Protocol and Statistical Analysis Plan

# Phase II Open-Label Trial of Tacrolimus/Methotrexate and Tocilizumab for the Prevention of Acute Graft-Versus-Host Disease After Allogeneic Hematopoietic Stem Cell Transplantation

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# PHASE II OPEN LABEL TRIAL OF TACROLIMUS/METHOTREXATE AND TOCILIZUMAB FOR THE PREVENTION OF ACUTE GRAFT VERSUS HOST DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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## PROTOCOL SYNOPSIS

### Phase II Study of Tacrolimus Methotrexate and Tocilizumab for the Prevention of Acute Graft-versus-Host Disease after Allogeneic Hematopoietic Cell Transplantation

| | |
|---|---|
| **Principal Investigator:** | William R. Drobyski, MD; Marcelo Pasquini, MD |
| **Study Design:** | This is a phase II open label trial designed to evaluate the efficacy of Tacrolimus, Methotrexate and Tocilizumab (Tac/MTX/Toc) in preventing graft versus host disease (GVHD) compared to a contemporary control from the CIBMTR. |
| **Primary Objective:** | The primary end point of the study is to compare grade II-IV aGVHD-free survival at Day 180 between recipients of Tac/MTX/Toc to contemporary CIBMTR controls. |
| **Secondary Objectives:** | Compare, grades II-IV and III-IV aGVHD, chronic GVHD, neutrophil and platelet engraftment, transplant related mortality (TRM), disease relapse or progression, progression-free and overall survival. Phase 2 specific outcomes include incidence of grades ≥3 toxicities according to CTACAE v4, incidence of infections, and proportion of donor chimerism, immune reconstitution, and production of pro-inflammatory cytokines. |
| Exploratory Objectives: | Compare depressive symptoms, anxiety, fatigue, sleep, and pain among allogeneic HCT recipients receiving tocilizumab to a control group of allogeneic HCT recipients who did not receive prophylactic tocilizumab (UW control cohort). To describe gene expression and Rap1 prenylation among allogeneic HCT recipients receiving prophylactic tocilizumab. |
| **Eligibility:** | Eligible patients are 18 years and older undergoing HCT for treatment of acute leukemia in complete morphologic remission; chronic myelogenous leukemia, other myeloproliferative disorders and myelodysplasia with less than 5% blasts in bone marrow; and chemotherapy sensitive lymphoproliferative diseases. Patients must receive a busulfan-based conditioning regimen and have related or unrelated peripheral blood or bone marrow donor. Sibling donor must be a 6/6 match for HLA-A, B and DRB1 or unrelated donor must be a 8/8 match at HLA- |

| | |
|---|---|
| | A, -B, -C and DRB1. Patients are excluded if they have a history of intolerance or allergy to Tocilizumab; or if they received rituximab or other monoclonal antibodies as part of the conditioning regimen. Eligibility from the control cohort includes patients who received their first allogeneic transplant at a US center, for the same indications and conditioning regimens as listed above, from a HLA match related or unrelated donor with bone marrow or peripheral blood graft in the years of 2010 to 2013. Controls should also receive tacrolimus and methotrexate as the sole GVHD prophylaxis. Donor type of the controls will be matched to that of patients enrolled in the clinical trial. |
| **Treatment Description:** | Patients enrolled in the clinical trial will receive tacrolimus per standard of care at doses to maintain therapeutic levels and continued until at least Day 90 post-transplant. Methotrexate will be dosed at 15 mg/m$^2$ Day +1 and 10mg/m$^2$ Days +3, +6 and +11. Tocilizumab will be administered intravenously at a dose of 8 mg/kg at Day -1. |
| . | |
| **Accrual Objective:** | 35 patients will be accrued to the clinical trial and at most 200 CIBMTR controls will be included in the analysis. |
| **Accrual Period:** | The estimated accrual period is 2 years. |
| **Study Duration:** | Patients will be followed for 12 months following initiation of therapy. |

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# 1.0 BACKGROUND AND RATIONALE

## 1.1 Acute Graft versus Host Disease

Graft versus host disease (GVHD) is the major complication associated with allogeneic hematopoietic cell transplantation (HCT). A prominent characteristic of GVHD is the presence of a proinflammatory milieu that is attributable to conditioning regimen-induced host tissue damage as well as secretion of inflammatory cytokines [e.g. interleukin-1 (IL-1), tumor necrosis alpha-α (TNF-α), interferon-γ (IFN-γ), interleukin-6 (IL-6)] by alloactivated donor T cells and other effector cell populations.[1-3] These cytokines perpetuate GVHD through direct cytotoxic effects on host tissues,[4-6] activation and/or priming of immune effector cells,[7] and differentiation of proinflammatory T cell populations (i.e. $T_H1$ and $T_H17$ cells) from naïve T cell precursors.[8,9] This inflammatory environment is also promoted by the absence of an effective regulatory T cell (Treg) response as both a relative and an absolute decline of Tregs in the peripheral blood and target tissues has been demonstrated in a majority of studies.[8,10-12] The strong association between a proinflammatory milieu and the absence of an effective counter regulatory response suggests that the inflammatory environment prevents and/or inhibits Treg reconstitution during GVHD. How this occurs, however, is not well understood.

Up to 30% of the recipients of HCT from HLA-match donors develop at least grade II aGVHD despite immunosuppressive prophylaxis.[13] The occurrence of moderate (grade II) or severe (grade III or IV) aGVHD after HCT results in increased treatment related mortality (TRM), which precludes improvement in overall survival.[14] T-cells in the graft play a key role in the pathogenesis of aGVHD. During the past 20 years, a combination of a calcineurin inhibitor (tacrolimus or cyclosporine (CSA)) and methotrexate (MTX) has been standard therapy for prevention of GVHD. However, despite use of these agents, the incidence of grade II-IV GVHD is 35-50%[15] The risk increases with unrelated or partially HLA-matched donors due to the greater genetic disparity between the donor and the host. Furthermore, acute GVHD incidence and severity is augmented in older aged patients who increasingly comprise the majority of adult patients that need an allogeneic stem cell transplant for the treatment of their hematological malignancy. Thus, new therapeutic treatment strategies are desperately needed for the prevention of acute GVHD.

