CASE COMPREHENSIVE CANCER CENTER

STUDY NUMBER: CASE 6Z13

STUDY TITLE: Tacrolimus, mini-dose Methotrexate and Mycophenolate Mofetil versus Tacrolimus and Methotrexate for the Prevention of Acute Graft-versus-Host-Disease (NCT01951885)

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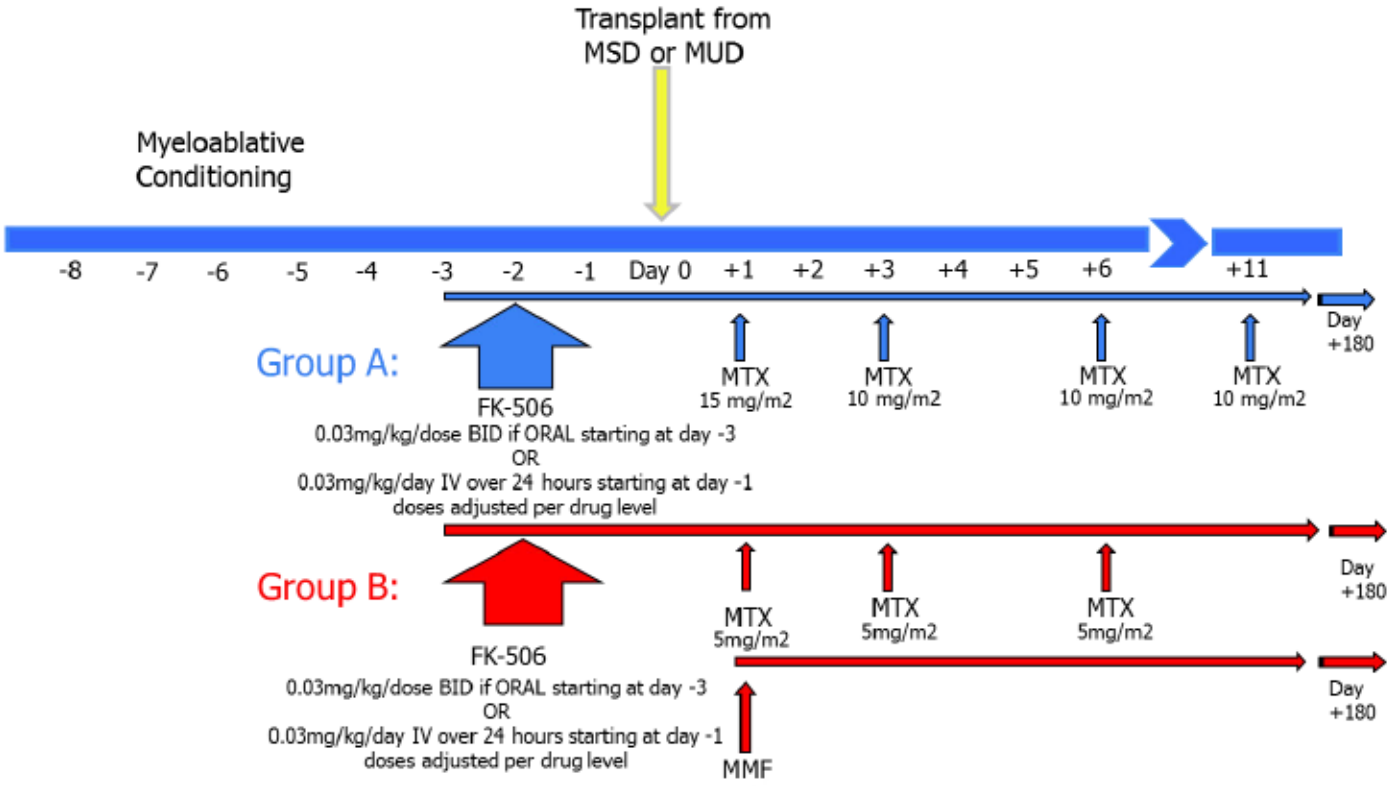
AGENTS: **Methotrexate, Mycophenolate Mofetil, Tacrolimus**

SCHEMA:


Transplant from
MSD or MUD
Myeloablative
Conditioning
-8 -7 -6 -5 -4 -3 -2 -1 Day 0 +1 +2 +3 +4 +5 +6 +11
Day
+180
Group A:
FK-506
0.03mg/kg/dose BID if ORAL starting at day -3
OR
0.03mg/kg/day IV over 24 hours starting at day -1
doses adjusted per drug level
MTX
15 mg/m2
MTX
10 mg/m2
MTX
10 mg/m2
MTX
10 mg/m2
Group B:
FK-506
0.03mg/kg/dose BID if ORAL starting at day -3
OR
0.03mg/kg/day IV over 24 hours starting at day -1
doses adjusted per drug level
MTX
5mg/m2
MTX
5mg/m2
MTX
5mg/m2
MMF
Day
+180
Day
+180

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1.0 INTRODUCTION

1.1 Allogeneic Hematopoietic Cell Transplant

Allogeneic hematopoietic cell transplantation (HCT) is an effective therapy for a variety of hematologic malignancies and bone marrow failure syndromes. However, it carries a significant risk for treatment-related mortality, primarily due to infection, conditioning regimen-related toxicities, and graft versus host disease (GVHD); which continues to limit its utility. The risk of transplant-related mortality (TRM) is influenced by several factors, including patient age, disease remission status, number of prior chemotherapy regimens, co-morbid diseases, and conditioning regimen intensity. Since the 1980's, several advances have reduced the risk of TRM in patients undergoing allogeneic HCT; including more effective approaches for prevention of infection, enhancements in HLA typing and matching, and GVHD prophylactic regimens [1]. Despite this however, TRM remains high and GVHD remains a major cause of morbidity and mortality amongst allogeneic HCT patients. Even with prophylactic measures, the incidence of acute GVHD is estimated to be 40-50% among patients from HLA identical sibling donors and up to 75% in patients receiving unrelated transplants. Treatment for established GVHD can be difficult, with a mortality rate of 60-80% in patients with grades 3-4 acute GVHD. Approximately 40% of patients with acute GHVD have a durable response to corticosteroid therapy, and there has been little change in this response rate over the past 20 years despite changes to GVHD treatment regimens [2]. Thus the use of improved and effective prophylaxis is of utmost importance.

1.2 GVHD Prophylaxis

Currently, GVHD prophylaxis is based on a calcineurin inhibitors (cyclosporine (CsA) or tacrolimus (Tac) also called FK-506) and a short course of methotrexate (MTX). Other options include steroids, mycophenolate mofetil (MMF), and sirolimus. While the combination of Tac and MTX has largely been shown to be superior in the prevention of aGVHD compared to other regimens, toxicities from these regimens are a cause of significant morbidity after allogeneic transplant.

Methotrexate has had a long history of use as a component of GVHD prophylaxis agents in HCT. Initially used as a single agent, it has been shown to be complementary in combination with calcineurin inhibitors cyclosporine and tacrolimus. However, it also may increase the risk of other complications such as mucositis, delayed neutrophil and platelet engraftment and contribute to hepatic and renal toxicities. Mucositis can be severe and debilitating necessitating the need for total parenteral nutrition (TPN) and furthering tissue damage contributing to GVHD. As a result of severe mucositis, doses of MTX must often be held, which may further decrease the efficacy of GVHD prophylaxis.

Tacrolimus is an immunosuppressive macrolide lactone that blocks the earliest steps of T-cell activation by inhibiting the calcium-dependent signal transduction pathway. Although the mechanism of action, pharmacokinetics, and side-effect profile of tacrolimus are similar to those of cyclosporine, its immunosuppressive potency in vitro is 50 to 200 times greater than

that of cyclosporine. Large phase III studies comparing Tac/MTX versus CsA/MTX for both matched related and unrelated donors have been performed and demonstrate superiority of Tac/MTX [3-4].

