystis carinii and fungal infections. Patients enrolled on BMT CTN Protocol #0101 (Fungal Prophylaxis) should continue on their assigned study drug.

## 2.5 GVHD Scoring

Weekly GVHD organ stage scores, overall clinical grade, biopsy information for GVHD and relevant differential diagnosis will be recorded and reported to the DCC. Organ involvement, biopsy information, staging, differential diagnosis, and GVHD therapy will be documented in the medical record using the BMT CTN GVHD scoring stamp or equivalent.

Symptoms of chronic GVHD, if present, will be reported according to the CTN MOP (Chapter 2) and reported on the GVHD symptom record.

Patients receiving alternative GVHD therapy (progression, PR or no CR at Day 28) should still have weekly scoring performed through week 8 and follow-up for all study endpoints.

### 2.5.1 GVHD Scoring

An example acute GVHD weekly data record (stamp) is shown below.

**Clinical Acute GVHD Assessment**

Date _____ Patient ID _____ Karnofsky/Lansky _____

| | CODES | | | | | | DIFFERENTIAL DIAGNOSIS | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | | GVHD | Drug Rxn | Cond Reg | TPN | Infect | VOD | Other |
| Skin | ☐ | ☐ | ☐ | ☐ | ☐ | | % body rash:____ | ☐ | ☐ | ☐ | | ☐ | | _____ |
| Lower GI | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | Vol: _____ | ☐ | ☐ | ☐ | ☐ | ☐ | | _____ |
| Upper GI | ☐ | ☐ | | | | | | ☐ | ☐ | ☐ | ☐ | ☐ | | _____ |
| Liver | ☐ | ☐ | ☐ | ☐ | ☐ | | Max bili: _____ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | _____ |

**Treatment:** ☐ CSA ☐ Tacrolimus ☐ Pred ☐ Methylpred ☐ Ontak
☐ Pentostatin ☐ MMF ☐ Etanercept ☐ Other _____

***Code Definitions:***

Skin:
0 No rash
1 Maculopapular rash, < 25% of body surface
2 Maculopapular rash, 25-50% of body surface
3 Generalized erythroderma
4 Generalized erythroderma with bullous formation and desquamation

Lower GI (Diarrhea):
0 None
1 ≤ 500 mL/day or < 280 mL/m$^2$
2 501-1000 mL/day or 280- 555 mL/m$^2$
3 1001-1500 mL/day or 556- 833 mL/m$^2$
4 > 1500 mL/day or > 833 mL/m$^2$
5 Severe abdominal pain with or without ileus, or stool with frank blood or melena

Upper GI:
0 No protracted nausea and vomiting
1 Persistent nausea, vomiting or anorexia

Liver (Bilirubin):
0 < 2.0 mg/dl
1 2.1-3.0 mg/dl
2 3.1-6.0 mg/dl
3 6.1-15.0 mg/dl
4 > 15.1 mg/dl

Signature _____

### 2.5.2 Chronic GVHD

Patients developing sign/symptoms of chronic GVHD (CGVHD) will have symptoms recorded on the CGVHD scoring form at the scheduled follow-up visits.

### 2.5.3 Studies of GVHD Biology

Laboratory studies to investigate biologic parameters of response and differential response to the four study drugs will be included in this clinical trial.

- Skin biopsies (pre and on Day 28 of study therapy) for clinical diagnostic pathology, immunopathology, and other investigations (Appendix C-4) are to be performed.
- Immunophenotyping: Cytokine assays to correlate with GVHD response and cytokine gene polymorphisms will be examined (Appendix C-1).
- Proteomics (Appendix C-6) of sera collected before and after therapy will be performed.

## Definite and Possible Manifestations of Chronic GVHD

| Organ System | Definite manifestations of chronic GVHD | Possible manifestations of chronic GVHD |
|---|---|---|
| Skin | Scleroderma (superficial or fasciitis), lichen planus, vitiligo, scarring alopecia, hyperkeratosis pilaris, contractures from skin immobility, nail bed dysplasia | Eczematoid rash, dry skin, maculopapular rash, hyperpigmentation, hair loss |
| Mucous membranes | Lichen planus, non-infectious ulcers, corneal erosions/non-infectious conjunctivitis | Xerostomia, keratoconjunctivitis sicca |
| GI tract | Esophageal strictures, steatorrhea | Anorexia, malabsorption, weight loss, diarrhea, abdominal pain |
| Liver | None | Elevation of alkaline phosphatase, transaminitis, cholangitis, hyperbilirubinemia |
| GU | Vaginal stricture, lichen planus | Non-infectious vaginitis, vaginal atrophy |
| Musculoskeletal/ Serosa | Non-septic arthritis, myositis, myasthenia, polyserositis, contractures from joint immobilization | Arthralgia |
| Hematologic | None | Thrombocytopenia, eosinophilia, autoimmune cytopenias |
| Lung | Bronchiolitis obliterans | Bronchiolitis obliterans with organizing pneumonia, interstitial pneumonitis |

From BMT CTN MOP Chapter 2

# CHAPTER 3

## 3 STUDY ENDPOINTS

### 3.1 Primary Endpoint

The primary endpoint is proportion of CR at Day 28 of therapy. **CR is defined as resolution of all signs and symptoms of GVHD in all evaluable organs in comparison to Day 1 scoring.** For a response to be scored as CR at Day 28 or later, the participant must still be in CR on that day and have had no intervening salvage therapy for an earlier progression, PR or NR.

3.1.1 Staging and Grading of Acute GVHD

**Staging***

| | Stage 0 | Stage 1 | Stage 2 | Stage 3 | Stage 4 |
|---|---|---|---|---|---|
| Skin | No rash | Rash < 25% BSA | 25-50% | > 50% Generalized erythroderma | Plus bullae and desquamation |
| Gut | < 500 mL diarrhea/day | 501-1000 mL/day | 1001–1500 mL/day | > 1500 mL/day | Severe abdominal pain & ileus |
| UGI | | Severe nausea/vomiting | | | |
| Liver | Bilirubin ≤ 2 mg/dl | 2.1-3 mg/dl | 3.1-6mg/dl | 6.1-15mg/dl | > 15 mg/dl |

**Grading Index of Acute GVHD***

| | Grade A | Grade B | Grade C | Grade D |
|---|---|---|---|---|
| Skin | 1 | 2 | 3 | 4 |
| Gut | 0 | 1-2 | 3 | 4 |
| Upper GI | 0 | 1 | | |
| Liver | 0 | 1-2 | 3 | 4 |

*[See Appendix A, Endnote 28]

## 3.2 Secondary Endpoints

### 3.2.1 Proportion of Partial Response (PR), Mixed Response (MR), No Response (NR) and Progression

**Partial response (PR) is defined as improvement in one or more organs involved with GVHD symptoms without progression in others.** For a response to be scored as PR at Day 28 or later, the participant must still be in PR on that day and have had no intervening salvage therapy for an earlier progression, PR or no response (NR). **Mixed response (MR) is defined as improvement in one or more organs with deterioration in another organ manifesting symptoms of GVHD or development of symptoms of GVHD in a new organ. Progression is defined as deterioration in at least one organ without any improvement in others. No response (NR) is defined as absence of any improvement or progression as defined.** Scoring of PR, MR, NR and progression are in comparison to the participant's GVHD status (score) on Day 0 of the study.

### 3.2.2 Proportion of Primary Treatment Failures

No response, progression, administration of additional therapy for GVHD, or mortality by Day 14 post-initiation of treatment will be considered a primary treatment failure.

### 3.2.3 GVHD Flares

Flares are defined as any increase in symptoms of or therapy for acute GVHD after an initial response (i.e., progression from an earlier CR or PR). The rate of flares prior to Day 90 will be examined.

### 3.2.4 Immunosuppression Discontinuation

Immunosuppression discontinuation will be assessed by Day 90, Day 180 and Day 270. It is defined as the discontinuation of corticosteroids and all additional immunosuppressives, except cyclosporine or tacrolimus, for treatment of acute GVHD without subsequent flare by Day 90 post-initiation of therapy and later by discontinuation of all immunosuppressive medications, including cyclosporine or tacrolimus. Note that discontinuation of MMF will be 4 weeks later than other study drugs even in patients with CR.