## 1.2 Interleukin 6

IL-6 is a pleiotrophic cytokine that is produced by a variety of cell types, including T cells, B cells, fibroblasts, endothelial cells, monocytes and keratinocytes.[16] IL-6 is of particular interest with respect to GVHD biology since it occupies a unique position at the crossroads where the fate of naïve T cells to become either regulatory cells or proinflammatory T cells is determined. In the presence of IL-6 and transforming growth factor-β (TGF-β), naïve T cells differentiate into $T_H17$ cells, whereas in its absence these same cells are induced to become Tregs.[117,18] Furthermore, IL-6 produced by dendritic cells after activation through Toll-like receptors is able to inhibit the suppressive function of natural Tregs.[19,20] Thus, IL-6 appears to have a pivotal role in directing the immune response towards an inflammatory phenotype and away from a regulatory response. The potential importance of IL-6 in GVHD is also supported by clinical studies that have shown that patients with elevated plasma levels of IL-6,[21,22] as well as those with a recipient or donor IL-6 genotype that results in increased IL-6 production,[23,24] have an increased incidence and severity of GVHD.

Signaling through IL-6 occurs by the binding to a low affinity IL-6 receptor (IL-6R) which together induces homodimerization of gp130 and subsequent transduction of the intracellular signal.[25] This membrane-bound IL-6R, however, is expressed only on hepatocytes and hematopoietic cells. Notably, the IL-6R can also be shed from the membrane generating a soluble form of the receptor which can complex with IL-6 and induce an intracellular response in cells that lack the membrane-bound IL-6R through a process called trans-signaling.[26,27] Interference with the actions of IL-6 by administration of an IL-6R antibody that prevents binding of the cytokine to its receptor has been shown to be effective in the treatment of a variety of inflammatory disease such as rheumatoid arthritis,[28,29] amyloidosis,[30] and colitis.[31]

### 1.3 **Tocilizumab**

Tocilizumab (Actemra™) is a humanized anti-IL-6 receptor antibody that blocks IL-6 signaling and has been FDA-approved for the treatment of severe active rheumatoid arthritis. It has been shown to have remission-inducing efficacy in patients with moderate to severe rheumatoid arthritis, systemic juvenile idiopathic arthritis, and multicentric Castlemans' disease.[32,33] A pilot phase I/II study in patient with active Crohn's disease also suggested benefit when administered on an every two week basis.[34] Recent studies in a murine model of GVHD, has shown that treatment with an anti-IL-6R antibody is able to significantly reduce GVHD-associated mortality and pathological damage.[35] One of the mechanisms by which this occurs is through the enhanced reconstitution of regulatory T cells that express the transcription factor foxp3. Reconstitution of both natural and induced Tregs has been demonstrated in animals treated with this antibody, suggesting that the increase in overall numbers of Tregs is responsible, in part, for the attenuation in GVHD severity. A recent case report demonstrated that administration of Tocilizumab was effective at significantly reducing the severity of GVHD in the GI tract as determined by a marked reduction in the volume of diarrhea.[36] Notably, this patient had failed multiple prior therapies, including high dose steroids, infliximab, budesonide, photopheresis, sirolimus, and mycophenolate mofetil. Furthermore, Drobyski et al[37] published a series of patients who received Tocilizumab for steroid refractory acute GVHD. Responses were observed in four of six patients with severe acute GVHD that had failed to respond to first, and in most cases, second line therapies. Collectively, these studies support the premise that Tocilizumab has activity in GVHD.

### 1.4 **IL-6 blockade in GVHD prophylaxis**

Whether blockade of IL-6 signaling has efficacy in the prevention of GVHD has not been critically examined. In a preliminary unpublished report, Kennedy et al[38] analyzed IL-6 levels during HCT and demonstrated that levels peaked at day 7 post transplant and only returning to pre-transplant level by day 30. This study demonstrated the same pattern of IL-6 levels in the related and unrelated HLA matched transplants, myeloablative regimens were associated with higher day 7 peaks of IL-6 in the serum compared to reduced intensity conditioning. Kennedy et al subsequently used an anti-IL-6 humanized monoclonal antibody with cyclosporine and methotrexate as GVHD prophylaxis in 48 recipients of HLA matched HCT. The mAb was administered as a single dose day -1 of transplant. The rates of grade II-IV and III-IV acute

GVHD were 11.1% and 5.6%, respectively. There were no reports of graft failure in this analysis.

### 1.5 **Rationale**

The current clinical trial has the objective to compare the combination of tacrolimus, methotrexate and tocilizumab (Tac/MTX/Toc) as GVHD prophylaxis to a contemporary control. The study will build upon the experience from Kennedy et al, by further exploring IL-6 blockade in GVHD prevention.

## 2.0 STUDY DESIGN

This is a phase II open label trial designed to evaluate the efficacy of Tac/MTX/Toc in preventing graft versus host disease (GVHD). Outcomes of patients on this clinical trial will be compared to those of contemporary controls from the CIBMTR.

### 2.1 **Primary Objective**

The primary objective of this study is to compare the probabilities of Grade II-IV acute GVHD-free survival at Day 180 post-transplant between recipients of Tac/MTX/Toc and contemporary controls who received Tac/MTX based GVHD prophylaxis.