Mycophenolate mofetil (MMF) has been utilized in an effort to improve GVHD prophylaxis and reduce toxicity. MMF is an ester prodrug of the immunosuppressant mycophenolic acid. Mycophenolic acid inhibits inosine monophosphate dehydrogenase, resulting in the blockade of de novo purine synthesis, thereby limiting proliferation of lymphocytes. MMF is effective in preventing GVHD in animal models, and the combination of a calcineurin inhibitor and MMF is more effective than either agent by itself [5]. The CsA and MMF or Tac and MMF combinations are commonly used as GVHD prophylaxis in patients undergoing reduced intensity transplants. MMF in combination with CsA has been compared to MTX and CsA in 2 small prospective randomized studies for GVHD prophylaxis in patients undergoing myeloablative transplant [6-7]. These studies demonstrated several benefits in the MMF arm, including less severe mucositis, shorter time to neutrophil and platelet engraftment, a reduction in use of TPN and narcotic analgesia, and shorter hospitalization times. The combination of Tac and MMF has been evaluated as GVHD prophylaxis in adults in single-arm phase II trials which have shown the regimen to be well tolerated [8-9].

1.3 Clinical Data to Date

The group at M.D. Anderson Cancer Center has developed a mini-dose methotrexate regimen (5 mg/m^2 IV on days 1, 3, 6, and 11) in an effort to reduce mucosal and hepatic complications and have published small studies on its use in combination with both CsA and Tac [10-11]. These studies have demonstrated the safety and efficacy of this regimen in HLA matched and 1 antigen mismatched unrelated donors, with an incidence of grade 2-4 acute GVHD of 33-59% and grade 3-4 acute GVHD of 17%. Tac and mini-dose MTX (5mg/m^2 IV on days 1, 3, and 6 only) has been further tested in combination with a third agent [12-13]. Mizumoto et al used a combination of Tac, mini-dose MTX and MMF as GVHD prophylaxis in reduced-intensity transplants and demonstrated a safe and well tolerated profile with a low incidence of severe grade 3-4 GVHD (5%). While these studies have demonstrated the safety and efficacy of these regimens, there have been no studies directly comparing mini-dose MTX and standard dose MTX based GVHD prophylactic regimens.

2.0 OBJECTIVES

2.1 Primary Objectives

- To determine the incidence, length and severity of mucositis from Day 0 until day of discharge or Day +28 (whichever comes first) after allogeneic bone marrow transplantation comparing Tac, mini-dose MTX and MMF with Tac and MTX.

- To study rates of neutrophil and platelet engraftment comparing Tac, mini-dose MTX and MMF versus Tac and MTX

- To study the incidence of acute graft-versus-host disease in HLA matched related and unrelated allogeneic bone marrow transplantation using a myeloablative preparative regimen comparing Tac, mini-dose MTX and MMF with Tac and MTX.

2.2 Secondary Objectives

- Length of hospitalization/time to discharge
- Use of TPN (total parenteral nutrition)
- Length of time on continuous infusion narcotics
- Incidence of infection in the first 100 days post-transplantation
- Incidence of hepatotoxicity in first 100 days post-transplantation
- Incidence of nephrotoxicity in first 100 days post-transplantation
- Incidence of pulmonary toxicity in first 180 days post-transplantation
- Incidence and severity of grade II-IV and III-IV acute GVHD
- Incidence and severity of chronic GVHD
- One year relapse-free and overall survival

3.0 STUDY DESIGN

3.1 Study Design

This is a prospective randomized trial to determine the effectiveness of different doses of GVHD prophylaxis on mucositis, engraftment and aGVHD. Study consists of two study groups of 50 subjects each.

Group A will receive Tac and MTX (15 mg/m^2 day +1, 10 mg/m^2 day +3, +6, +11).
Group B will receive Tac, Mini-dose MTX (5 mg/m^2 on day +1, +3, +6) and MMF.

3.2 Number of Subjects

We plan to enroll 50 subjects in each group for a total of 100 subjects.

3.3 Replacement of Subjects

Patients who drop out prior to day 100 study endpoints can be evaluated, will be replaced so that there will be 50 evaluable patients included in the study in each group.

3.4 Expected Duration of Subject Participation

3.4.1 Duration of Therapy

In the absence of graft versus host disease, treatment may continue until day 180 at the discretion of the investigator or until one of the following criteria applies:

- The investigator considers it, for safety reasons, to be in the best interest of the patient.

- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

- Patient decision to withdraw from treatment (partial consent) or from the study (full consent),

- Pregnancy during the course of the study for a child-bearing participant

- Death

- The PI reserves the right to temporarily suspend or prematurely discontinue this study. If the rate of graft failure, which will be monitored continuously throughout the study, exceeds 11% (approximately 20% higher than what we typically expect in our prior experience) in either arm at 6 months, we will halt accrual in order to perform further analysis. Graft failure is defined as having one of the following criteria in absence of relapse:
  - Failure to reach ANC > 500/ul for 3 consecutive days in patient surviving a minimum of 28 days
  - Decrease of ANC to < 100/ul for at least 3 consecutive determinations after initial engraftment without recovery before relapse, second HCT or death
  - < 1% donor T cells as assessed by a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) or FISH studies or VNTR of the patient and donor on day +28 or beyond

The date and reason for discontinuation must be documented. Every effort should be made to complete the appropriate assessments.

3.4.2 **Duration of Follow Up**

Patients will be followed for 1 year after T-0 (date of infusion of HCT) or until death, whichever occurs first.

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

4.0 PATIENT SELECTION

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. The

checklist must be completed for each patient and must be signed and dated by the treating physician.

4.1 Inclusion Criteria

Patients must meet all of the following inclusion criteria to be eligible for enrollment

- Patients must have one of the following documented diseases:

  Chronic Myelogenous Leukemia
  Chronic Lymphocytic Leukemia
  Multiple Myeloma
  Myelodysplasia
  Myeloproliferative disorder
  Non-Hodgkin's Lymphoma
  Hodgkin's Disease
  Acute Myelogenous Leukemia
  Acute Lymphoblastic Leukemia
  Acute Biphenotypic Leukemia

- Patients must be undergoing a myeloablative allogeneic hematopoietic cell transplant with one of the following conditioning regimens (see APPENDIX IV for TBI administration guidelines):
  - Busulfan (≥ 12.8 mg/kg IV or PO) and cyclophosphamide (≥ 120 mg/kg)
    - Busulfan dose may be adjusted according to pharmacokinetics targeting a daily AUC of 5000 µmol-min/L, per institution standard of practice.
  - TBI (≥ 1200 cGy) and Etoposide (60 mg/kg)
  - TBI (≥ 1200 cGy) and cyclophosphamide (120 mg/kg)

- Patient's disease status should meet criteria as outlined by institutional master protocol.
- Patient's donor must be a related or unrelated HLA 8/8 allele-level match (HLA-A, B, C and DRB1).
- Age 0-70
- Adult patients must have an ECOG performance status of 0 or 1. Pediatric patients must have Lansky score ≥ 60%
- Patients must have a life expectancy of 100 days.
- Patients must sign written informed consent.

4.2 Exclusion Criteria

- Patients who have undergone any prior transplant.
- Patients who are seropositive for human immunodeficiency virus (HIV).

- Patients with any medical illness or concurrent psychiatric illness which, in the investigators' opinion, cannot be adequately controlled with appropriate therapy.
- Patients who are pregnant or lactating.

4.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

5.0 REGISTRATION

All subjects who have been consented are to be registered in the OnCore Database. For those subjects who are consented, but not enrolled, the reason for exclusion must be recorded.

All subjects will be registered through Cleveland Clinic and will be provided a study number by the Study Coordinator.

5.1 Randomization

Patients will be randomized 1:1 to one of the two GVHD prophylaxis regimens using blocked randomization with random block sizes. The randomization list will be generated before the start of the study by the study statistician of the study using a SAS randomization program; the list will be kept in a secured location which will only be accessible to the study coordinator. Randomization will be stratified by TBI in the preparative regimen (yes, no), donor relationship (related, unrelated), and source of hematopoietic cells (PBSC, marrow).

6.0 TREATMENT PLAN

6.1 Agent Administration

6.1.1 Tacrolimus (Tac) Administration:

Patients in both group A and B will receive Tac 0.03 mg/kg/day intravenously beginning on day -1 or tacrolimus 0.03mg/kg/dose BID orally beginning on day -3. If Tac is administered intravenously, it will be given over 24 hours and will be converted to oral administration 2 times a day when the patient has engrafted and/or can tolerate oral medication.

Levels of Tac will be obtained to maintain a recommended target serum level of 5-15 mg/mL or per institution guidelines. Dose adjustments of Tac will be made according to Section 7.1.1 below.

In the absence of GVHD, Tac may start to be tapered approximately at day +100 and should be tapered off by day +180, per investigator's discretion.