### 3.2.5 Chronic GVHD

Chronic GVHD is defined per the BMT CTN Manual of Procedures (MOP) Chapter 2. The incidence of chronic GVHD at 9 months will be computed for each treatment arm.

### 3.2.6 Survival

Overall survival at Days 180 and 270 will be computed for each treatment arm.

### 3.2.7 Systemic Infections

All microbiologically documented infections occurring within three months of initiation of therapy will be reported by site of disease, date of onset, and severity. For definitions see the BMT CTN MOP. Post-transplant lymphoproliferative disorder will be reported through 9 months post-initiation of therapy.

# CHAPTER 4

## 4 PATIENT ENROLLMENT AND EVALUATION

### 4.1 Enrollment and Randomization

Patients will be registered using the BMT CTN Advantage Electronic Data Capture (EDC). The following procedures shall be followed:

1. An authorized user at the clinical center completes the initial screening by entering patient demographics and Segment A information (inclusion/exclusion criteria) of the Eligibility Form within 48 hours of diagnosis of acute GVHD.
2. If the patient is eligible, a study number and random treatment assignment is generated.
3. A visit schedule based on treatment start date is displayed for printing.

If a connection is interrupted during a randomization session, the process is completely canceled and logged. A backup manual registration and randomization system will also be available to provide for short-term system failure or unavailability.

#### 4.1.1 Randomization

Patients will be randomized within 48 hours of diagnosis of acute GVHD. Patients who have received MMF for GVHD prophylaxis, within 7 days of GVHD onset, will be randomized in a 1:1:1 ratio to receive ONTAK, pentostatin or etanercept. Patients who have not received MMF for GVHD prophylaxis will be randomized in a 2:1:1:1 ratio (MMF and the other three arms respectively) to one of the four treatment arms. Treatment should be initiated as soon as possible after randomization. A maximum of 48 hours is allowable.

No stratification factors will be used. Strata that were considered included: Grade B versus Grades C/D, age, unrelated donor, single organ versus multi-organ GVHD, full versus non-ablative conditioning, GVHD prophylaxis with a calcineurin inhibitor. However, none of these consistently identify GVHD patient cohorts with different CR rates. Therefore, it was decided that no stratification factors would be used in this trial.

### 4.2 Study Monitoring

#### 4.2.1 Follow-up Schedule

The Follow-up Schedule for scheduled study visits is outlined in Table 4.2.1. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User's Guide.

**Follow-up Assessments**: The timing of follow-up visits is based on the date of randomization. Following randomization, the Transplant Center can print a Patient Visit Schedule listing target

dates for assessments. Weeks 1-8 visits are primarily for acute GVHD scoring. The subsequent visits are for follow-up reports.

**Criteria for Forms Submission**: Criteria for timeliness of submission for all study forms are detailed in the Data Management Handbook and User's Guide. Forms that are not received at the DCC within the specified time will be considered delinquent. Transplant Centers can view past due forms via the Web-based data entry system. A missing form will continue to appear until the form is entered into the DCC's master database, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Data Management Handbook and User's Guide.

**Reporting Patient Deaths:** The Recipient Death Information must be entered into the web-based data entry system within 24 hours of knowledge of a patient's death. If the cause of death is unknown at that time, it need not be recorded at that time. However, once the cause of death is determined, the form must be updated.

**CIBMTR Data Reporting:** All transplant centers participating in BMT CTN protocols are required to register all of their transplant patients, not just those enrolled on BMT CTN protocols, with the CIBMTR, using standard CIBMTR Preregistration and Transplant Essential Data Forms (instructions available at www.ibmtr.org). CIBMTR Day 100 Report Forms (including the Core, Graft and Disease Inserts) and CIBMTR Follow-up Forms (including the Core and Disease Inserts) are required for patients enrolled on BMT CTN protocols and will be submitted directly to the CIBMTR at the times specified in the Data Management Handbook and User's Guide.

**Table 4.2.1**
**FOLLOW-UP SCHEDULE**

| Assessment Time | Target Day[1] (Days Post-Enrollment) |
|---|---|
| 1 week | 7 days |
| 2 weeks | 14 days |
| 3 weeks | 21 days |
| 4 weeks | 28 days |
| 5 weeks | 35 days |
| 6 weeks | 42 days |
| 7 weeks | 49 days |
| 8 weeks | 56 days |
| 90 days | 90 days |
| 120 days | 120 days |
| 6 months | 180 days |
| 9 months | 270 days |

[1.] Target day range = ±2 days up to Week 8, and ±14 days for Days 90, 120, and ±28 days for 6 and 9 months, post-randomization.

4.2.2 Assessments

All assessments are considered standard-of-care unless identified below by "*."

Pre-Randomization

1. Recommended biopsy of involved tissue*
2. History and physical exam including height and weight
3. Pregnancy test (if applicable)
4. Complete acute GVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, weight and liver function tests
5. CBC with differential, platelet count
6. Liver function tests (bilirubin, alkaline phosphatase, AST, ALT) plus creatinine
7. Tacrolimus/cyclosporine level
8. Samples for laboratory studies (see Appendix C)*

Post-Randomization

1. Recommended skin biopsy if skin involved on Day 28 (see Appendix C-4)*

2. Karnofsky or Lansky performance status on Days 28, 90, 180 and 270
3. Complete acute GVHD staging and grading information including assessments of rash, diarrhea, nausea/vomiting, weight and liver function tests weekly until Day 56, and 3, 4, 6 and 9 months, creatinine weekly until Day 28, and recommended creatinine on Days 35, 42, 49, 56, and 3, 4, 6, and 9 months.
4. Chronic GVHD evaluation (if present) 3, 4, 6 and 9 months
5. CBC with differential, platelet count weekly until Day 56
6. Toxicity evaluation weekly until Day 56
7. Steroid dose weekly until Day 56, and 3, 4, 6 and 9 months
8. Tacrolimus/cyclosporine levels weekly until Day 28
9. MMF levels in weeks 1 and 2 (4 levels at time 0, 1, 2, 6 hours on oral MMF; 0, 2, 4, 6 hours if on IV MMF) (see Appendix C-5)*
10. Samples for laboratory studies on Days 0, 14, 28 and 90 (see Appendix C)*
11. Infections through Day 90 and occurrence of Post-transplant Lymphoproliferative Disorder (PTLD) through Day 270.

### 4.2.3 Weekly GVHD Monitoring

GVHD scoring will be performed weekly for 8 weeks from study entry. Days 1, 28 and 56 ($\pm$7 days) scoring must be performed by direct observation at the Transplant Center.

### 4.2.4 Serious Adverse Event (SAE) Reporting

Serious Adverse Events will be consistent with BMT CTN procedures (BMT CTN Administrative Manual of Procedures, Chapter 6) and reported through an expedited Adverse Event (AE) reporting system. Serious and unexpected Grades 3-5 adverse events should be reported within three working days. Deaths are to be reported within 24 hours. Other SAEs will be tracked periodically as defined in the Form Submission Schedule, and 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 Grade 3-5 unexpected adverse experiences on an annual basis.

## Table 4.2.2 REQUIRED ASSESSMENTS

| | (Day 0) | Days Post Randomization | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Baseline | **7** | **14** | **21** | **28** | **35** | **42** | **49** | **56** | **90** | **120** | **180** | **270** |
| Suggested biopsy of involved tissue (+ extra skin biopsy Days 1 & 28[1]) | X | | | | X | | | | | | | | |
| History and physical exam | X | | | | | | | | | | | | |
| Pregnancy test (if applicable) | X | | | | | | | | | | | | |
| Karnofsky/Lansky performance status | | | | | X | | | | | X | | X | X |
| Acute GVHD evaluation | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Chronic GVHD evaluation | | | | | | | | | X | X | X | X | X |
| CBC with differential, platelet count | X | X | X | X | X | X | X | X | X | | | | |
| Basic chemistry (creatinine) | X | X | X | X | X | X[2] | X[2] | X[2] | X[2] | X[2] | X[2] | X[2] | X[2] |
| Liver function tests (alkaline phosphatase, bilirubin, AST, ALT) | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Toxicity evaluation | | X | X | X | X | X | X | X | X | | | | |
| Steroid dose | | X | X | X | X | X | X | X | X | X | X | X | X |
| Tacrolimus/cyclosporine levels | X | X | X | X | X | | | | | | | | |
| MMF levels (see Appendix C-5[1]) | | X | X | | | | | | | | | | |
| Blood and serum for ancillary laboratory studies (see Appendix C[1]) | X | | X | | X | | | | | X | | | |

[1] Research procedures beyond that required for usual care.
[2] Recommended

# CHAPTER 5

## 5 STATISTICAL CONSIDERATIONS

### 5.1 Study Design Synopsis

This study is a multi-center, randomized two-stage Phase II trial designed to evaluate several frontline treatments for acute GVHD. After 20 patients per treatment are evaluable (stage I), a stopping rule for lack of efficacy will be applied, so that future patients may be randomized to the most promising treatments. The remaining patients will be randomized in stage II to the treatments passing through stage I, to a maximum of 65 patients per arm or 180 patients total.