### 2.2 **Secondary Objectives**

Secondary objectives of the study are to compare grades II-IV and III-IV aGVHD, chronic GVHD, neutrophil and platelet engraftment, transplant related mortality (TRM), disease relapse or progression, progression-free and overall survival between Tac/MTX /Toc and Tac/MTX CIBMTR controls. Additionally, secondary objectives include description of incidence of grades ≥3 toxicities according to CTACAE v4, incidence of infections, and proportion of donor chimerism, immune reconstitution, and production of pro-inflammatory cytokines among patients who receive Tac/MTX/Toc.

### 2.3 **Exploratory Objectives**

The primary objective of this ancillary study is to compare levels of depressive symptoms, anxiety, fatigue, sleep, and pain between allogeneic HCT recipients who received prophylactic tocilizumab to a control group of allogeneic HCT recipients who did not receive tocilizumab (UW control cohort). Additionally, exploratory objectives include assessing gene expression and Rap1 prenylation levels.

## 2.4 **Patient Eligibility**

### 2.4.1 Inclusion Criteria (Tocilizumab Arm)

1. Age ≥18years
2. Patients with acute leukemia, chronic myelogeneous leukemia, myeloproliferative disease and myelodysplasia with less than 5% of blasts in the bone marrow.
3. Patients with chronic lymphocytic leukemia/small lymphocytic lymphoma, Non-Hodgkin Lymphoma or Hodgkin Disease with chemosensitive disease at time of transplant.
4. Planned conditioning regimens including combination of busulfan and fludarabine or busulfan and cyclophosphamide.
5. Transplantation with T-cell-replete grafts
6. Bone marrow or mobilized peripheral blood cell grafts
7. Patients must have either a sibling donor (6/6 match at HLA-A, B and DRB1) or a unrelated donor (8/8 match at HLA-A, -B, -C and -DRB1).
8. Cardiac function: Ejection fraction at rest >45% for myeloablative conditioning or >40% for reduced intensity conditioning.
9. Estimated creatinine clearance greater than 50 mL/minute (using the Cockcroft-Gault formula and actual body weight)
10. Pulmonary function: DLCO ≥40% (adjusted for hemoglobin) and FEV1≥50%
11. Liver function: total bilirubin < 1.5 x the upper limit of normal and ALT/AST < 2.5x the upper normal limit.
12. Signed informed consent.

### 2.4.2 Exclusion criteria (Tocilizumab Arm)

1. Prior allogeneic HCT
2. Karnofsky Performance Score <70%
3. Patients with uncontrolled bacterial, viral or fungal infections (currently taking medication and with progression of infectious disease or no clinical improvement) at time of enrollment.
4. Prior intolerance or allergy to Tocilizumab
5. Use of rituximab, alemtuzumab, ATG or other monoclonal antibody at time of conditioning regimen.
6. History of diverticulitis, Crohn's disease or ulcerative colitis
7. History of demyelinating disorder
8. Pregnant and lactating women
9. Patients with a history of rheumatologic disorders who have previously received Tocilizumab

### 2.4.3 Eligibility for the Control Arm

Patients in the control arm will be identified from patients reported to the CIBMTR from U.S centers. Control patients will be required to satisfy similar eligibility requirements as patients

being enrolled in the clinical trial. Patients will need to fulfill the same inclusion criteria for the clinical trial according to Section 2.3.1, plus the following:

1. Receive Tac/MTX as the sole GVHD prophylaxis approach
2. Receive the same regimens as specified in Table 2.5
3. Year of transplant from 2010 to 2013

Exclusion criteria for the controls:

1. Karnofsky Performance Score < 70%

Data for all eligible patients will be used to constitute the control database for this study.

## 2.5 Donor Selection

Donor selection will be done according to the eligibility of this trial following the standard operating procedures from MCW BMT program.

## 2.6 Treatment Plan

It is recommended that adjusted ideal body weight be used when calculating conditioning regimen chemotherapy doses. One exception is fludarabine, which uses actual body weight.

**Ideal Body Weight (IBW) will be calculated per institutional standards.**

### 2.6.1 Conditioning Regimens

Eligible patients will receive either a myeloablative or reduced intensive conditioning according to the decision of the treating physician. Regimens allowed in this protocol are outlined in table 2.5.

**TABLE 2.5: CONDITIONING REGIMENS[1]**

| Reduced Intensity Conditioning | Myeloablative Conditioning |
|---|---|
| **Fludarabine/Busulfan (Flu/Bu2)**<br>• Fludarabine (120-180 mg/m$^2$)<br>• Busulfan (≤ 6.4 mg/kg IV) | **Fludarabine/Busulfan[a] (Flu/Bu)**<br>• Busulfan (12.8-16 mg/kg )<br>• Fludarabine (120-180 mg/m$^2$) |
| | **Busulfan[a]/Cyclophosphamide (Bu/Cy)**<br>• Busulfan (12.8 -16 mg/kg )<br>• Cyclophosphamide (120 mg/kg) |

[a] Bu = PO doses will be adjusted to maintain BU Css at 900±100 ng/ml.

2.6.1.1 Fludarabine and busulfan (Flu/Bu2)

The recommended Flu/Bu regimen is the following:

- Days -6 to -2: Flu (30 mg/m$^2$/day, total dose of 150 mg/m$^2$)
- Days -5 to -4: Busulfan (3.2mg/kg/day IV for 2 days, total dose of 6.4mg/kg, respectively)

The sequence of fludarabine and busulfan administration in RIC regimens will be done according to MCW BMT standing operating procedures.