6.1.2 Mycophenolate Mofetil (MMF) Administration:

Patients in group B only will receive MMF beginning on day +1. Patients ≥40 kg will receive MMF 1000 mg twice a day. MMF should be given orally twice a day. IV formulation may be used if the patient cannot tolerate oral route. Patients < 40 kg will receive MMF 45 mg/kg/day (15 mg/kg three times a day). MMF may be given orally or intravenously as per institutional protocol

In the absence of GVHD, MMF may be tapered after day +45 and stopped by day +100 at the discretion of the physician and per institutional guidelines for taper.

6.1.3 Methotrexate (MTX) Administration:

Group A

MTX will be given at 15 mg/m^2 IV on day +1. Then MTX will be given at 10 mg/m^2 on days +3, +6 and +11. Dose adjustments of MTX will be made according to Section 7.1.3 below.

If patient < 10 kg then MTX will be given at 0.5 mg/kg IV on day +1. Then MTX will be given at 0.33 mg/kg on days +3, +6 and +11.

Group B

MTX will be given at 5 mg/m^2 IV on days +1, +3 and +6.

If patient < 10 kg then MTX will be given at 0.17 mg/kg on days +1, +3 and +6.

6.2 General Concomitant Medications and Supportive Care Guidelines

Patients should receive full supportive care throughout the study including transfusions of blood and blood products, cytokines, antibiotics, antiemetics, Mesna, bladder washes and/or hydration to prevent hemorrhagic cystitis, etc. when appropriate.

The following therapies and/or medications should be avoided during the study period:

- Amiphostine
- IL-11(Neumega®)
- Sucralfate in suspension form (use of sucralfate tablets is not proscribed)
- Povidone-iodine rinses
- Other investigational agents
- Cytotoxic drugs other than those specified in the protocol (Exception: Intrathecal methotrexate or cytosinarabinoside for subjects with central nervous system involvement)
- ATG suppression/Campath, or Rituximab as a prophylactic agent for GVHD (may only be used to treat aGVHD severity grades 3-4 refractory to steroids)

Patients will not be removed from study if taking one of the above medications but the medication, dose, dates taken, reason for taking it, etc. will be recorded.

Below are general guidelines but may vary depending on institutional and program guidelines.

6.2.1. **Growth Factor Support** - Colony stimulating factors (G-CSF)

No growth factor support will be routinely given unless indicated per standard institutional guidelines.

6.2.2 **Anemia**

Transfusions of red blood cells are indicated for the symptomatic management of anemia. An attempt will be made to maintain the hemoglobin > 8.0 g/dl. All blood products must be irradiated (1500 – 3000 cGy) and leukoreduced by filtration.

6.2.3 **Thrombocytopenia**

Prophylactic platelet transfusions should be given to maintain the platelet count >10,000/ul or above a platelet level at which signs of bleeding are known to occur, whichever is greater. All aspirin-containing drugs are contraindicated.

6.2.4 **Antimicrobial, Antifungal, Antiviral Prophylaxis**

Antibacterial, antifungal, pneumocystis jiroveci, and antiviral prophylaxis is recommended and will be used according to institutional and program guidelines.

CMV surveillance and empiric treatment will be based on institutional and program guidelines.

6.2.5 **Fever:**

Aggressive diagnostic and therapeutic management of fever in the neutropenic patient is mandatory. The following are recommended guidelines for the management of fever, but may also be treated according to institutional and program guidelines. At the onset of fever (38ºC), the patient should be thoroughly examined and cultured. Broad-spectrum antibiotics should be initiated to include coverage of gram-positive (including staphylococcus) and gram-negative (including pseudomonas) organisms. Voriconazole should be used empirically for persistent fevers (>3 days).

6.2.6 **Mouth Care**

A mouth care protocol per institution preference is recommended to be employed using a combination of mouthwash and other anti-bacterial solutions to minimize the risk of mucositis and dissemination of oral pathogens.

6.2.7 Venous access

Triple lumen Hickman catheters or Broviac catheters in pediatric patients, will be recommended to facilitate intravenous administration of drugs and the use of parenteral hyperalimentation.

6.2.8 Nutrition

Initiation of TPN will be left to the discretion of the attending physician; however general guidelines should be used as follows:

- In adult patients, it is recommended that the TPN support team should be consulted regarding potential initiation of TPN if:
  - there is less than 50% of enteral intake for > 7 days
  - patient has severe grade 3-4 WHO mucositis for > 5 days
- In pediatric patients, TPN is recommended if the child:
  - has less than 50% of enteral intake for > 3 days
  - has lost >10% of his admission weight
  - has severe grade 3-4 WHO mucositis that is expected to last more than 5 days
  - is showing signs of nutritional deficiency with a prealbumin < 15

6.2.9 Antiemetics

Antiemetics will be used based on institution and program guidelines. Standard antiemetic therapies include ondansetron, lorazepam, prochlorperazine and metoclopramide which can be considered either as scheduled or as needed medication.

6.2.10 Management of Acute Graft-Versus-Host Disease:

If acute graft-versus-host disease develops, the primary team will evaluate acute GVHD stage and grade and start the patient on prednisone or equivalent intravenous methylprednisone (MP) dose based on overall grade. Refer to Appendix I for Acute GVHD Grading Scale. Biopsy should be obtained whenever possible.

The goal of glucocorticoid therapy is to control the acute manifestations of GVHD and then to taper the doses of prednisone (or MP) as soon as possible. Literature demonstrates that prolonged use of high-dose glucocorticoids for the treatment of acute GVHD is associated with an increased risk for infection, relapse and death.

Treatment of <u>mild acute GVHD</u>

- includes rash < 50% of the total body surface area (skin < stage 3 and not progressing rapidly within 6-24 hours), anorexia, nausea, emesis, or diarrhea < 1.0 L/day (GI stage 1 or less) and no liver involvement (stage 0)
- can treat patient with prednisone at 1mg/kg (or equivalent MP), along with budesonide at 3mg orally twice daily and beclomethasone 1mg orally four times daily depending on patients upper and lower gastrointestinal symptoms

- recommended duration of 50 days with a taper over 1-2 weeks [14]
- Suggested duration and dosing of initial prednisone (or MP) treatment is at least 10 days with a subsequent taper according to patient response. If the patient has a rapid response to therapy, then the steroid taper may occur more rapidly every 3 days as suggested in Table A below:

TAPER SCHEDULE A (mild GI-tract GVHD with minimal diarrhea)

Day	AM dose (mg/kg)	PM dose (mg/kg)
1-10	0.5	0.5
11	0.25	0.25
13	0.125	0.125
15	0.0625	0.0625
17-79 *	Continue 0.0625	0
80	stop	0

* Day 50 Budesonide/belcomethasone may be stopped or tapered rapidly, over 1-2 weeks.

- If the patient has a slower response and improvement in his symptoms, it is recommended to taper every 5 days , as suggested in Table B below:

TAPER SCHEDULE B (mild GI-tract GVHD with 10-20 ml/kg diarrhea; max. 500-1000 ml/day)

Day	AM dose (mg/kg)	PM dose (mg/kg)
Before taper	0.5	0.5
1	0.5	0.4
6	0.5	0.3
11	0.5	0.2
16	0.5	0.1
21	0.5	0
26	0.4	0
31	0.3	0
36 – till day X	0.2	0
Day of taper	Mg/kg/day (AM dose only)	
1	0.2 (not to exceed 20 mg)	
2	0.1 (not to exceed 10 mg)	
3	0.25 (not to exceed 25 mg)	
4	0.05 (not to exceed 5 mg)	
5	0.3 (not to exceed 30 mg)	
6	0	
7	0.2 (not to exceed 15 mg)	
8	0	
9	0.15 (not to exceed 10 mg)	
10	0	
11	0.1 (not to exceed 5 mg)	
12	STOP	

Treatment of more than mild grades of acute GVHD

- Includes rash covering > 50% of the body surface area (skin stage 3) or progressing rapidly within 6-24 hours, diarrhea ≥ 1 L day or 556-833 ml/m2/day for pediatric patients (GI stage 2), or serum bilirubin ≥ 2.0 mg/dL in the absence of non-GVHD causes for hyperbilirubinemia, or ≥ 3.0 mg/dL if other causes of hyperbiliruibinemia are present besides GVHD
- Treated with 2mg/kg/day of prednisone or equivalent MP
- Suggested duration of initial prednisone (or MP) is 14 days followed by a taper over 6 weeks

In the case of non-responsive, or steroid refractory aGVHD (defined as no response after 5 days of high dose steroids), second line GVHD therapies will be based on physician discretion, including stopping of study medications and withdrawal from the study.