### 5.2 Accrual

It is estimated that nine months of accrual, or approximately 20 patients per month, will be necessary to enroll the targeted sample size. Only Core Centers will enroll patients on this study.

### 5.3 Randomization

Randomization will occur separately in two strata. Stratum 1 consists of patients who had received MMF previously as GVHD prophylaxis and are not eligible to receive MMF again as GVHD treatment, while stratum 2 consists of patients who did not receive MMF previously as GVHD prophylaxis. Patients in stratum 1 will be randomized in equal proportions to the other three treatment arms. Patients in stratum 2 will be randomized in unequal proportions to all four arms (initially 40% to MMF and 20% to each of the other three arms). This unequal allocation was chosen to target approximately equal sample sizes per arm (45) by the end of the study, assuming 33% of patients will belong to stratum 1. Randomization in each stratum will occur using two sets of permuted blocks of varying sizes. One set of permuted blocks will determine MMF vs. not MMF for patients in stratum 2, while the other set will determine which of the other three arms the patient is assigned to. After 80 patients have been enrolled and followed up for the primary endpoint, two things will be assessed. First a stopping rule will be applied as described below to determine whether any of the arms should be closed for lack of efficacy. Second, the proportion of patients entering stratum 1 will be assessed. If the MMF treatment arm is closed for lack of efficacy, then we will revert to equal randomization for any remaining treatment arms. Otherwise, based on the number of non-MMF arms remaining (i.e. not closed for lack of efficacy) and the proportion of patients entering stratum 1, we will adjust the allocation probability P to MMF treatment in stratum 2 according to the following formula:

$$P=(k*i+3*(180-i)-4*k*y)/(3*(k+1)*(180-i)*(1-r)),$$

where r=observed percent of MMF prophylaxis use so far, i=80 is the number accrued, k is the number of non-MMF treatment arms remaining, and y is the number assigned to MMF treatment. This formula is obtained by setting the expected final number of patients assuming allocation of P in stratum 2 to MMF treatment equal to the expected final number of patients in

each of the other treatment arms. Some examples of the new allocation rates are given in the table below, as a function of the observed MMF prophylaxis rate and the number of remaining arms. Also given are the expected sample sizes after 80 patients and after 180 patients in both the MMF arm and a representative non-MMF arm.

| **Evaluated at** | | **After 80 Patients** | | | **After 180 Patients** | |
|---|---|---|---|---|---|---|
| MMF Rate, r | # of non-MMF arms remaining, k | # MMF | # other trt | New P | # MMF | # other trt |
| 0.2 | 3 | 26 | 18 | 0.243 | 45 | 45 |
| 0.2 | 2 | 26 | 18 | 0.354 | 54 | 54 |
| 0.2 | 1 | 26 | 18 | 0.578 | 65 | 65 |
| 0.33 | 3 | 21 | 20 | 0.352 | 45 | 45 |
| 0.33 | 2 | 21 | 20 | 0.478 | 53 | 53 |
| 0.33 | 1 | 21 | 20 | 0.732 | 65 | 65 |
| 0.45 | 3 | 18 | 21 | 0.498 | 45 | 45 |
| 0.45 | 2 | 18 | 21 | 0.645 | 53 | 53 |
| 0.45 | 1 | 18 | 21 | 0.938 | 65 | 65 |
| 0.5 | 3 | 16 | 21 | 0.580 | 45 | 45 |
| 0.5 | 2 | 16 | 21 | 0.738 | 53 | 53 |
| 0.5 | 1 | 16 | 21 | 1.000 | 60 | 65 |

## 5.4 Primary Endpoint

The primary endpoint for the trial is the proportion of complete response to acute GVHD therapy at Day 28.

## 5.5 Primary Hypothesis

Each arm will be compared to the historical rate of 35% CR at Day 28 of therapy. This will be done by computing a "Bayesian posterior probability of efficacy," i.e., the Bayesian posterior probability that the response rate is > 35%, using a Beta (3.5, 6.5) prior distribution. This prior has a mean of 35% and a "weight" of 10 patients. If this posterior probability of efficacy is > 90%, the treatment is considered a candidate for further investigation based on the primary endpoint. No statistical comparisons between the arms will be performed: the purpose of the trial is only to identify treatments for further investigation through comparisons to a historical control of corticosteroids alone and is underpowered for comparisons among the arms.

## 5.6 Study Design

Stage I: Randomize patients in two strata as described above until 80 patients are evaluable for the primary endpoint. After 80 patients are evaluable, apply the following stopping rule for lack of efficacy: if the Bayesian posterior probability of efficacy for a particular arm is < 20%, close the arm to further accrual. This Bayesian stopping rule is equivalent to stopping if the number of patients alive in CR at Day 28 is less than or equal to 4 out of 20.

Stage II: Continue randomizing patients after modifying the randomization scheme as described above. If the MMF treatment arm is dropped for lack of efficacy, then the randomization will be done equally among all remaining treatment arms. If not, then the randomization probabilities will be recomputed according to the rate of MMF prophylaxis use in the first 80 patients as well as the number of remaining non-MMF treatment arms that passed successfully through stage I. This randomization will be done up until a maximum of 65 per group or 180 patients have been accrued. At the end of the study, a treatment identifying a good candidate will be based on the primary endpoint if the Bayesian posterior probability of efficacy is > 90%.

This Bayesian rule corresponds to a cutoff in terms of numbers of patients alive in CR at Day 28, which depends on the final enrolled sample size and therefore the number of treatment arms passing through stage I. Sample cutoffs are given in Table 5.6 below.

**Table 5.6**
**CUTOFFS FOR PATIENTS ALIVE IN CR AT DAY 28**

| Number of Treatments Passing through Stage I | Final n per Treatment Group | Declare Treatment a Good Candidate if # of CRs is ≥ x |
|---|---|---|
| 1 | 65 | 29 |
| 2 | 65 | 29 |
| 3 | 52 | 24 |
| 4 | 45 | 21 |

## 5.7 Power and Operating Characteristics of Study Design

This design, including the stopping rules for lack of efficacy as well as stopping for mortality discussed in Section 5.8, was simulated for several configurations of true success rates, which represent improvements over the historical rate of 0%, 10%, 15%, and 20%. Table 5.7 below gives the configurations of true CR rates considered, with bold entries denoting effective treatments relative to the historical control rate of 35%. Note that because there are multiple decisions being performed on the various treatments, there is no simple definition of power or type I error. Therefore, for each configuration of success rates, several measures of power and type I error are presented which are appropriate for a design with multiple arms. Also note that these operating characteristics apply for the situation when the true proportion r in stratum 2 is 33%. Deviations from this will have minimal impact on the operating characteristics because the final sample sizes are similar regardless of r.