2.6.1.2 Busulfan and fludarabine (Flu/Bu4)

The recommended Bu/Flu regimen is the following:

- Days -5 to -2: Busulfan (~3.2 mg/kg/day for 4 days with targeted Bu Css 900±100 ng/mL)
- Days -5 to -2: Flu (30 mg/m$^2$/day, total dose of 120 mg/m$^2$)

Busulfan dosing for myeloablative doses will follow pharmacokinetics to target a concentration at steady state of 900±100 ng/mL. Busulfan will be administered every 6 hours and starting dose may range from 0.8mg/kg to 0.9mg/kg or according to the test dose pharmacokinetics if it is done.

2.6.1.3 Busulfan and cyclophosphamide (Bu/Cy)

The recommended Bu/Cy regimen is the following:

- Days -7 to -4: Busulfan (~3.2 mg/kg/day for 4 days with targeted Bu Css 900±100 ng/mL)
- Days -3 to -2: Cy (60 mg/kg/day, total dose of 120mg/kg).

Busulfan dosing for myeloablative doses will follow pharmacokinetics to target a concentration at steady state of 900±100 ng/mL. Busulfan will be administered every 6 hours and starting dose may range from 0.8mg/kg to 0.9mg/kg or according to the test dose pharmacokinetics if it is done.

2.6.2 Hematopoietic Cell Transplantation

Bone marrow and mobilized peripheral blood progenitor cells are the graft sources in this study.

2.6.2.1 Peripheral Blood Progenitor Cells PBPC) Mobilization and Collection

PBPC mobilization and collection will be done according to standard operating procedures from MCW BMT program. It is recommended the following mobilization and collection:

- Donors will receive G-CSF (filgrastim, Amgen) at a dose of ~10 mcg/kg/day subcutaneously for 5 consecutive days. Daily dose will not exceed 1200 mcg/day, and volume per injection site will not exceed 2.0 mL. G-CSF should be administered at approximately the same time each day. The fifth dose will be given at least one hour prior to apheresis.
- Apheresis will begin on Day 5 of G-CSF administration. The recommended method for apheresis is through a continuous-flow apheresis device and ideally bilateral peripheral venous access. Donors with insufficient peripheral access will undergo placement of a central venous catheter.

Target CD34 cell doses are between 5-10 x $10^6$ per kg recipient body weight.

The transportation of the PBPC product from unrelated donors shall be done in accordance with NMDP Standards.

2.6.2.2 Bone marrow collection

Bone marrow donors should undergo harvest on Day 0. Either general or regional (epidural, spinal) anesthesia may be used. The bone marrow cell dose recommended is approximately 4 x $10^8$ nucleated cells per kg of recipient body weight. This dose will be unattainable for many recipients because of donor and/or recipient factors, e.g., body size mismatches. The volume of marrow shall not exceed 20 mL per kg donor weight. The estimated cell dose and a planned donor marrow volume shall be agreed upon by the donor and transplant centers for unrelated donors and between the transplant physician and cell processing laboratory for related donors before initiation of the transplant conditioning regimen.

Processing of bone marrow for reduction of volume, plasma, red blood cells, or fat, will be performed by the transplant center cell processing laboratory according to standard operating procedures from MCW BMT program.

2.6.2.3 PBPC and Marrow Infusion

PBPC or BM grafts will be infused through an appropriate central catheter, according to standard operating procedures from MCW BMT program at Day 0. For recipients of related donor PBPC, whose donors require a third day of collection (Day +1), these cells will be infused separately from the Day –1 and Day 0 collections on Day +1.

### 2.6.3 Tacrolimus/Methotrexate/Tocilizumab

Tacrolimus

Tacrolimus will be given per standard operating procedures from MCW BMT program,. Subsequent dosing will be based on blood levels. The dose should be adjusted accordingly to maintain a suggested level of 5-15 ng/mL. The dose of tacrolimus may be switched to oral at a 1:4 dose equivalence and rounded to the nearest 0.5 mg at the discretion of the treating physician. If patients are on medications which alter the metabolism of tacrolimus (e.g. azoles), the initial starting dose and subsequent doses should be altered as per institutional practices. Tacrolimus taper can be initiated at a minimum of 90 days post HSCT if there is no evidence of active GVHD. The rate of tapering will be done according institutional practices but patients should be off tacrolimus by Day 180 post HSCT if there is no evidence of active GVHD.

Methotrexate

Methotrexate will be administered, per institutional practices, at the doses of 15 mg/m$^2$ IV bolus on Day +1, and 10 mg/m$^2$ IV bolus on Days +3, +6 and +11 after hematopoietic stem cell infusion. Dose reduction of MTX due to worsening creatinine clearance after initiation of conditioning regimen, high serum levels or development of oral mucositis is allowed according to institutional practices.

Tocilizumab

Tocilizumab will be administered intravenously at a dose of 8 mg/kg (maximum dose of 800mg) once on the Day-1 approximately 24 hours prior to the estimated time of the hematopoietic stem cell infusion. The infusion will be administered over 60 minutes through a dedicated IV line and must not be administered by IV bolus.

## 2.7 **Supportive Care**

All supportive care will be given according to standard operating procedures of the MCW BMT program.

### 2.7.1 Growth Factors

G-CSF or other growth factors will not be used preemptively in patients enrolled in this clinical trial. The use of growth factor for treatment of delayed engraftment is allowed according to physician preference.

### 2.7.2 Prophylaxis Against Infections

Patients will receive infection prophylaxis according to standard operating procedures of the MCW BMT program. Infection prophylaxis will include, but is not limited to, agents or strategies (e.g., PCR screening and preemptive therapy) to reduce the risk of bacterial, herpes simplex, CMV, HHV-6, EBV, Pneumocystis jiroveci, and fungal infections:

- Antifungal therapy: Prophylaxis with fluconazole or other antifungal agents can be given as per standard operating procedures of the MCW BMT program. Fluconazole, voriconazole and other azoles are expected to increase serum tacrolimus levels, therefore, dosages of tacrolimus should be adjusted accordingly.
- CMV: CMV monitoring through nucleic acid amplified testing (NAAT) will be done weekly starting Day 21 through Day 63 post-transplant and then at day 100, or at anytime if there is clinical suspicion. Any reactivation and/or CMV disease will be captured in this study.