Tacrolimus and mycophenolate (in the appropriate treatment group) should otherwise be continued during the treatment of acute GVHD.

If started on prednisone, Pneumocystis prophylaxis will begin as well, with either aerosolized pentamidine or oral trimethaprim-sulfamethoxazole (Bactrim). If prednisone is > 40 mg, prophylactic anti-fungal should be started.

6.3 Duration of Therapy

Study medications will be given up to 180 days. Adjustments will be made according to 7.1 below. In the absence of aGVHD, taper can begin at day 100 for Tac and Day 45 for MMF according to guidelines in 6.1.1 and 6.1.2 above.

After 180 days of therapy, it will be at the discretion of the investigators how these patients are to be treated if graft versus host disease develops. They may continue Tac and/or MMF or other therapeutic agents to treat graft-versus-host disease.

7.0 DOSING DELAYS / DOSE MODIFICATIONS

7.1 Adjustment of Study Medications:

Any GVHD prophylaxis medication change will be recorded along with the reason it was reduced or withheld.

7.1.1 Tacrolimus

Levels of tacrolimus will be drawn twice per week while hospitalized then weekly or monthly thereafter unless a change in medication or renal function might result in an acute change in level. The target serum level for tacrolimus is 5-10 mg/mL. Dose adjustments are based on clinical judgment of the treating physician after considering clinical toxicity, serum levels, GVHD, concomitant drug use and the rate of rise or decline of the serum level.

7.1.2 **Mycophenolate Mofetil**

MMF has been associated with GI toxicity, including nausea, vomiting, abdominal pain and diarrhea, not related to GVHD. If symptoms occur, work up for GVHD should occur. A dose reduction may be instituted for 48-72 hours. If GI toxicity does not resolve within 48-72 hours of stopping the study drug, toxicity is likely not related to the study medication and the drug should be restarted. MMF has also been associated with myelosuppresion. If the ANC remains or decreases to less than 1000/ul after engraftment, MMF may be reduced or held at the physician's discretion. Dose adjustments are based on the clinical judgment of the treating physician after considering clinical toxicity and GVHD.

7.1.3 **Methotrexate**

MTX WILL NOT be dose adjusted or held for neutropenia. MTX may be held or dose reduced if creatinine clearance is decreased by > 50% baseline, if the patient is experiencing clinical signs of veno-occlusive disease, and if the patient has severe mucositis (oral ulceration, significant edema) compromising respiratory function or necessitating paretneral hyperalimentation. Further guidelines for dose adjustments for liver and renal toxicity are as follows:

Dose adjustments for liver toxicity:

Bilirubin (mg/dl)	MTX dose
< 3.0	100%
3.1 -5.0	50%
> 5.0	hold MTX

Dose adjustment for renal toxicity:

Creatinine clearance (ml/min)	MTX dose
> 50	100%
10 – 50	50%
< 0	hold MTX

When creatinine clearance is ≤ 60 ml/minute, administer Leucovorin at a dose of 1:1 (Leucovorin:Methotrexate) IV once, 24 hours after the dose of MTX.

Patients with pleural, pericardial or ascetic effusion(s) must be monitored closely for increased duration of MTX toxicity (mucositis and renal insufficiency) and considered for leucovorin therapy.

8.0 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

The following is a list of AEs (Section 8.1) and the reporting requirements associated with observed AEs (Sections 8.3 and 8.4).

The clinical course of each event will be followed until resolution, stabilization, or until it is has been determined that the study treatment or participation is not the cause.

Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

8.1 Adverse Events and Potential Risks

Please refer to the package insert(s) for the comprehensive list of adverse events. Many of these toxicities are recognized toxicities of transplantation and will not be considered adverse effects in the context of this study. For example, re-admissions are common after allogeneic transplantation, and reasons for readmissions most commonly include fever with or without infection. Unless inpatient hospitalizations are thought to be due to one of the study medications, they will not be reported as serious adverse events. Transplant related adverse events which may be influenced by GVHD prophylaxis will be specifically evaluated per secondary objectives (e.g. incidence of renal, liver, and pulmonary toxicities).

8.1.1 Tacrolimus

There are many well described toxic effects of tacrolimus. Primary toxicities in large controlled trials were reversible renal insufficiency, thrombotic microangiopathy, hypertension, hyperglycemia, hypomagnesaemia, hypokalemia, opportunistic infection, and neurologic toxicity. In addition there may be an infusional toxicity from the diluent.

8.1.2 Mycophenolate Mofetil

The use of mycophenolate mofetil has been associated with myelosuppresion, increased risk of opportunistic infection and GI toxicity including diarrhea, abdominal pain and vomiting, not related to GVHD. Gastrointestinal bleeding and bowel perforation have been described infrequently.

8.1.3 Methotrexate

The most frequently reported adverse reactions associated with methotrexate use as GVHD prophylaxis include ulcerative stomatitis, leukopenia and suppressed hematopoiesis, hepatic and nephrotoxicity, nausea, and abdominal distress. Other frequently reported adverse effects are malaise, fatigue, chills and fever, and dizziness. Methotrexate may be associated with increased rates of pulmonary complications after transplantation.

8.2 Drug Interactions

8.2.1 Methotrexate

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

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

8.2.2 **Mycophenolate Mofetil**

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 mycophenolic acid (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.

8.2.3 **Tacrolimus**

Tacrolimus is extensively metabolized by the cytochrome P-450 (CYP3A4) system.
Care should be taken when drugs or other substances that are metabolized by CYP3A4 are administered concomitantly with tacrolimus. Grapefruit juice and other drinks containing bergamottin (*Fresca, Squirt, Sunny Delight)* reduces the CYP3A4-mediated metabolism of tacrolimus and may increase its levels.

Drugs that may increase tacrolimus blood concentrations include:

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

Drugs that may decrease tacrolimus concentrations include:

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

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

8.2 Definitions

8.2.1 Adverse Events

An **adverse event** (AE) is any unfavorable or unintended event, physical or psychological, associated with a research study, which causes harm or injury to a research participant as a result of the participant's involvement in a research study. The event can include abnormal laboratory findings, symptoms, or disease associated with the research study. The event does not necessarily have to have a causal relationship with the research, any risk associated with the research, the research intervention, or the research assessments.

Adverse events may be the result of the interventions and interactions used in the research; the collection of identifiable private information in the research; an underlying disease, disorder, or condition of the subject; and/or other circumstances unrelated to the research or any underlying disease, disorder, or condition of the subject.

External adverse events are adverse events experienced by subjects enrolled in multicenter clinical trials at sites other than the site(s) over which the Institutional Review Board has jurisdiction.

Internal adverse events are adverse events experienced by subjects enrolled at the site(s) under the IRB's jurisdiction for either multicenter or single-center research projects.

8.2.2 **The significance of an adverse event** is used to describe the patient/event outcome or action criteria associated with events that pose a threat to a patient's life or functioning (i.e., moderate, severe or life threatening). Based on the National Cancer Institute Guidelines for the Cancer Therapy Evaluation Program, severity can be defined by the following grades of events:

Grades 1 are mild adverse events (e.g., minor event requiring no specific medical intervention; asymptomatic laboratory findings only; marginal clinical relevance).

Grades 2 are moderate adverse events (e.g., minimal intervention; local intervention; non-invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation).

Grades 3 are severe and undesirable adverse events (e.g., significant symptoms requiring hospitalization or invasive intervention; transfusion; elective interventional radiological procedure; therapeutic endoscopy or operation).

Grades 4 are life threatening or disabling adverse events (e.g., complicated by acute, life-threatening metabolic or cardiovascular complications such as circulatory failure, hemorrhage, sepsis; life–threatening physiologic consequences; need for intensive care or emergent invasive procedure; emergent interventional radiological procedure, therapeutic endoscopy or operation).

Grades 5 are fatal adverse event resulting in death.