First we report the marginal probability of identifying treatment arm 1 as a good candidate. This can be interpreted as the power for arm 1 individually, if arm 1 is effective, and the type I error rate for arm 1 individually if arm 1 is not effective. Next we report the probability that all effective treatments are identified as good candidates and no ineffective treatments are identified, which can be interpreted as a "perfect" decision on all treatments. Third, we present the probability that at least one effective treatment is identified as a good candidate and no ineffective treatments are identified. This can be interpreted as a "perfect" decision on the ineffective treatments and a "good" decision on the effective treatments (i.e. we correctly

identified at least one good candidate). Finally, we present the probability that at least one ineffective treatment is identified as a good candidate, while none of the effective treatments are identified. This can be interpreted as a type I error and is an undesirable result. Results are based on 10000 simulated datasets for each scenario. Note that this two-stage design has approximately 75% marginal probability or power to detect an improvement of 15% in the CR rate over the historical control rate, and more than 90% marginal power to detect a 20% improvement. Also, if there are two or more effective treatments with 50% CR rates, the design has at least 80% power to identify at least one of them as a good candidate while not identifying any ineffective treatments.

**Table 5.7**
**POWER AND OPERATING CHARACTERISTICS**

| **True CR Rates (Effective arms denoted in bold)** | | | | **Probability of Identifying as Good Candidates:** | | | |
|---|---|---|---|---|---|---|---|
| **Arm 1** | **Arm 2** | **Arm 3** | **Arm 4** | **Arm 1** | **All effective treatments and no ineffective treatments** | **At least one effective treatment and no ineffective treatments** | **No effective treatments and at least one ineffective treatment** |
| 0.35 | 0.35 | 0.35 | 0.35 | 7% | - | - | 27% |
| **0.45** | 0.35 | 0.35 | 0.35 | 49% | 40% | 40% | 10% |
| **0.50** | 0.35 | 0.35 | 0.35 | 75% | 59% | 59% | 5% |
| **0.55** | 0.35 | 0.35 | 0.35 | 91% | 72% | 72% | 2% |
| **0.50** | **0.50** | 0.35 | 0.35 | 74% | 47% | 80% | 1% |
| **0.50** | **0.50** | **0.50** | 0.35 | 72% | 35% | 91% | 0% |
| **0.50** | **0.50** | **0.50** | **0.50** | 72% | 27% | 99% | - |

Frequentist confidence intervals (CI) will also be constructed for the CR rate at Day 28. For a treatment which is declared a good candidate ($\geq$ 29/65 or 24/52 or 21/45 CRs), a 90% CI will have a lower bound of at least 34% and a 95% CI will have a lower bound of at least 32%.

## 5.8 Stopping Guidelines

At the end of stage I, lack of efficacy stopping guidelines will be implemented separately for each arm. If the Bayesian posterior probability of efficacy for a particular arm is < 20%, it will be recommended that the arm be closed to further accrual. For lack of efficacy, an arm will be closed if the posterior probability is $\leq$ 20% that the CR rate at Day 28 is greater than 35%. This is equivalent to stopping for lack of efficacy if 4 or fewer of the first 20 patients are CRs. Once the stopping criteria have been met for a particular arm, no more patients will be randomized to that arm.

After 20 patients have been accrued, this stopping rule for lack of efficacy results in the following stopping probabilities as a function of the true CR rate.

**Table 5.8**
**STOPPING GUIDELINES**

| N Evaluable | Stop if # of CRs are | Probability of Stopping for Lack of Efficacy | | | |
|---|---|---|---|---|---|
| | | CR=15% | CR=25% | CR=35% | CR=45% |
| 20 | ≤ 4 | 83% | 42% | 12% | 2% |

5.8.1 Stopping Guidelines for Mortality

Mortality of 25% at Day 28 of therapy would be considered unexpected and an unacceptable number of excess deaths compared to the anticipated and historical rate of approximately 10%.

Therefore, a stopping rule for Day 28 mortality will be applied after every 10 patients are accrued per group. A particular arm will close at interim look t if the number of deaths on that arm is >= $x_t$., e.g., if four or more deaths in the first 10 patients or six or more deaths in the first 20 patients, etc. as detailed in Table 5.8.1 below. Looking after every 10 patients per group corresponds approximately to looking at mortality every 2 months starting with the third month (i.e. after months 3, 5, 7, 9), assuming an accrual rate of 20 patients/month. If one or more arms should close after stage I, we will review the mortality data slightly more frequently.

The stopping rules and probabilities of stopping at each interim look are given in the table below. Note that the final sample size per arm is variable due to the stopping rule for lack of efficacy; therefore, the cumulative stopping probabilities are also given for cumulative sample sizes of 40 and 60. In summary, a treatment with a 10% mortality rate, which is what is expected based on historical data, will have a 2.6% chance of triggering the stopping rule for mortality. If that mortality rate for a treatment under investigation should increase to 25%, a 15% increase in mortality over the historical rate, we will have a 65.2% chance of triggering the stopping rule within 40 patients and a 76.8% chance of triggering the stopping rule within 65 patients.

**Table 5.8.1**
**STOPPING PROBABILITIES FOR VARIOUS MORTALITY RATES AT DAY 28**
(Stopping rule is defined as stop if the number of deaths at look t is $\geq x_t$)

| Look (t) | Total N | $x_t$ | Probabilities of Stopping at Look t for True Mortality Rates of | | | | |
|---|---|---|---|---|---|---|---|
| | | | p = 0.1 | 0.15 | 0.2 | 0.25 | 0.3 |
| 1 | 10 | 4 | 1.4% | 5.3% | 12.7% | 22.8% | 35.2% |
| 2 | 20 | 6 | 0.7% | 4.2% | 11.1% | 20.4% | 26.4% |
| 3 | 30 | 8 | 0.3% | 2.7% | 7.5% | 13.2% | 15.0% |
| 4 | 40 | 10 | 0.2% | 1.8% | 5.8% | 8.8% | 9.0% |
| 5 | 50 | 12 | 0.0% | 1.6% | 4.7% | 6.5% | 5.4% |
| 6 | 60 | 14 | 0.0% | 1.0% | 3.8% | 5.1% | 3.2% |
| Cumulative probability of stopping after 40 patients | | | 2.6% | 14.0% | 37.1% | 65.2% | 85.6% |
| Cumulative probability of stopping after 60 patients | | | 2.6% | 16.6% | 45.6% | 76.8% | 94.2% |

If lack of efficacy or mortality rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures.

## 5.9 Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Between group comparisons will be performed for continuous variables via a t-test and for categorical variables, via the chi-square test. Covariates considered will include age (recipient and donor), sex, type of donor (related versus unrelated), graft source (marrow versus peripheral blood versus umbilical cord blood), HLA match/mismatch, GVHD Grades B versus C/D at enrollment, single versus multiorgan GVHD, skin GI, liver involvement, CMV seropositivity in recipient or donor, use of CSA versus tacrolimus prophylaxis, and day of onset of GVHD.

## 5.10 Endpoints and Analysis Plan

The primary endpoint is the proportion of CR at Day 28.

The secondary endpoints are:

- Proportion of PR, mixed response, no response, and progression at Day 28
- Proportion of treatment failures by Day 14
- Incidence of GVHD flares before Day 90
- Incidence of discontinuing immune suppression without flare by Days 90, 180 and 270 of therapy
- Incidence of chronic GVHD within 9 months
- Survival at 6 and 9 months after initiation of therapy

- Incidence of systemic infections within 3 months of therapy
- Incidence of EBV lymphoma within 9 months of therapy

In addition to the estimation of the Bayesian posterior probabilities as discussed above, estimates and 95% confidence intervals will be constructed for all endpoints, separately for each study group. These may include point estimates, Kaplan-Meier survival curves, or cumulative incidence curves depending on the secondary endpoint of interest. Acceptance of a particular study agent as sufficiently promising for future Phase III testing will include efficacy in the primary study endpoint as well as the secondary endpoints. Toxicities, secondary infections and 9-month survival may be of particular importance in assessing the therapeutic index for each study agent.

# APPENDIX A

# REFERENCES

# APPENDIX A

# REFERENCES

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# APPENDIX B

# INFORMED CONSENTS AND EDUCATIONAL MATERIAL

## INFORMED CONSENT TO PARTICIPATE IN RESEARCH

## ASSENT TO PARTICIPATE IN RESEARCH

## CLINICAL INFORMATION FOR RESEARCH PROTOCOL

# *Informed Consent to Participate in Research*

We invite you to participate in this research study. About 180 patients will participate at up to 27 centers around the country. Your study participation will last about 9 months. This is a study for patients who have a new diagnosis of graft-versus-host disease (GVHD) after receiving an allogeneic (donor) stem cell transplant.