2.7.3 Intravenous Immune Globulin (IVIG)

IVIG administration will be according to standard operating procedures of the MCW BMT program

## 2.8 **Participant Risks**

2.7.1 Busulfan

Busulfan side effects include:
- Cardiovascular: Tachycardia (rapid heart rate), High blood pressure, Low blood pressure
- Neurologic: Dizziness, Headache, Insomnia, Seizures
- Gastrointestinal: Abdominal discomfort, Constipation, Diarrhea, Heartburn, Nausea and vomiting
- Hematologic: Low blood counts
- Endocrine and Metabolic: High magnesium and phosphorus levels in the blood, High sugar levels in the blood, Irregular or no menstrual cycles,
- Miscellaneous: Fluid retention, Lack of appetite, Mouth sores, Running nose, Skin rashes, Cough, Hepatic Veno-occlusive disease (damage to the liver which can be life-threatening), Infertility, Cataracts, Lung fibrosis (scarring)

2.7.2 Fludarabine

Fludarabine side effects include:
- Neurologic: Sensitivity to light, Numbness or tingling in your fingers or toes
- Gastrointestinal: Nausea and vomiting, Diarrhea, Loss of appetite
- Hematologic: Low white blood cell count and increased risk of infection, Low platelet count and increased risk of bleeding, Low red blood cell count and increased need for red cell transfusions
- Endocrine and Metabolic: Fatigue
- Miscellaneous: Trouble seeing or problems with your eyes, Lung failure or pneumonia (may be permanent), Shortness of breath, Confusion, Coma

2.7.3 Cyclophosphamide

Cyclophosphamide side effects include:

- Gastrointestinal: Diarrhea, Loss of appetite, Nausea, Vomiting
- Hematologic: Low blood counts, Suppression of the immune system
- Endocrine and Metabolic: Damage to male (testes) and female (ovaries) sex glands, Infertility, Irregular or no menstrual cycles
- Miscellaneous: Fluid retention, Hair loss, Bleeding and/or irritation in the bladder, Inflammation of the heart muscle (heart failure), Shortness of breath, Allergic reaction, Lung fibrosis (scarring), Serious skin rashes

### 2.7.4 Tacrolimus

Tacrolimus side effects include:

- Cardiovascular: hypertension
- Neurologic: confusion, dizziness, insomnia, seizures, tremors, changes in how clearly one can think, headaches, trouble seeing or problems with your eyes (may be permanent)
- Gastrointestinal: nausea, vomiting, stomach pain or feeling of indigestion, liver problems
- Hematologic: microangiopathic hemolytic anemia, thrombocytopenia
- Endocrine and metabolic: hypomagnesemia, hypokalemia, hypocalcemia, hyperlipidemia
- Miscellaneous: unwanted hair growth, renal insufficiency (reversible or permanent), infections and post-transplant lymphoproliferative disorders, swelling of the hands or feet with a burning sensation , numbness and tingling of the hands or feet, sensitivity to light, muscle cramps

### 2.7.5 Methotrexate

The reported adverse reactions associated with methotrexate use as GVHD prophylaxis include:

- Neurologic: fever, dizziness, chills, undue fatigue, blurred vision, changes in how clearly one can think, headaches
- Gastrointestinal: ulcerative stomatitis, nausea, vomiting, abdominal distress, diarrhea, loss of appetite
- Hematologic: leucopenia, anemia, thrombocytopenia and suppressed hematopoiesis (leading to infection)
- Miscellaneous: abnormal liver blood tests, liver damage, kidney failure, mouth sores, greater risk of sunburn, allergic inflammation of the lung with fever, cough and feeling short of breath, hair loss, skin reactions (rash, itching), Redness of eyes and maybe itching but not serious (conjunctivitis)

### 2.7.6 Tocilizumab

Tocilizumab side effects include:

- Hematologic: Decreased white blood cells with risk of infections, decreased platelets
- Respiratory: upper respiratory tract infections, nasopharyngitis, bronchitis
- Gastrointestinal: mouth ulceration, upper abdominal pain, gastritis, gastrointestinal perforations.
- Hepatic: transaminases elevation
- Neurologic: headache, dizziness
- Cardiovascular: hypertension
- Dermatologic: skin rash, increase in cholesterol
- Miscellaneous: Hypersensitivity reactions and infusion reactions

## 2.9 **Study Drug Information**

**Study Agent:** Actemra® (Tocilizumab)

Classification: Immunomodulatory

### 2.9.1 Clinical Pharmacology

Tocilizumab is a recombinant humanized interleukin-6 receptor inhibiting monoclonal antibody. Tocilizumab binds to both soluble and membrane bound IL-6 receptors and results in the blockade of interleukin-6 signaling through these receptors.

#### 2.9.1.1 Pharmacokinetics

Pharmacokinetic studies indicate that tocilizumab undergoes biphasic elimination from the circulation. In rheumatoid arthritis patients treated with 4 and 8 mg/kg every 4 weeks, the central volume of distribution was 3.5 L and the peripheral volume of distribution was 2.9 L with a volume of distribution at steady state of 6.4 L. Tocilizumab dosed at 8 mg/kg resulted in a mean steady state area under the curve (AUC), minimum concentration (Cmin) and a maximum concentration (Cmax) of 35 $\pm$ 15 mg·hr/ml, 9.74 $\pm$ 10.5 mcg/ml and 183 $\pm$ 85.6 mcg/ml respectively. Tocilizumab AUC, Cmin and Cmax increased with increasing body weight with a 86% higher exposure in patients greater than 100 kg. As a result, doses exceeding 800 mg (max dosing weight 100 kg) per infusion are not recommended.