8.2.3 Serious Adverse Events

A serious adverse event (SAE) is any adverse experience occurring at any dose that results in any of the following outcomes:

- Results in **death.**
- Is a **life-threatening** adverse experience. The term life-threatening in the definition of serious refers to an adverse event in which the subject was at risk of death at the time of the event. It does not refer to an adverse event which hypothetically might have caused death if it were more severe.
- Requires **inpatient hospitalization or prolongation of existing hospitalization**. Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following expectations is met:
    - The admission results in a hospital stay of less than 12 hours OR
    - The admission is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study) OR
    - The admission is not associated with an adverse event, please refer to Section 8.1.
    - However it should be noted that invasive treatment during any hospitalization may fulfill the criteria of "medically important" and as such may be reportable as a serious adverse event dependant on clinical judgment. In addition where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.
- Results in **persistent or significant disability/incapacity**. The definition of disability is a substantial disruption of a person's ability to conduct normal life's functions.
- Is a **congenital anomaly/birth defect**.
- Is an **important medical event**. Important medical events that may not result death, be life-threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood disease or disorders, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.2.4 Expectedness

Adverse Events can be Expected or Unexpected.

An expected adverse event is an event previously known or anticipated to result from participation in the research study or any underlying disease, disorder, or condition of the

subject. The event is usually listed in the Investigator Brochure, consent form or research protocol.

An unexpected adverse event is an adverse event not previously known or anticipated to result from the research study or any underlying disease, disorder, or condition of the subject.

8.2.5 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study drug. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study drug.
- Probable – The AE is likely related to the study drug.
- Possible – The AE may be related to the study drug.
- Unlikely – The AE is doubtfully related to the study drug.
- Unrelated – The AE is clearly NOT related to the study drug.

8.3 Reporting Procedures for All Adverse Events

All participating investigators will assess the occurrence of AEs throughout the subject's participation in the study. Subjects will be followed for toxicity for 30 days after treatment with tacrolimus or mycophenolate has been discontinued, tapered or until death, whichever occurs first. If patient continues on tacrolimus or mycophenolate for treatment of continued GVHD past the endpoint of study (1 year after infusion of HCT), adverse events will no longer be reported. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

Toxicities of the blood/bone marrow, gut, liver and skin will be evaluated separately since they are likely to be affected by GVHD. GVHD severity will be determined clinically, however biopsies of affected are strongly encouraged whenever possible.

Grade 4-5 serious adverse events will be reported according to the local IRB's policies and procedures.

The investigator is responsible for ensuring that serious adverse events observed by the investigator or reported by the subject which occur after the subject has started treatment with the first study drug (day -1) are fully recorded in the subject's case report form, subject's medical records, and/or any other institutional requirement. Specific toxicities, including laboratory values and events outlined in secondary objectives will be captured per protocol; and will not necessarily be considered as part of adverse event reporting as these are expected toxicities post transplant, but may be influenced by GVHD and GVHD prevention approaches. Source documentation must be available to support all adverse events.

The investigator will provide the following for serious adverse events:

- Description of the event
- Grade of toxicity
- Attribution of relatedness to the investigational agent

In this study, descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 available at http://ctep.cancer.gov will be utilized for AE reporting.

Investigative sites will report adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events.

8.4 Serious Adverse Event Reporting Procedures

Serious adverse events that occur beginning with the first day of the study drug administration, during treatment, or within 30 days of the last dose of treatment, or up to study end point must be reported to the Cleveland Clinic Principal Investigator.

Investigative sites will report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting serious adverse events.

8.4.1 FDA Reporting

N/A

8.5 Data Safety Toxicity Committee

It is the ***Case Comprehensive Cancer Center*** Principal Investigator's responsibility to ensure that ALL serious adverse events are reported to the Case Comprehensive Cancer Center's Data Safety Toxicity Committee. This submission is simultaneous with their submission to other Regulatory body.

9.0 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 8.0.

9.1 Investigational Agents

N/A

9.2 Commercial Agents

Agents are listed in alphabetical order.

9.2.1 **Methotrexate** (NSC #740)

Chemical name: Methotrexate

Formulation:	Methotrexate is available as the sodium salt for parenteral use in 20 mg, 50 mg, and 1 g vials as the lyophilized powder. Liquid methotrexate sodium injection products, and tablets for oral administration are also available.
Toxicity:	Adverse reactions include dizziness, drowsiness, general body discomfort, headache, loss of appetite, mild hair loss, mucositis, nausea, vomiting, diarrhea, myelosuppression, skin rashes, itching, pigmentation changes, impaired renal and hepatic function.
Manufacturer:	Immunex
Supplier:	Patient or 3rd Party
Mechanism of Action:	Methotrexate is an antimetabolite and has its principle mechanism of action the competitive inhibition of dihydrofolate reductase.

9.2.2 **Mycophenolate** (NSC #61443)

Chemical name:	Mycophenolate mofetil
Formulation:	Mycophenolate is available in capsule form, 250 mg or tablets of 50mg. Liquid forms are also available and come in 500 mg vials (20 mL).
Toxicity:	Adverse reactions include anxiety, back pain, constipation, diarrhea, dizziness, headache, loss of appetite, leukopenia, sepsis, nausea, vomiting, tremor, trouble sleeping and increased incidence of infections.
Manufacturer:	Roche
Supplier:	Patient or 3rd party
Mechanism of Action:	Mycophenolate is rapidly absorbed following oral and IV administration and hydrolyzed to form MPA, which is the active metabolite. MPA is a potent, selective, uncompetitive and reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), and therefore inhibits the de novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Because T- and B-lymphocytes are critically dependent for their proliferation on de novo synthesis of purines whereas other cell types can utilize salvage pathways, MPA has potent cytostatic effects on lymphocytes. MPA inhibits proliferative responses of T- and B-lymphocytes to both mitogenic and allospecific stimulation.

9.2.3 **Tacrolimus** (NSC #77865)

Chemical name:	FK506
Formulation:	Capsule, oral: 0.5 mg, 1 mg, 5 mg. Injection, solution: 5 mg/ml (1 ml).

Toxicity:	Adverse reactions include back pain, constipation, diarrhea, dizziness, headache, joint pain, loss of appetite, nausea, stomach pain or upset, trouble sleeping, vomiting, weakness.
Mechanism of Action:	Binds to the FKBP-12 protein and forms a complex with calcium-dependent proteins, thereby inhibiting calcineurin phosphatase activity and resulting in decreased cytokine production. This agent exhibits potent immunosuppressive activity and prevents the activation of T-lymphocytes in response to antigenic or mitogentic stimulation.

10.0 CORRELATIVE / SPECIAL STUDIES

10.1 Research Sample Repository

The purpose of this correlative study is to improve our understanding of GVHD by storing samples of blood/tissue of the recipient to use in various laboratory studies.

10.1.1 Background and Rational:

GVHD is a complex disease which arises when donor T cells respond to genetically defined and disparate proteins on host cells; the most important of which are human leukocyte antigens (HLAs), which are highly polymorphic and are encoded by the major histocompatibility complex (MHC). GVHD is often associated with a graft versus leukemia (GVL) effect, but separating the two is often a challenge. The pathogenesis underlying GVHD is a complex, multi-step process. There are many factors which are not fully understood which determine whether GVHD occurs and the strength of the reaction. These include the type and properties of transplanted T cells, histocompatibility antigen mismatching, interactions between T cells and endothelial cells, signaling events occurring between immune cells, induction of pro-inflammatory cytokines, and amount of B lineage-specific progenitor cells. Acute GVHD requires the early trafficking of donor naive T cells to recipient secondary lymphoid tissue where they undergo activation and expansion, and their subsequent migration to target organs where they elicit injury [15-17]. The study of these processes through laboratory studies including the measurement of cytokine production and cytokine receptors, determination of hematopoietic cell subtypes by flow cytometry, and determination of HLA antigen types by genotyping may help elucidate and separate the detrimental and beneficial effects conferred by donor-derived T cells and improve our understanding of GVHD.

10.1.2 Patient Selection:

Cleveland clinic patients enrolled in the trial may be asked to participate in this study to collect blood and tissue samples for laboratory research purposes.

10.1.3 **Research Plan/Study Parameters:**

Research in graft versus host disease depends on the ready availability of samples of blood, tissue (skin, GI tract, and liver biopsies) and bone marrow of transplant recipients and donors for *in vitro* studies. Assays may include phenotypic analysis by flow cytometry and histochemical methods, molecular biologic determination of specific DNA and RNA species, and gene expression analysis.

In addition, repositories of plasma, serum, DNA, RNA and whole cell preparations may be invaluable in retrospective analysis. Correlation of clinical data, such as age, sex, laboratory results, cytogenetics, bone marrow morphology, treatment, progression, co-morbidities, etc. may be used as needed. This data will only be linked to research sample number.