GVHD is a medical condition where the donor cells attack and damage your tissues after you have a transplant. GVHD can cause:

- Skin rashes,
- Intestinal problems like feeling sick to your stomach (nausea), throwing up (vomiting), diarrhea, or,
- Liver damage like hepatitis or jaundice.

GVHD can also increase your risk of infection. Sometimes, GVHD can be controlled with treatments that use corticosteroids (like prednisone). More often, patients need long-term drug therapy to control their GVHD symptoms and to suppress the immune system. Long-term drug therapy has risks and side effects. Because of the risks of continued GVHD, new drugs to control GVHD will be tested in this study.

All four drugs being tested in this study are approved by the US Food and Drug Administration (FDA) for treating diseases other than GVHD. This study will test whether these new drugs can help treat GVHD better than the standard therapy of steroids alone. One of the study drugs is given either orally, or if you cannot take pills, then it will be given by IV infusion. The other 3 study drugs are given either by IV or by injection under the skin.

This consent form tells you about the study. The study investigators have found that you are eligible to participate in this study, and this form must be signed before any treatment related to the study is given to you. The doctors in charge of this study (the investigators) or other staff will also discuss this study with you and answer any questions you might have. Before you decide to join this study, please read this information and ask any questions about things you do not understand. Some patients find it helpful to have a family member or friend with them to help ask questions and listen to information.

This study will give more information to doctors about future treatment choices for GVHD. Importantly,

- You will not be paid to be in this study.
- You or your insurance company will pay for all medical bills for your treatment.
- You will not be charged for research tests.
- You will also face the same risks and benefits as any other transplant patient.

Before you decide to join the study, please read the information below. Feel free to ask questions to understand your rights and protections. It is your choice to take part in this study. **You and your doctor will discuss other treatment options if you decide not to be in this study.**

**Your Name:**_____

**Title of Research Study:** Initial Systemic Treatment of Acute GVHD: A Phase II Randomized Trial Evaluating Etanercept, Mycophenolate Mofetil (MMF), ONTAK and Pentostatin in addition to Corticosteroids

**Principal Investigator:** Daniel Weisdorf, M.D., University of Minnesota, MMC 480, Minneapolis, MN 55455, 612-624-3101

**Transplant Center Principal Investigator:** _____

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

The drugs in this study were donated by the companies that made them, and these companies also gave some financial support to help pay the costs of this study. These companies did not plan or design this study. In addition, they will not have a part in analyzing the results of this study.

**The Purpose of the Study**

The study is to test new treatments for acute Graft-versus-Host Disease (GVHD). GVHD is a medical condition where the donor graft attacks and damages your tissues after you have a transplant. If you have limited GVHD of the skin or only GVHD in your upper gastrointestinal (GI) tract, your doctor may decide that you need systemic treatment, though not all patients receive systemic treatment for this type of GVHD. By offering you this study, your doctor recommends that you get additional treatment.

Four new drugs are being tested:

- Etanercept (ENBREL®)
- Mycophenolate Mofetil (MMF) (CellCept®)
- Denileukin Diftitox (ONTAK®)
- Pentostatin (Nipent®)

In this study, use of one of these four drugs, along with prednisone or methylprednisolone, may increase the control of your GVHD. It is uncertain whether use of any of these new drugs will improve control of GVHD.

Each drug will be given along with the standard therapy of prednisone or methylprednisolone which is the usual therapy for acute GVHD. There is no placebo or sugar pill treatment in this trial. Everyone in the study will receive prednisone or methylprednisolone plus one of the four new drugs, unless you are already receiving MMF; then only one of the other 3 drugs will be assigned to you. All patients in the study will be randomly assigned (like flipping a coin) to receive one of the four new study drugs with some statistical adjustment to ensure that about the same number of patients will receive each of the new study drugs.

**What will be done if you take part in this study?**
In addition to receiving prednisone plus the assigned study drug, for your routine care (outside this study), you will be watched closely for signs and symptoms of:

- GVHD,
- Changes in your blood counts,
- Changes in liver and kidney function, and
- Any signs of infection.

For the study, the GVHD signs and symptoms will be recorded at least once a week for the first 8 weeks. The exam at week 4 and week 8 must be done at the transplant center. Other weekly exams and tests may be done at the transplant center, or at a local doctor's office if your condition improves and it is closer to where you live.

If you are randomly chosen to receive MMF, you will have additional blood samples drawn to study the actions of this drug in your body. You will have 5 mL (about 1 teaspoon) of blood drawn at 4 different times (total of 20 mL) on one day between Days 3 and 7 and again on another day between Days 10 and 14 (for a total of 40 mL). This blood will be drawn from an existing central venous catheter or a temporary peripheral venous catheter.

## STUDY DRUGS

### What are the possible discomforts and risks?

### Infections

Because GVHD is caused by an immune attack on your tissues from the transplanted donor cells, all treatments for GVHD include drugs to suppress (turn off) this immune attack.

The risk of infection is increased in patients with GVHD and those taking immune suppressing corticosteroids like prednisone or methylprednisolone, the standard therapy for GVHD. All four study drugs can also increase your chance of infection. Therefore, you will take several protective antibiotics and be watched carefully for any infections while you are being treated for

GVHD. Tell your doctors promptly if you get a fever, chills, a cough or any other symptoms that might be part of an infection.

**Side Effects of Study Drugs**

All drugs can have side effects, both the standard therapy (steroids) and the new drugs being tested in this study. Your doctors will watch you carefully for any side effects and will modify your treatment if they develop. Experience with pediatric patients treated with these agents for GVHD is limited.

**Etanercept (ENBREL):** Etanercept is an anti-inflammatory and immune suppressive drug.

Etanercept can cause certain side effects. Some patients develop redness or soreness at the injection sites. Sometimes headache, dizziness, or rash can occur. A few people develop fever, upper respiratory tract symptoms (like a cold with cough, stuffy nose or sinusitis) and rarely some serious infections have developed in people taking etanercept. Rarely, blood counts can be lowered in people taking etanercept.

In this study, the drug will be given by injection under the skin (subcutaneously) twice weekly for up to four weeks.

**Mycophenolate Mofetil (MMF) (CellCept):** MMF is a potent immunosuppressive drug that blocks the growth of lymphocytes (immune cells), which can cause GVHD.

MMF can cause certain side effects. Occasionally it can lower the blood counts. More frequently, it can cause nausea, vomiting or diarrhea. Rarely, serious gut injury can occur.

In this study, it is given twice daily as capsules or a liquid. It may be continued for 8-10 weeks or as long as GVHD is active.

**Denileukin Diftitox (ONTAK):** ONTAK is an immune suppressive drug, but has also been used to treat certain kinds of lymphocyte cancers called T cell lymphomas.

ONTAK can cause certain side effects. Most patients develop a headache, skin rash, fever, chills, muscle aches, and joint aches while the infusion is given or within a day of each infusion. Dyspnea (shortness of breath), chest tightness, and flushing of the skin may occur. Slowing the infusion may minimize these side effects. Occasionally, acute allergic-like reactions with itching, temperature, chills, or a rash can develop. These side effects may be relieved by temporarily stopping and then restarting the infusion and giving medications before the dose of ONTAK like acetaminophen (Tylenol) or diphenhydramine (Benadryl) to try to prevent these reactions.

In a few patients, ONTAK can cause fluid accumulation (edema). This can lead to swelling in the tissues, legs, or rarely fluid in the lungs or low blood pressure. If given in higher doses than in this study, anorexia (loss of appetite), upset stomach with nausea, vomiting or diarrhea can occur. Rarely, liver problems or low blood counts can develop.

In addition, new information has suggested that rarely, vision changes or vision loss can occur in patients treated with ONTAK.

In this study, ONTAK will be given intravenously (over approximately 60 minutes) Days 1, 3, 5 of the first week and repeated in the third week (Days 15, 17, 19) of your treatment.

**Pentostatin (Nipent):** Pentostatin is an immune suppressive drug that is sometimes used as an anticancer chemotherapy drug as well.