The total clearance of Tocilizumab is concentration-dependent and is represented by the both the linear clearance and the nonlinear clearance. Upon saturation of the non-linear clearance pathway, the main determining factor is linear clearance. The reported linear clearance in the pharmacokinetic studies is estimated to be 12.5 mL/h. The concentration dependent half-life is up to 11 days for the 4 mg/kg dose and up to 13 days for the 8 mg/kg dose every 4 weeks at steady state.

### 2.9.2 Special Populations

Pharmacokinetic analysis in adult rheumatoid arthritis patients did not demonstrate a change in kinetics based on age, gender or race. The effects of renal and hepatic impairment have not been assessed.

### 2.9.3 Drug Supply and Storage

Tocilizumab is commercially available, but will be paid for by the study. Single use vials containing Tocilizumab, preservative free, sterile concentrate solutions (20 mg/ml) for IV infusion are available in the following sizes: 80 mg, 200mg, and 400 mg. The solution is colorless to pale yellow, with a pH of approximately 6.5.

Vials should be stored under refrigeration at 2°C to 8°C (36°F to 46°F). Do not freeze. Protect the vials from light by storage in the original package until time of use. Inspect vials visually for particulates and discoloration prior to use and discard if particulates or discoloration is noted.

### 2.9.4 Preparation

Using aseptic technique, utilize a 100 ml bag of 0.9% sodium chloride injection USP and withdraw a volume equal to the volume of the tocilizumab solution required for the dose. Next, withdraw the calculated volume of tocilizumab solution necessary for the dose from the vials and slowly inject the tocilizumab to the infusion bag. The final solution volume should be 100ml. Gently invert the IV bag to mix the solution. Inspect the prepared IV solution for particulates.

### 2.9.5 Storage of prepared IV solution

The solution may be stored under refrigeration or at room temperature for up to 24 hours and should be protected from light.

### 2.9.6 Warning and Precautions

#### 2.9.6.1 Serious Infections

The product information labeling contains a black box warning regarding the risk of serious infection. Most patients who developed these infections were taking concomitant immunosuppressants such as Methotrexate or corticosteroids. Serious infections leading to hospitalization or death, including tuberculosis, bacterial, invasive fungal, viral and other opportunistic infections have occurred in patients receiving tocilizumab. Viral reactivation and cases of herpes zoster exacerbation were reported in clinical trials. It is recommended that patients are tested for latent tuberculosis before and during use of tocilizumab. If a serious infection develops, Tocilizumab should be withheld until the infection is controlled.

2.9.6.2 Gastrointestinal perforations

Events of GI perforation have been reported in clinical trials, primarily as complications of diverticulitis. Patients presenting with new onset abdominal symptoms should be evaluated promptly.

2.9.6.3 Laboratory Parameters

Neutropenia, decreases in platelets, transaminase elevations, and increases in lipid parameters (total cholesterol, LDL and triglycerides) have been reported in relation to the use of tocilizumab.

2.9.6.4 Drug Interactions

Elevated levels of IL-6 and other cytokines have been associated with reduced expression of some cytochrome (CYP) P450 enzymes. Tocilizumab, through IL-6 inhibition, has the potential to affect expression of multiple CYP enzymes by restoring their activity to a higher level than that in the absence of tocilizumab. Monitoring of drugs that are metabolized by CYP's with narrow therapeutic index or where the dose is individually adjusted is advised. Caution should be exercised when tocilizumab is coadministered with CYP3A4 substrate drugs where decrease in effectiveness is undesirable, e.g., oral contraceptives, atorvastatin etc. The effect of tocilizumab on CYP450 enzymes may occur within approximately 2 weeks of starting therapy and persist for several weeks after stopping therapy. A list of CYP drug interactions can be found at this site http://medicine.iupui.edu/clinpharm/ddis/

## 3.0 STUDY ENDPOINTS

### 3.1 Primary Endpoint

The primary endpoint of this phase II clinical trial is to compare the probabilities of grade II-IV acute GVHD-free survival at Day 180 post-transplant between recipients of Tac/MTX/Toc and CIBMTR controls who received Tac/MTX alone. Development of grade II-IV acute GVHD or death is considered event for this composite endpoint. Patients who are alive without ever experiencing grade II-IV acute GVHD will be censored at the last follow-up.

### 3.2 Secondary Endpoints

3.2.1 Acute GVHD

Cumulative incidences of grade II-IV and III-IV acute GVHD will be determined. Acute GVHD will be graded according to the Appendix A. The time of onset of acute grades II-IV and III-IV acute GVHD will be recorded, as well as the maximum grade achieved. This endpoint will be evaluated through 180 days post HSCT. Rates of grade II-IV and III-IV will be compared to the CIBMTR controls. Within the acute GVHD endpoint, the proportion of patients with visceral involvement (liver or gut) will be described.

3.2.2 Chronic GVHD

The cumulative incidence of chronic GVHD will be determined. Assessment of chronic GVHD will occur up to one year post HSCT. Rates of chronic GVHD will be compared to the CIBMTR controls

3.2.3 Hematologic Recovery

Hematologic recovery will be assessed according to neutrophil and platelet counts recovery after HSCT. Neutrophil recovery is defined as achieving an absolute neutrophil count (ANC) $\geq 500/mm^3$ for three consecutive measurements on three different days. The first of the three days will be designated the day of neutrophil recovery. The competing event is death without neutrophil recovery.

Platelet recovery is defined by two different metrics: the first day of a sustained platelet count $>20,000/mm^3$ or $>50,000/mm^3$ with no platelet transfusion in the preceding seven days. The first day of sustained platelet count above these thresholds will be designated the day of platelet engraftment.