Recipients will have 10ml of peripheral blood obtained by venipuncture in 1 green top heparinized tube for a baseline specimen prior to transplantation. If a bone marrow specimen is performed, a 10 ml aspirate sample in a green top tube will be collected at that time. After transplantation, 10-20 ml (in 1-2 green top heparinized tubes) of peripheral blood may be obtained around transplant day +100 (+/-14 days), +180 (+/-30 days)(or time of immunosuppression withdrawal), and +365 (+/-30 days) through the patient's central venous catheter or by venipuncture. Additional blood samples from patients may be collected at time of clinical diagnosis or biopsy-proven GVHD.

If possible, with the development of GVHD some of the biopsy specimens from the organ involved (including skin, liver, or GI tract biopsy) may also be further assessed if there is adequate tissue.

For those patients who require a donor lymphocyte infusion (DLI) or subsequent infusion of additional hematopoietic stem cells post-transplant a follow-up peripheral blood sample will be obtained prior to procedure as well as again around day +100 (+/-14 days), and +365 (+/-30 days) after re-infusion.

The collection of ALL blood AND tissue specimens for the sample repository will only be collected when clinical specimens are collected to minimize patient discomfort.

10.1.4 **Specimen Handling:**

Tissue:
Biopsy material (bone marrow or GVHD affected tissue) may be frozen in liquid nitrogen and stored indefinitely at -80ºC for molecular genetic studies if required as deemed by specific study. Otherwise, routine specimen fixation (in formalin and other appropriate fixates) for conventional histology and immunohistochemistry/FISH, preservation in tissue culture media for flow cytometry, and analysis of fresh sterile tissue for cytogenetics. Post-procurement processing on both frozen and formalin-fixed paraffin-embedded tissues will be performed. Derivative DNA and RNA may be extracted on these tissue samples for relevant assays for investigators.

Blood:
The protocol for blood processing and storage is as follows: Peripheral blood will be obtained in green top heparinized tubes. For each 10 ml of whole blood, approximately 4-5 ml of serum or plasma can be derived. These will be divided into 0.5 ml aliquots. Excess mononuclear cells will be frozen and stored in aliquots for flow cytometric analysis or DNA extraction. Glycerol will be added to a final concentration of 10% (w/v) as a cryoprotectant and specimens will be frozen at -80°C in aliquots of 10 ml and stored until use.

Specimens will be collected after the patient signs the written, informed consent. Specimens will only be obtained at the time routine clinical specimens are collected to minimize patient discomfort. Patients may refuse collection of specimens at any time. Specimens will be stored indefinitely or until a patient withdraws from the study.

DNA/blood samples from all BMT donors are stored in a registry under IRB 09-591 Allogen Laboratories Registry/Database. These specimens/data may be shared/tested/combined with this study to gain additional information.

10.1.5 **Privacy Protection:**

Specimens allocated to this research project will be assigned a research ID number, which will be the only identifier provided to investigators when they are given specimens. The key linking the research ID number with the patient identification (name, CCF ID number and date of birth) will be located in OnCore which is a secured, password protected database. Occasionally chart review will be necessary. For this purpose, a Co-Investigator will be provided with patient name and ID number for the purpose of abstracting pertinent historical information. The information will be recorded with only the research ID number.

10.1.6 **Types of Laboratory Studies to Be Performed:**

Blood, surgical specimens and bone marrow from patients may be utilized for diverse purposes. DNA/blood samples from all BMT donors are stored in a registry under IRB 09-591 Allogen Laboratories Registry/Database. These specimens/data may be shared to gain additional information.

This includes studies of hematopoiesis and immune reconstitution, measurements of cytokine production and cytokine receptors; determination of hematopoietic cell subtypes by flow cytometry and identification of T cell repertoire and antigens. Some studies may be genetic in nature, as for example determination of HLA antigen types by genotyping. Cells as well as serum are often banked for several years for future studies, the nature, which cannot be precisely predicted. However, the following list consists of examples of research that is currently performed on blood, surgical specimens and bone marrow:

- Using Flow cytometry analysis for proteins on the surface or inside of cells.
- Using flow cytometry and cell culture techniques to identify lymphocyte types and their function in disease including TCR usage and cytokine secretion phenotype.

- Measuring immunity to malignant cells, normal cells, viruses, bacteria, and fungi using flow cytometry, PCR techniques, cytokine release assays, proliferation assays and cytotoxicity assays. Some techniques will require culture of cells for several weeks.
- Detection of viral DNA or RNA.
- Correlation of clinical data, such as age, sex, laboratory results, cytogenetics, bone marrow morphology, treatment, progression, transfusion requirements, past medical history, co-morbidities, etc. as needed for future projects. These data will only be linked to the research sample number.
- Gene expression analysis.
- Immunohistochemistry for proteins expressed in or on the surface of cells.
- Analysis of chromosomal defects using array-based comparative and other molecular methods.
- Analysis of discrete changes in the DNA sequences derived from blood, tissue, and bone marrow using high density SNP arrays:
    - SNP genotyping in blood and marrow
    - SNP array-based kayotyping in blood and marrow

These studies do not include any therapeutic manipulations.

10.1.7 **Risks:**

The risks of this trial are minimal to patients. Transplant patients will have central venous catheters in place from which most of their blood specimens can be obtained, thus avoiding the potential minor discomfort from venipuncture. No bone marrow aspirations/biopsies or surgeries/tissue biopsies will be performed solely for research purposes. They will only be performed if already medically indicated.

11.0 STUDY PARAMETERS AND CALENDAR

11.1 Study Parameters

11.1.1 Pre-Study

All evaluations must be completed < 42 days prior to administration of myeloablative conditioning regimen given for allogeneic hematopoietic stem cell transplant.

- Informed consent
- Demographics
- Medical history
- Complete physical exam
- Disease assessment of tumor or site involved including bone marrow biopsy and aspirate
- Performance status (ECOG for adults, Lansky for pediatrics)
- Laboratory studies:
    - Complete blood count (CBC) with differential

    - Hepatic enzymes: AST,ALT, alkaline phosphatase, total bilirubin, creatinine
    - ß-HCG for women of childbearing potential
    - PT/PTT
- Pulmonary function testing with DLCO if pt > 6 years of age
- CMV screening
- HIV screening

See Study Parameters Table for specific time points for study assessments

11.1.2 During **hospitalization for Transplant**

- Laboratory studies:
    - Complete blood count (CBC) with differential
    - Hepatic enzymes: AST,ALT, alkaline phosphatase, total bilirubin, creatinine
    - PT/PTT
- CMV DNA testing
- Mucositis grading
- Acute GVHD monitoring
- Organ toxicity assessment
- Peripheral blood chimerisms (approximately 1, 2, 3, 6, 9 and 12 months or as clinically indicated)

See Study Parameters Table for specific time points for study assessments

11.1.3 **Post hospitalization for Transplant to End of Study**

Same assessments as above (during hospitalization for transplant); See Study Parameters Table for specific time points for study assessments

11.1.4 **Study Parameters Table**

Required Assessments	Pre-study (< 42 days prior Allo-HSCT)	During Initial Hospitalization for Allo-HSCT	Post Hospitalization for Allo-HSCT to Study End Point
Tumor characteristics / Site of involvement	X		As clinically indicated
ECOG / Lansky	X		Q visits until 1 year
Bone Marrow Aspirate / Biopsy	X		Day 100 (optional) or as clinically indicated
CBC and diff	X	Daily until ANC >500, plt >20,000, then Q weekly until normal	Q weekly until day 100 and then at at follow up visits
Hepatic enzymes (AST, ALT, Alk Phos, Total Bili)	X	Daily until discharge	At follow up visits
Creatinine	X	Daily until discharge	At follow up visits
PT/PTT	X	Q weekly	As clinically indicated
CMV DNA	X	Q weekly	Weekly until D 100, then as clinically indicated
HIV screening	X		
PFT studies (> 6yo)	X		day 100 and as needed
PB chimerism		X (if patient is still in hospital at any time point)	Approximately 1, 2, 3, 6, 9, 12 months or as clinically indicated
HCG (female of childbearing age)	X		
Tac levels		2X / week or as clinically indicated	As clinically indicated
Acute GVHD Monitoring		1X / week starting D +7	Q weekly until day 100
Chronic GVHD monitoring			After D 100, at least every 3 months
Mucositis grading		3X / wk D 0 to D +28 or D/C (which ever occurs first)	
Organ Toxicity assessment		Q weekly	Q visits until 1 year

11.1.5 **Study Assessments**

Engraftment

Neutrophil engraftment will be measured by the number of days to reach a neutrophil count of greater than or equal 500/ul for three consecutive laboratory values obtained on different day. The day of engraftment will be the first day of the three consecutive laboratory values

Platelet engraftment will be measured by the number of days to reach a platelet count of greater than or equal 20,000/ul for three consecutive laboratory values obtained on different days independent of platelet transfusions the prior 7 days. The day of engraftment will be the first day of the three consecutive laboratory values.