Pentostatin can cause certain side effects. Up to half of the patients develop skin rash, nausea, vomiting, anorexia (loss of appetite), mouth sores, or diarrhea, although these side effects usually occur when patients are given higher dosages of the drug than you will receive on this study. Rarely, patients have kidney trouble. In a few patients treated with higher dosages of pentostatin, low platelet counts, anemia, or increased need for transfusions have developed. Rarely, low white blood cell counts can occur. A small number of patients also develop temporary abnormalities in their liver function blood tests.

In this study, pentostatin will be given intravenously (over 15-30 minutes) on Days 1, 2, 3, of the first week and repeated in the third week on Days 15, 16 and 17 of your treatment.

**Voluntary Participation**

You are free to choose to participate or decline participation now or at any time in your treatment course. If you decline to participate now or withdraw your consent after treatment is started, standard GVHD therapy will be available to you. None of your rights are waived by enrolling in this research study and therapy for any medical conditions will continue even if you withdraw from the study. Your doctors have made themselves available to answer any of your questions about participation in this study and will continue to do so throughout your treatment. Throughout the study, the researchers will tell you of new information that might affect your decision to remain in the study. If you have additional questions about the study or about your rights as a participant in this study, you may contact [the Principal Investigator at your transplant center]. If you have any questions or concerns, please ask your doctors or the study coordinators.

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

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

- The study is cancelled by the Food and Drug Administration (FDA) or the National Institutes of Health (NIH).

**Benefits**

Although this study cannot be guaranteed to be of benefit to you, it is hoped that your taking part may help in the treatment of your GVHD. A possible advantage of this study is that one of the study drugs may treat GVHD better than the others. However, you may not benefit from this treatment. It is also hoped that the information learned from this study will help your doctors treat patients in the future who get GVHD.

**Compensation**

In the event that this research activity results in an injury, treatment will be available including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to your insurance company. If you think you have suffered a research related injury, let the study physicians know right away. Unexpected side effects or accidents might result in your getting sicker than anticipated in the course of this treatment. All available medical care will be provided to you, but you and your insurance company (3rd party payer) are responsible for the costs of all such care.

**Confidentiality**

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

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

You will be given a copy of this form.

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

a. Purpose: As a research participant, I authorize the Principal Investigator and the researcher's staff to use and disclose my individual health information for the purpose of conducting the research study entitled *Initial Systemic Treatment of Acute GVHD: A Phase II Randomized Trial Evaluating Etanercept, Mycophenolate Mofetil (MMF), Denileukin Diftitox (ONTAK), and Pentostatin in Combination with Corticosteroids*

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

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

(*list hospitals, clinics or providers from which health care information can be requested)*

_____

_____

_____

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

- Principal Investigator and the researcher's staff
- Dr. Dan Weisdorf, Study Chairperson and staff/laboratories at University of Minnesota
- Staff/laboratories identified in the protocol for the evaluation of other laboratory samples; e.g., Dr. Pamela Jacobson/University of Minnesota and Dr. Brian Nickoloff/Loyola University of Chicago
- National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI), both of the National Institutes of Health (NIH), study sponsors
- Blood and Marrow Transplant Clinical Trials Network (BMT CTN), data coordinating center
- U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)

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

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

f. Right to Revoke: I can change my mind and withdraw this authorization at any time by sending a written notice to the Principal Investigator to inform the researcher of my decision. If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about me will be collected by or disclosed to the researcher for this study.

g. Potential for Re-disclosure: My individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected. Examples include potential disclosures for law enforcement purposes, mandated reporting or abuse or neglect, judicial proceedings, health oversight activities and public health measures.

h. This authorization does not have an expiration date.

**Blood Samples for Research**
You will have tests to measure the effect of GVHD and the drugs on your immune system.

For these tests you will need to give a blood sample (3-5 teaspoonsful) at Days 0, 14 and 28 of the study, and 1 teaspoonful on Day 90. If your GVHD comes back, you will also need to give 1 teaspoonful of blood.

Additionally, to understand how certain genes may affect the development of GVHD in you, some genes that affect inflammation hormones (cytokines) will be studied in your blood or in a swab of the tissues in your mouth.

**Skin Samples for Research**
GVHD can cause a skin rash. To study how GVHD may affect your skin and the genes in your skin cells, a small skin sample (skin biopsy) will be taken before you start treatment and another 28 days after you start treatment for your GVHD. The skin samples are taken from an area where you currently have a rash. Each sample is a 1/8" (one-eighth of an inch) circle of skin.

These skin samples are not required for your treatment, but will be used to study what happens to your skin during treatment. This may better explain how acute GVHD injures the skin cells and the effect of treatment by the different study drugs.

The samples collected for research purposes will be sent to laboratories that have contracts with the National Marrow Donor Program (NMDP) to conduct these research tests. They will be labeled with unique codes that do not contain information that could identify you. A link to this code does exist. The link is stored at the Data Coordinating Center for the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). The staff at the laboratories where your samples are being tested do not have a link to this code. Your samples will be stored at these laboratories until the entire sample has been used for the research tests or until the end of the study.

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

**Research Samples are Optional**
It is your choice to volunteer to give these extra blood and skin samples. Your blood samples and skin biopsy will be collected confidentially (your name will not be attached to them). Only the study doctors or personnel working with them will study your skin and blood samples.

If you agree to allow your blood and skin samples to be used for research, you can change your mind later. If you do, please contact [the Principal Investigator at your transplant center] in writing to state that you are withdrawing permission for your blood or your skin biopsy to be used for research. His mailing address is on the first page of this consent form. Any unused blood or biopsy tissue 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.

Please indicate your choice(s) below:

Blood Samples

_____ I give permission to use additional samples of my blood for GVHD-related research.

_____ No, I do not wish samples of my blood to be used for additional research.

Skin Samples

_____ I give permission to use additional sample of my skin for GVHD-related research.

_____ No, I do not wish samples of my skin to be used for additional research.

**Patient's Signature:**_____

**Date:**_____

**Statement of Consent**

I consent to participate in this study treatment plan for GVHD including the use of prednisone plus one of four study drugs that will be assigned to me in random fashion. I have asked questions that I have now and have received answers. I know that I can ask additional questions in the future.

Signature Date

I have explained in full the details of this study treatment plan for acute GVHD and answered as best as possible all questions that were asked.

Signature of Counseling Physician Date

# *Assent to Participate in Research (Ages 6 to 17 years old)*

**1. Title of Research Study**
Initial Systemic Treatment of Acute GVHD: A Phase II Randomized Trial Evaluating Etanercept, Mycophenolate Mofetil (MMF), ONTAK and Pentostatin in addition to Corticosteroids

**2. Purpose of the Study**
After your donor transplant, you have developed a new problem called graft-vs-host disease (GVHD). The donor cells are attacking your tissues and can cause skin, stomach, or liver trouble. Your doctors will give you standard drugs to treat GVHD (like prednisone), but want to test whether one of four new drugs can treat GVHD even better. The four drugs (etanercept, ONTAK, MMF, or pentostatin) all can treat some diseases. Some patients with GVHD have gotten better using these drugs.

Your doctors want you to join a study where you will get only one of these drugs in addition to the standard one, prednisone. Your doctors will watch you carefully and check to see if your GVHD is improving or if any of the drugs are causing new problems.

These treatments may cause infections, so your doctors will watch you closely for fever or chills. If you feel sicker, tell your doctors.

If you have any questions don't hesitate to ask your doctors and tell them whether you understand their answers.

Your parents (or a guardian) are also asked for their permission for you to join this treatment study.

I agree to be in the GVHD study.

_____ _____
Patient's Signature Date

_____ _____
Doctor's Signature Date

# CLINICAL INFORMATION FOR RESEARCH PROTOCOL

(Appropriate for Ages 6 – 17)

After your transplant, you have developed a new problem called graft vs. host disease (GVHD). The donor cells are attacking your tissues and can cause skin, stomach, or liver trouble. Your doctors will give you standard drugs to treat GVHD (prednisone), but want to test whether adding one of four new drugs can treat GVHD even better. The four drugs (etanercept, ONTAK, MMF or pentostatin) can treat some diseases, but have not been well studied for patients with GVHD. Some patients with GVHD have gotten better using these drugs. If you join the study, you will receive prednisone, a medicine given to all patients with GVHD. In addition, the doctors would randomly choose (like flipping a four-sided coin) which of the four study drugs you will receive, unless you are already receiving MMF; then it would be like a 3-sided coin assigning you one of the other three new drugs.