Cumulative incidence of hematologic recovery will be compared with CIBMTR controls.

3.2.4 Transplant Related Mortality

The cumulative incidence of TRM will be estimated at Days 100, 180, and 1 year after HSCT. An event for this endpoint is death without evidence of disease progression or recurrence. Disease progression or recurrence will be considered competing event. Cumulative incidence of TRM will be compared with CIBMTR controls.

3.2.5 Disease Relapse or Progression

Relapse is defined by either morphological or cytogenetic evidence of acute leukemia or MDS consistent with pretransplant features, or radiologic evidence of lymphoma, documented or not by biopsy. Progression of disease applies to patients with lymphoproliferative diseases (lymphoma or chronic lymphocytic leukemia) not in remission prior to transplantation. The event is defined as increase in size of prior sites of disease or evidence of new sites of disease, documented or not by biopsy.

***Acute leukemia and MDS*** – Relapse will be diagnosed when there is:

- Reappearance of leukemia blast cells in the peripheral blood; or,
- >5% blasts in the bone marrow, not attributable to another cause (e.g. bone marrow regeneration)
- The appearance of previous or new dysplastic changes (MDS specific) within the bone marrow with or without falling donor chimerism; or
- The development of extramedullary leukemia or leukemic cells in the cerebral spinal fluid or
- The reappearance of cytogenetic abnormalities present prior to transplantation

***Lymphoproliferative Diseases*** – Relapse or progression will be diagnosed when there is:

- Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased FDG uptake in a previously unaffected site will only be considered relapsed or progressive disease after confirmation with other modalities. In patients with no prior history of pulmonary lymphoma, new lung nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the PET without histologic confirmation.
- At least a 50% increase from nadir in the sum of the product diameters of any previously involved nodes, or in a single involved node, or the size of other lesions (e.g., splenic or hepatic nodules). To be considered progressive disease, a lymph node with a diameter of the short axis of less than 1.0 cm must increase by ≥ 50% and to a size of 1.5 x 1.5 cm or more than 1.5 cm in the long axis.

  Lesions should be PET positive if observed in a typical FDG-avid lymphoma or the lesion was PET positive before therapy unless the lesion is too small to be detected with current PET systems (<1.5 cm in its long axis by CT).

- In addition to the criteria above, patients with CLL who present in complete remission prior to transplantation may fulfill the relapse definition if there is reappearance of circulating malignant cells that are phenotypically characteristic of CLL.

**Institution of any therapy to treat persistent, progressive or relapsed disease, including the withdrawal of immunosuppressive therapy or donor lymphocyte infusion, will be considered evidence of relapse/progression regardless of whether the criteria described above were met.**

Rates of disease progression or relapse will be compared to CIBMTR controls.

### 3.2.6 Disease Free Survival

The event for this endpoint is relapse/progression or death. The time to this event is measured from transplant to death or relapse/progression, whichever comes first. Patients who are alive and disease free will be censored at the last follow-up. Disease-free survival will be compared with CIBMTR controls.

### 3.2.7 Overall Survival

The event for this endpoint is death from any cause. The time to this event is measured from the time of transplant to death from any cause or for surviving patients, to last follow-up. Overall survival rates among recipients of Tac/MTX/Toc will be compared to CIBMTR controls.

### 3.2.8 Incidence of Infections

The incidence of definite and probable viral, fungal and bacterial infections will be tabulated for recipients of Tac/Mtx/Toc. The cumulative incidence of CMV reactivation by nucleic acid amplification test (NAAT) in the first 100 days post HSCT will be described. All Grade 2 and 3 infections will be reported.

### 3.2.9 Toxicities

All grades ≥ 3 toxicities according to CTCAE, version 4 will be tabulated for recipients of Tac/Mtx/Toc. The proportion of patients developing grade ≥ 3 AE will be reported.

### 3.2.10 Donor cell Chimerism

Chimerism will be evaluated using sorted whole blood in CD3 and CD33 fractions. For the purpose of this protocol, mixed chimerism is defined as the presence of donor cells, as a proportion of total cells to be < 95% but > 5% in the bone marrow or peripheral blood. Full donor chimerism is defined as ≥ 95% of donor cells. Mixed and full chimerism will be evidence of donor cell engraftment. Donor cells of ≤ 5% will be considered as graft rejection. The proportion of patients with each level of chimerism described above will be described as part of this outcome for recipients of Tac/Mtx/Toc. For sorted blood cell fractions, CD3+ donor cell chimerism will be used to define the donor/recipient chimerism status.

### 3.2.11 Immune Reconstitution

Quantitative assessments of peripheral blood CD3, CD4, CD8, CD19 and CD56 positive lymphocytes will be done through flow cytometric analysis at Days 28, 100,180 and 365 after transplant. Results will be tabulated according to time from transplant. Detailed immune reconstitution assessments are outlined in appendix C. Approximately 30 mL in green top tubes (sodium heparin) will be drawn for each assessment. Study team will notify Dr. Carolyn Taylor's lab prior to study draws.

### 3.2.12 Levels of Pro-inflammatory Cytokines

Serum will be analyzed for IL-4, IL-6, IL-10, IL-13, IL-17, interferon-gamma, and tumor necrosis alpha using a Bio-Plex Human Cytokine Assay Kit (Bio-Rad) at baseline, days +7, +14, and +28. Soluble IL-6 receptor levels will also be assessed at the same time points using an ELISA assay. Results will be tabulated according to time from transplant. Approximately 10 mL in red top tubes will be drawn for each assessment. Study team will notify Dr. Carolyn Taylor's lab prior to study draws.