Acute Graft-Versus-Host Disease
Acute graft-versus-host disease will be monitored daily and will be documented 1X / week from day +7 while patient is hospitalized for transplant. After discharge from hospital, Acute GVHD will be monitored and documented at a minimum of every 21 days till day +100 (or more if clinically indicated). Acute graft-versus-host disease will be graded according to the table shown in Appendix 1. Skin, gastrointestinal, and/or liver biopsies will be obtained, when appropriate, to facilitate the diagnosis. The type and duration of immunosuppressive treatment given for aGVHD will be recorded.

Chronic Graft-Versus-Host Disease
After day 100, patients will be assessed at least every 3 months (+/- 14 days) for cGVHD. cGVHD will be graded as mild, moderate, or severe based on the National Institutes of Health Consensus Development Project according to the table shown in Appendix II. The type and duration of immunosuppressive treatment given for cGVHD will be recorded.

Mucositis
Mucositis will be graded 3 times per week (Mon-Wed-Fri) by the mid-level provider or transplant physician from day 0 until day +28 or day of discharge (whichever occurs first) according to the WHO grading scale shown in Appendix III.

Total Parenteral Nutrition (TPN)
Start and stop dates of TPN will be recorded.

IV Pain Medications
Start and stop dates for the need for continuous IV infusion of narcotics for severe mucositis pain in the form of intravenous infusion or patient controlled analgesia (PCA) will be recorded.

Length of Hospitalization
Length of hospitalization will be measured from date of transplant to date of discharge.

Organ Toxicity
Information regarding renal, hepatic and pulmonary function will be collected at study entry and will be assessed throughout the time that the patient remains on GVHD prophylaxis. Patients will be assessed at least weekly until day 100 post transplant and then at follow up visits for 1 year post transplant for organ toxicity.

A patient will be considered to have liver dysfunction should the patient have a total bilirubin $\geq$ 3X the upper limit of normal and AST, ALT or alkaline phosphatase $\geq$ 5X the upper limit of normal.

Hepatic veno-occlusive disease will be diagnosed according to criteria set forth by McDonald et al [18]. Patient must have the presence, before day 20 after transplant, of at least two of the following features:

- Bilirubin greater than or equal to 2 mg/dL
- Hepatomegaly and right upper quadrant pain
- Ascites and/or unexplained weight gain greater than 2% from baseline.

A patient will be considered to have renal dysfunction should the patient have a serum creatinine $\geq$ 3X the baseline value, creatinine clearance or GFR $\leq$ 40% of baseline or dialysis dependence.

A patient will be considered to have pulmonary toxicity based on the following criteria (using CTCAE v4):

- Bronchopulmonary hemorrhage, grade 3 or higher
- Pulmonary edema, grade 3 or higher
- Respiratory failure, grade 4 or higher

Overall Survival and Relapse-Free Survival
Both overall survival and relapse-free survival will be assessed one year from the day of stem cell infusion, T-O or death, which ever is first. One-year-relapse-free and overall survival will be defined by CIBMTR criteria for individual diseases. Suspicion for relapse should be confirmed by bone marrow biopsy and date of biopsy should serve as date of relapse. Biopsy is strongly encouraged but not required if there are compelling medical reasons why biopsy is not feasible or unobtainable or if relapse is otherwise evident and confirmed by peripheral blood flow cytometry.

Evaluation of Chimerism
Donor chimerism will be assessed by analysis of single-tandem repeats on whole marrow and in lymphocyte enriched or sorted CD3+ T cells and CD33+ granulocytes from the blood depending on institution practice. Blood will be obtained for donor chimerism "BME analysis" at approximately 1, 2, 3, 6, 9 and 12 months or as clinically indicated.

Infections

Documented bacterial, viral and fungal infections will be recorded for 1 year after transplantation.

12.0 MEASUREMENT OF EFFECT

12.1 Incidence, Length and Severity of Mucositis

The incidence, length and severity of mucositis from day 0 to day 28 (or discharge date if earlier) after allogeneic bone marrow transplantation comparing Tac, mini-dose MTX and MMF with Tac and MTX will be determined.

12.2 Rate of Neutrophil and Platelet Engraftment

Number of days to engraftment, for both neutrophil and platelet after allogeneic bone marrow transplantation comparing Tac, mini-dose MTX and MMF with Tac and MTX will be determined.

12.3 Incidence of Acute GVHD

The incidence and severity of grade II-IV and III-IV acute GVHD after allogeneic bone marrow transplantation comparing Tac, mini-dose MTX and MMF with Tac and MTX will be determined.

12.4 **Secondary objective parameters** will also be compared in the Tac, mini-dose MTX and MMF with the Tac and MTX:

- Length of hospitalization
- Length of use of TPN
- Incidence of infection in the first 100 days post-transplantation
- Incidence of hepatotoxicity in the first 100 days post-transplantation
- Incidence of nephrotoxicity in the first 100 days post-transplantation
- Incidence of pulmonary toxicity in the first 180 days post-transplantation
- Incidence and severity of chronic GVHD
- One year relapse-free survival
- Overall survival
- Chimerism results

13.0 RECORDS TO BE KEPT / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 8.0 (Adverse Events: List and Reporting Requirements).

13.1 Data Reporting

The OnCore Database will be utilized, as required by the Case Comprehensive Cancer Center, to provide data collection for accrual entry OnCore is a Clinical Trials Management System housed on secure servers maintained at Case Western Reserve University. OnCore properly used is compliant with Title 21 CFR Part 11. Access to data through OnCore is restricted by user accounts and assigned roles.

Data will be entered into the Cancer Center Clinical Trials Oncore Database. The Investigator will ensure that the records and documents pertaining to the conduct of the study and the distribution of the protocol therapy, that is copies of CRFs and source documents (original documents, data, and records [e.g., hospital records; clinical and office charts; laboratory notes; SAE reports]) be retained by the Investigator for as long as needed to comply with national and international regulations (generally 2 years after discontinuing

clinical development). The records of this study will be kept private. Information that may be gained from this study will be used for research and educational purposes only. In any report that might get published, no information will be included that would allow for identification of the patient. At any time the Investigator may be subject to a field audit by regulatory authorities (e.g., FDA, TPP, EMEA) in order to validate the participation of subjects in the study. Therefore, careful attention should be paid to seeing that all study documents/records are complete, accurate, and filed and retained by the Investigator.

13.2 Regulatory Considerations

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

13.2.1 Written Informed Consent

Provision of written informed consent must be obtained prior to any study-related procedures. The Principal Investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study as well as the subject's financial responsibility. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The original, signed written Informed Consent Form must be kept with the Research Chart in conformance with the institution's standard operating procedures. A copy of the signed written Informed Consent Form must be given to the subject.

13.2.2 Subject Data Protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), a subject must sign an authorization to release medical information to the sponsor and/or allow the sponsor, a regulatory authority, or Institutional Review Board access to subject's medical information that includes all hospital records relevant to the study, including subjects' medical history.

13.2.3 Retention of Records

The Principal Investigator of The Cleveland Clinic supervises the retention of all documentation of adverse events, records of study drug receipt and dispensation, and all IRB correspondence for as long as needed to comply with national and international regulations. No records will be destroyed until the Principal Investigator confirms destruction is permitted.

13.2.4 Audits and Inspections

Authorized representatives of the sponsor, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the Center to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and

accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements.

13.2.5 **Data Safety and Monitoring Plan**

This protocol will adhere to the policies of the Case Comprehensive Cancer Center Data and Safety Monitoring Plan in accordance with NCI regulations.

14.0 STATISTICAL CONSIDERATIONS

Considering the potential for missed doses of methotrexate or other drug, this investigation will be based on an intent to treat analysis. This study hopes to show a reduction in the incidence of severe mucositis and time to neutrophil and platelet engraftment while not increasing the incidence or severity of acute GVHD in the allogeneic HCT population.