The first drug, etanercept, is given in a shot under the skin twice a week for four weeks. Sometimes the skin spot where the shot was given can get red or sore and sometimes people get headaches or dizzy.

The second drug, MMF, is given twice daily as pills or a liquid. If you can't take any pills it can be given through a vein (IV) or your Hickman catheter. It sometimes causes stomach upset or diarrhea and sometimes lowers blood counts.

The third drug, ONTAK, is given through your IV or through your Hickman catheter three days a week the 1st and 3rd week of treatment. Sometimes people get a headache, skin rash, fever, or chills. Sometimes people have some breathing trouble or swelling of their feet.

The fourth drug, pentostatin, is given through you IV on Days 1, 2, 3, and 15, 16, 17 of your GVHD treatment. It can cause a rash, stomach trouble, or diarrhea. It can lower blood counts in some people.

You will only receive one of the study drugs along with prednisone. Your doctors will watch you carefully for any side effects and to see how well your GVHD is responding to the treatment. Any of these drugs can increase your chance of getting an infection, so your doctors will watch you carefully for fever or other problems as well.

Any time you want, you can ask questions of your doctors or your parents. Make sure you understand their answers and ask more questions if you don't.

While you are treated for GVHD, you will have exams and blood tests to check how well you are doing. If you have a rash, the doctors may want a small skin biopsy (numbing the skin and taking a 1/8" circle of skin) to look at under the microscope. This will be used to study how the skin GVHD is changing.

The doctors want to improve treatments for GVHD and hope that this study will identify new drugs that are a better treatment than prednisone alone.

# APPENDIX C

# LABORATORY PROCEDURES

# APPENDIX C

# LABORATORY PROCEDURES

## ANCILLARY LABORATORY STUDIES

Additional laboratory testing will be performed to characterize the immunologic responses to the various study agents and these are listed below.

### *C-1. IMMUNOPHENOTYPING*

Lymphocyte phenotyping: at baseline (before therapy), Day 14 and Day 28 of study treatment. Peripheral blood lymphocyte phenotyping will be performed including, but not limited to, enumeration of CD3, 4, 8, 25, 69, 20, $CD4^{+}25^{+}$.

The lymphocyte phenotyping will be performed on freshly drawn cells at the transplant center and recorded as cells of each subset phenotype per mL of blood.

### *C-2. CYTOKINE ANALYSIS*

Serum for cytokines IL-2, TNF, IFN-gamma will be collected on Days 0, 14 and 28 of therapy. Additionally, serum samples will be analyzed by mass spectroscopy for proteomics analysis. A 7mL peripheral blood sample will be collected in a red top tube, spun, aliquoted into three 1.8 mL cryovials and frozen at -20° C at the transplant center and then batch shipped quarterly on dry ice to the BMT CTN NIH sample repository for distribution and assay.

### *C-3. CYTOKINE GENE POLYMORPHISM*

A 3 mL peripheral blood sample (for DNA) will be collected in an ACD-A anticoagulant-containing tube on Day 0 to analyze cytokine gene polymorphisms. This sample will represent donor DNA. To assess recipient cytokine genes, if available, pre-transplant recipient blood collected in an ACD-A anticoagulant-containing tube or an EDTA tube for DNA will be used or alternatively, a buccal swab will be collected for recipient DNA.

Blood will be aliquoted into two 1.8 ml cryovials and frozen at -20° C. The buccal swab should be allowed to dry at room temperature. Remove the tip of the swab and store in a 1.8 mL cryovial. Freeze cryovial at 20° C. Samples will be batch shipped quarterly on dry ice to the BMT CTN NIH sample repository.

## *C-4. SKIN BIOPSY -- IMMUNOPATHOLOGY AND GENE EXPRESSION*

It is recommended that a skin biopsy be collected on Day 0 and Day 28 for pathology, immunopathology for lymphoid cell infiltration, immunochemical analysis and gene expression profiling.

There are four aims in analyzing the skin biopsies obtained through this protocol:

(1) To identify the nature of the dermal infiltrate and examine the effects of the different treatments on the composition of the infiltrate.

(2) To analyze the endothelial adhesion molecules and chemokines expressed in the skin and determine how the different treatments impact these molecular mediators of lymphocyte trafficking.

(3) To examine the deposition and molecular structure of hyaluronic acid in GVHD in the skin and how different treatments modify the hyaluronic acid deposits.

(4) To compare the level of gene expression of immune response-associated gene products (e.g., cytokines) and characterize the changes in expression among different treatments within different cells present in skin (e.g., endothelial cells, infiltrating cells, keratinocytes, etc.).

Aims (1) and (2) will require immunohistochemistry of fixed sections using antibodies to subset-specific markers to define the infiltrating cells and to adhesion molecules and chemokines. Aims (3) and (4) will require frozen tissue, from which sections will be cut for hyaluronic acid studies (Aim 3) or for laser capture dissection of discrete cells within the skin followed by gene expression profiling by arrays (Aim 4).

**Sample Handling Requirements:**

At the time of clinical diagnosis of acute cutaneous GVHD (prior to initiating any therapy), two 4 mm punch biopsies will be obtained. One biopsy will be placed in buffered formalin for routine histopathology per local institutional practice. The second biopsy will be snap frozen in liquid nitrogen and sent for research studies. The freezing step will be performed as follows:

Note: The skin biopsy should be snap frozen immediately. If this is not possible, the skin sample must be transported from the clinic on a saline soaked gauze pad and snap frozen within 60 minutes of the procedure.

(1) The skin sample is placed in a 1.8 mL cryovial and the cryovial closed

(2) The cryovial is then immersed vertically into liquid nitrogen

(3) Once frozen in liquid nitrogen, the sample must be immediately transferred to a -80ºC freezer for long-term storage. For handling of the cryovial, it is imperative that only the very bottom of the tube or the very top of the cap is handled (i.e, do not touch the sides of the cylinder), so as to never transfer heat to the skin sample. All transfers between freezers must be performed quickly, to prevent thermal warming of the skin sample.

Skin biopsies are obtained again on Day 28 and handled precisely as described above.

The frozen skin samples will be batch-shipped quarterly on dry ice by overnight delivery to the laboratory of Dr. Brian Nickoloff at Loyola University of Chicago.

## *C-5. MYCOPHENOLATE PHARMACOKINETICS*

Added sampling for MMF and metabolite concentration and clearance calculations will be conducted during weeks 1 (between Days 3-7) and week 2 (Days 10-14) for patients assigned to receive MMF. Sampling will be done at hour 0 (pre-dose), 1, 2 and 6 hours for patients receiving oral MMF and hour 0, 2, 4 and 6 hours for patients receiving IV (infusion over 2 hours) MMF.

**Pharmacokinetic Methods:**

Pharmacokinetics will be studied twice in each subject following the initiation of MMF and corticosteroids for the treatment of acute GVHD. Patients will be sampled at steady-state once in week 1 (between Days 3-7) and again in week 2 (between Days 10-14) of MMF treatment. Steady-state is defined as a minimum of 4 identical mycophenolate mofetil doses given 12 hours apart.

Blood samples will be obtained at times 0, 1, 2, 6 hours following the administration of the oral dose and at 0, 2, 4 and 6 hours after the start of the IV infusion if receiving IV MMF. Intravenous MMF must be administered at a constant rate over 2 hours. Five mL of blood will be drawn at each time point from an existing central venous catheter or a temporary peripheral venous catheter.

Serum albumin, serum creatinine and total bilirubin will be measured, height and weight, medical history, and a detailed list of concomitant medications will be obtained on the day of each pharmacokinetic sampling. The pharmacokinetic form (included in the shipping box) will be completed for each pharmacokinetic sampling period.