Our current rate of severe (grade 3-4) mucositis is 41%. Ninety-four (47 in each group) would be needed to demonstrate a 25% improvement in mucositis to 16%, based on a one-sided test with 5% significance and 80% power.

Our current estimate of mean day to neutrophil engraftment is 19 days. One hundred patients (50 in each group) would be needed to demonstrate a 4 day improvement in time to neutrophil engraftment based on a one-sided test with 5% significance and 80% power.

Our current percent of patients achieving platelet engraftment by day 21 is 26%. Ninety-eight (49 in each group) would be needed to demonstrate a 25% improvement in platelet engraftment to 51%, based on a one-sided test with 5% significance and 80% power.

Our current rate of any acute GVHD by day 60 is 62%. If the hazard ratio between treatment arms is at most 1.7, then we will conclude that the new treatment does not significantly increase acute GVHD. Forty-five patients per group are needed to detect this hazard ratio using a one-sided non-inferiority log-rank test with 5% significance and 80% power.

Therefore, this study requires 100 patients to have a minimum of 80% power to address all of the primary objectives.

Overall survival and progression-free survival will be estimated using the Kaplan-Meier method and compared between patients receiving Tac and MTX versus Tac, mini-MTX, and MMF using the log-rank test. Hospital stay will be compared between groups using the Wilcoxon rank sum test. TPN and 100-day incidence of complications will be compared using the Chi-square test. Acute and chronic GVHD will be estimated using cumulative incidence methods and compared using the Pepe-Mori test.

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APPENDIX I

ACUTE GRAFT VS. HOST DISEASE GRADING SCALE

Acute GVHD Staging:

Stage	Skin	Liver	Intestine
0	No rash	Bilirubin ≤ 2 mg/dl	< 500 mL diarrhea per day
1	Maculopapular rash <25% of body surface	Bilirubin 2-3 mg/dl	>500-1000 mL diarrhea per day or persistent nausea/vomiting/anorexia
2	Maculopapular rash 25-50% of body surface	Bilirubin 3.1-6 mg/dl	>1000 -1500 mL diarrhea per day
3	Maculopapular rash >50% body surface area or Generalized erythroderma	Bilirubin 6.1-15 mg/dl	>1500 mL diarrhea per day
4	Generalized erythroderma with bullous formation and desquamation	Bilirubin >15 mg/dl	> 2000 mL diarrhea per day or severe abdominal pain +/-ileus

Acute GVHD Grading:

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

Conversion chart for staging the volume of Diarrhea in Pediatric Patients: (Source adapted from the modified keystone criteria GVHD reporting form) and the pediatric GUT GVHD staging is based on the CIBMTR/ CTN technical manual of practice version 2 (Sep, 2005).

STAGE	STOOL VOLUME
0	none or diarrhea < 280 ml/m^2/day
1	280-555 ml/m^2/day
2	556-833 ml/m^2/day
3	> 833 ml/m^2/day
4	severe abdominal pain, w/ or w/out ilius

APPENDIX II

CHRONIC GVHD SCORING: using NIH consensus criteria

	Score 0	Score 1	Score 2	Score 3
Performance Score: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1; KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, > 50% of waking hours out of bed (ECOG 2; KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, > 50% of waking hours in bed (ECOG 3-4; KPS or LPS < 60%)
SKIN Clinical Features: ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthysosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Pruritus ☐ Sclerotic features ☐ Hair involvement ☐ Nail involvement **% BSA involved:** ☐	☐ No symptoms	☐ < 18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ > 50% BSA **OR** deep sclerotic features "hide-bound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **WITH** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **WITH** major limitation of oral intake
EYES Mean tear test (mm): ☐ > 10 ☐ 6-10 ☐ < 5 ☐ Not done	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eye-drops < 3 x/day) **OR** asymptomatic signs of kerato-conjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring eye-drops > 3 x/day or punctual plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
GI TRACT	☐ No symptoms	☐ Symptoms such as	☐ Symptoms associated with	☐ Symptoms associated with significant weight

		dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (< 5%)	mild to moderated weight loss (5-15%)	loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
LIVER AST ____ ALT ____ AP ____ Tbili	☐ Normal LFT	☐ Elevated Bilirubin, AP, AST or ALT < 2x ULN	☐ Bilirubin > 3 mg/dl or bilirubin, enzymes 2-5x ULN	☐ Bilirubin or enzymes > 5x ULN
LUNGS **FEV1** ☐☐☐ **DLCO** ☐☐☐	☐ No symptoms ☐ FEV1 > 80% **OR** LFS =2	☐ Mild symptoms (SOB climbing one flight of stairs) ☐ FEV1 60-79% **OR** LFS 3-5	☐ Moderate symptoms (SOB after walking on flat ground) ☐ FEV1 40-59% **OR** LFS 6-9	☐ Sever symptoms (SOB at rest; requiring O2) ☐ FEV1 40-59% **OR** LFS 6-9
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs OR joint contractures, erythema thought due to fasciitis, moderated decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures WITH significant decrease of ROM **AND** significant limitation of ADL (unable to tie shoes, button shirts, dress self, etc.)
GENITAL TRACT	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic WITH advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

Other indicators, clinical manifestations or complications related to chronic GVHD.

- Check all that apply
- Assign a score to its severity (0-#) based on its functional impact where applicable
  - none = 0
  - mild = 1
  - moderate = 2
  - severe = 3

Esophageal stricture/web _____	Pericardial Effusion _____	Pleural Effusion(s) _____
Ascites (serositis) _____	Nephrotic syndrome _____	Peripheral Neuropathy _____
Myasthenia Gravis _____	Cardiomyopathy _____	Eosinophilia > 500ul _____
Polymyositis _____	Cardiac conduction defects _____	Coronary artery involvement involvement _____
Platelets < 100,000/ul _____	Progressive onset _____	

Others: (specify) _____

CHRONIC GVHD GRADING

MILD: involves only 1 or 2 organs or sites (except the lung), with no clinically significant functional impairment (maximum of score 1 in all affected organs or sites).

MODERATE: involves: (1) at least 1 organ or site with clinically significant but no major disability (maximum score of 2 in any affected organ or site) or (2) 3 or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). Or a lung score of 1 will also be considered moderate chronic GVHD.

SEVERE: indicates major disability caused by chronic GVHD (score of 3 in any organ or site). Or a lung score of 2 or greater will also be considered severe chronic GVHD.

APPENDIX III

WORLD HEALTH ORGANIZATION (WHO) MUCOSITIS GRADING SCALE

Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
No mucositis	Soreness +/- Erythema	Erythema, ulcers; ability to eat solids	Ulcers, requires liquid diet	Alimentation not possible.

The following additional guidelines may be used in the assessment of oral mucositis

- **Grade 1** may include buccal mucosal scalloping with or without erythema. No ulcers. Patient can swallow a solid diet.
- **Grade 2** may include ulcers with or without erythema. Patient can swallow a solid diet.
- **Grade 3** may include ulcers with or without (extensive) erythema. Patient is able to swallow a liquid, but not solid diet.
- **Grade 4** refers to mucositis to the extent that alimentation is not possible. If total parenteral nutrition was started for reasons other than mucositis, a determination of the subject's ability to swallow must be made using the above criteria.

APPENDIX IV

TBI ADMINISTRATION

Patients may be treated either in the AP/PA position and/or in the right and left lateral position. Compensators or blocks may be used to compensate for the thinner parts of the anatomy (neck, head, lower legs, and feet).

Total dose will be 1200-1320 cGy in 6-8 fractions over 3-4 days with at least six hours between fractions depending on institutional guidelines. Dose will be prescribed at the level of the umbilicus at midplane.

TBI will be delivered from either a linear accelerator or cobalt source at a dose rate of between 4 and 26 cGy/minute using energies of between 1 and 25 MV.

The skin dose should be at least 90% of the prescribed dose. If a higher energy beam (> 4 MV)is used for the TBI treatments, a beam spoiler should be used to accomplish this or thermoluminescent dosimetry data submitted showing that the skin dose is at least 90% of the prescribed dose.

Testicular boosts could be used for all males with ALL (and according to institutional practices for other diseases) depending on institutional guidelines. The testicular boost recommendation is single 400 cGy fraction with either electrons prescribed to D_{max} or photons prescribed to the midplane of the scrotum. If electrons are used, the energy for the testicular boost depends on the thickness of the testicles and is chosen so that the D90 corresponds to the posterior surface of the scrotum.