**MPA and MPAG Plasma Sample Processing and Storage:**

Blood samples (5 mL) will be collected in a 10 mL purple top tube containing EDTA anticoagulant and placed on wet ice. Optimally, samples will be processed within 30 minutes of withdrawal, however samples are stable for up to 4 hours at room temperature or 8 hours if refrigerated at 1-4 degrees C. Samples should be prepared by centrifugation at 3400 rpm x 10 minutes, plasma removed and divided equally between two 5 mL screw top plastic tubes and then frozen at -20 degrees C. Samples should be stored frozen at -20 degrees C and then batch shipped after the completion of week 1 and 2 for each patient, frozen on dry ice in the provided shipping container to the University of Minnesota.

Total MPA and MPAG, and unbound MPA will be measured with high-pressure liquid chromatography methods. Total MPA and MPAG concentrations are simultaneously measured. Unbound MPA concentrations are measured separately. The total MPA standard curve is prepared over the concentrations of 0.01 to 10 mcg/mL. MPAG standard curve is 1-100

mcg/mL. The unbound MPA standard curve is prepared over the concentrations of 0.0025 to 0.1 mcg/mL. Inter and intraday coefficients of variation are < 10% for both assays.

**Data Analysis:**

Exposure measures will be determined from four blood samples from which a 12-hour AUC will be calculated using the limited sampling model developed from data in a previous pharmacokinetic trial [40]. The primary exposure measures of interest are total and unbound MPA area under the curve (AUC), total and unbound MPA Cmax and Cmin, and free fraction. For each patient, an AUC will be calculated for each sampling period and will be used to investigate the interpatient variability, as measured by standard statistics such as standard deviation and coefficient of variation (CV). Graphs and correlations will be used to examine the distribution of values and bivariate relationships. The relationships between exposure measures and individual characteristics (age, gender, diagnosis, serum albumin, renal and hepatic function, site and grade of GVHD, height, weight, BSA) will be examined in order to identify potentially important variables that determine exposure. Intrapatient variability will be expressed by comparing the change in total exposure measures between the two sampling times. Clinical covariates (serum creatinine and albumin, total bilirubin, grade and site of GVHD at time of sampling) will be assessed for their relationship to changes in pharmacokinetic parameters. Exploratory analyses will be conducted to evaluate the relationship between acute GVHD resolution and MPA exposure.

If MMF is given by IV, it will be infused over two hours. Since the infusion pump must be shut off during pharmacokinetic sampling, the pump infusion rate should be changed accordingly to ensure complete delivery of dose over two hours. The line should be completely flushed at the end of the infusion to ensure administration of the entire dose. Mycophenolate samples may be drawn from either a peripheral or central line. If samples are drawn from the central line, infusion pumps on all lumens must be shut off during the draw and a waste drawn that is equivalent to the volume of catheter. For intravenous pharmacokinetics, blood will be drawn through a lumen is not being used for mycophenolate infusion. This will avoid contamination of sample. If possible, no medications or IV fluids should be co-infused with mycophenolate.

## *C-6. PROTEIN PROFILING*

Serum specimens will be obtained at the time of study entry (Day 0) and then again at Day 14, Day 28 and Day 90 or at the time of GVHD recurrence. These samples will be analyzed by mass spectrometry and time of flight analysis to determine if there are protein signatures associated with the onset GVHD and response to therapy.

The goals for the proteonomic analysis are:

1. To determine whether specific, predictable protein signatures in the serum or urine of patients experiencing acute GVHD
2. Identify whether response to treatment correlates with changes in serum protein signatures

3. To investigate whether proteomonic signatures at the time of recurrent GVHD are similar to that of the initial episode of GVHD
4. To determine whether protein signatures present after the start of therapy can predict treatment failure
5. To use proteonomic analysis to identify changes in novel proteins that may be involved in the pathogenesis of GVHD and are predictive for the onset of acute GVHD

**Sample Acquisition Times:**

Samples should be obtained at the below stated times:

- Study enrollment (Day 0)
- Day 14
- Day 28
- At the time of disease flare
- After steroid taper (Day 90)

**Sample Handling Requirements:**

On Days 0, 14, and 28, the collection and processing of the samples is the same as that for the cytokine analysis sample. **There is no need to collect this sample separately on Days 0, 14, and 28.**

On Day 90 or at time of GVHD recurrence, collect 5 mL peripheral blood in a red top tube, allow sample to clot for 30 minutes, then centrifuge at 900 x g or 2100 RPM. Extract serum carefully without disturbing the clot. Transfer and divide serum equally into two 1.8 mL cryovials and freeze at -20° C. Batch ship quarterly to the BMT CTN NIH sample repository, on dry ice.

## SCHEDULE OF LABORATORY EVALUATIONS

| Type of Sample | Method | Type of Storage | Dates Samples Obtained | Shipping Specifications | Test Location |
|---|---|---|---|---|---|
| Immunophenotyping | Peripheral blood lymphocyte phenotyping, including CD3, 4, 8, 25, 69, 20, $CD4^+$ $25^+$ on freshly drawn cells. | According to institutional practice | Day 0, Day 14 and Day 28 | N/A | Transplant Center |
| Cytokine Analysis and Protein Profiling | 7 mL peripheral blood sample (10 mL red top tube) placed upright and allowed to clot for approximately 30 minutes at room temperature, centrifuge at 2100 RPM[1]. Extract serum carefully without disturbing clot. Transfer and divide serum into three 1.8 mL cryovials. | Frozen at -20°C | Day 0, Day 14 and Day 28 | Shipped quarterly frozen on dry ice in a provided shipping container, FedEx priority overnight to the BMT CTN NIH sample repository | Dr. Susan Hsu's Laboratory, ARC Penn Jersey |
| Protein Profiling | 5 mL peripheral blood sample (10 mL red top tube) placed upright and allowed to clot for approximately 30 minutes at room temperature, centrifuge at 2100 RPM[1]. Extract serum carefully without disturbing clot. Transfer and divide serum into two 1.8 mL cryovials. | Frozen at -20°C | Day 90 and at the time of GVHD recurrence | Shipped quarterly frozen on dry ice in a provided shipping container, FedEx priority overnight to the BMT CTN NIH sample repository | Dr. Gary Nelsestuen's Laboratory, University of Minnesota |

Note:
[1]If necessary, samples can be stored at 2-8ºC overnight and centrifuged the next day. However, same day processing is preferred.

| Type of Sample | Method | Type of Storage | Dates Samples Obtained | Shipping Specifications | Test Location |
|---|---|---|---|---|---|
| Cytokine Gene Polymorphism | 3 mL peripheral blood sample (ACD-A anticoagulant yellow top tube or EDTA purple top tube) aliquoted into two 1.8 mL cryovials. Buccal Swab allowed to dry at room temperature and transferred into a 1.8 mL cryovial. | Frozen at -20°C | Pre-transplant (if blood available) and Day 0. Buccal Swab if pre-transplant blood not available | Shipped quarterly frozen on dry ice in a provided shipping container, FedEx priority overnight to the BMT CTN NIH sample repository | Dr. John Hansen's Laboratory, Fred Hutchinson Cancer Research Center |
| Skin Biopsy | Two 4 mm punch biopsies, one biopsy in buffered formalin for routine histopathology. The second snap frozen in liquid nitrogen for research studies. | Second biopsy frozen at -80°C | Day 0 and Day 28 | Shipped quarterly on dry ice to Loyola University | Dr. Nickloff's Laboratory, Loyola University |
| Pharmacokinetics (Patients assigned to MMF arm only) | 5 mL of peripheral blood sample (10 mL purple top tube containing EDTA anticoagulant) will be drawn at each timepoint, and medical information recorded. Sample placed on wet ice, then centrifuge for 10 minutes at 3400 RPM within 30 minutes of collection[2]. Remove plasma and divide equally between two 5 mL plastic tubes. | Frozen at -20°C | Week 1 and Week 2 (Sampling will be done at 0, 1, 2, and 6 hours) | Shipped after completion of week 1 and week 2 on dry ice to University of Minnesota | Dr. Pamala Jacobson, University of Minnesota |

Note:
[2]Optimally, samples will be processed within 30 minutes of collection; however, samples are stable for up to 4 hours at room temperature or 8 hours if refrigerated at 1-4ºC.

# APPENDIX D

# HUMAN SUBJECTS

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**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.