# 4.0 PATIENT ENROLLMENT AND EVALUATIONS

## 4.1 Patient evaluation and enrollment

Patients will be approached for this study after the decision to proceed with transplant is made and a suitable HLA-matched donor is identified. Transplant physicians will evaluate the patient eligibility onto this study. Eligible patients willing to participate in the trial will sign a MCW Institutional Review Board approved informed consent form. A Clinical Trials Office clinical research coordinator will record the documentation of patient consent and proceed with registration procedures.

All source documents that support eligibility including a signed informed consent/HIPAA and signed eligibility checklist, will be available, reviewed and eligibility verified.

At the point of registration, a member of the study team will register the patient in the electronic database, including demographics, consent and on-study information. The patient will be assigned a unique sequence number for the study. One of the co-Principal Investigators of the study, Drs. Drobyski or Pasquini, will be notified prior to enrollment. Pharmacy will be notified upon patient registration.

## 4.2 Guideline for serious adverse event reporting

Please refer to Appendix C for more details on adverse event reporting.

### 4.2.1 Monitoring the Progress of Trial and the Safety of Participants

This Phase II clinical trial will be monitored by the principal investigators (PI), William R Drobyski MD and Marcelo C. Pasquini, MD, MS. The PIs will review the outcome of the data for each individual patient on an ongoing basis. The PIs of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the Institutional Review Board. The PIs will ensure that the monitoring plan is followed and that all data required for oversight of monitoring are accurately reported, that all AEs are reported according to the protocol guidelines, and that any AEs reflecting patient safety concerns are appropriately reported.

### 4.2.2 Reporting of Adverse Events

The capture of toxicities and AEs in this protocol will follow the same approach and will be done in two levels. First, grades 3-5 AEs will be collected at different time points during the study period. Any AE that fulfills this criteria will be capture and the frequency of each organ toxicity tabulated. The DSMC and IRB will receive a summary of all the AEs captured in a log form on a quarterly to biennial basis depending the schedule of protocol review meetings. Additionally the PIs will review the frequency of toxicities in a quarterly basis.

Second, any AE that is unexpected and of grades 3 to 5 will required expedited report to the oversight committees (MCW IRB, MCW DSMC and FDA – if applicable for studies under IND). The collection grades 3-5 unexpected AEs is event driven and require a more comprehensive description of the event. The report for these AEs are termed Individual Case Safety Reports (ICSR) and include the following components: 1 summary cover sheet of the

event, 2 narrative of the event, 3 associated conditions, medications and diagnostic information, source documents with further explanation of the even and an evaluation of the event by one of the PIs. The PIs will review all these events and will determine if they require expedited reporting. If they are considered grades 3-5 and unexpected the report will be sent to the oversight committees within three days from acknowledgement of the event. If the event is not considered to fulfill criteria of grades 3-5 unexpected AE, it will be included in the DSMC and IRB reports for scheduled protocol reviews and will not require expedited reporting.

Reporting timelines:

1. **Fatal (grade 5) or Life Threatening** events must be reported within 24 hours but not later than 3 calendar day of the investigator's observation or awareness of the event.
2. **All other events grades 3-4 unexpected** events (non-fatal/non life-threatening) must be reported within 3 to 5 calendar days of the investigator's observation or awareness of the event.

This study will be reviewed by the Medical College of Wisconsin Cancer Center Data Safety Monitoring Committee (MCW CC DSMC). A summary of the MCW CC DSMC activities are as follows:

• Review the clinical trials for data integrity and safety
• Review all adverse events requiring expedited reporting as defined per protocol
• Review all DSM reports
• Submit a summary of any recommendations related to study conduct
• Terminate the study if deemed unsafe for patients

A copy of the MCW CC Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study PI twice annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary. Any available DSMC letters will be submitted to the IRB of record as required.

### 4.3 **Study monitoring**

The follow-up schedule for scheduled study visits is outlined in Table 4.3a.

**TABLE 4.3a: STUDY VISIT SCHEDULE**

| Study Visit | Target Day Post-Transplant* |
|---|---|
| Baseline | ≤ 42 days from conditioning |
| 1 week | 7 ± 6 days |
| 2 week | 14 ± 6 days |
| 3 week | 21 ± 6 days |
| 4 week | 28 ± 6 days |
| 5 week | 35 ± 6 days |
| 6 week | 42 ± 6 days |
| 7 week | 49 ± 6 days |
| 8 week | 56 ± 6 days |
| 9 week | 63 ± 6 days |
| 100 day | 100 ± 10 days |
| 4 month | 120 ± 10 days |
| 5 month | 150 ± 10 days |
| 6 month | 180 ± 14 days |
| 9 month | 270 ±14 days |
| 12 month | 365 ± 14 days |

*Research samples (Proinflammatory Cytokines, Immune reconstitution and ancillary samples) will be collected as close to the target day ± the window as possible, however, due to the research labs availability to run samples some samples may be drawn outside the above specified window. A note to file will document when this situation occurs.

### 4.3.1 Patient Assessments

Table 4.3b summarizes patient clinical assessments over the course of the study.

## TABLE 4.6b: PATIENT CLINICAL ASSESSMENTS

| Study Assessments/ Testing | Baseline | -6 | 7 | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 | 100 | 120 | 150 | 180 | 270 | 365 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| History, physical exam, weight and height* | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Karnofsky performance status and HCT-CI* | X | | | | | | | | | | | | | | | | |
| HLA typing (donor and recipient) * | X | | | | | | | | | | | | | | | | |
| (CBC[1], differential[1], platelet count[1], and CMP[2])* | X | | X | X | X | X | X | X | X | X | X | X | | | X | X | X |
| Estimated creatinine clearance[3]* | X | | | | | | | | | | | | | | | | |
| Infectious disease titers[4]* | X | | | | | | | | | | | | | | | | |
| EKG* and LVEF* | X | | | | | | | | | | | | | | | | |
| DLCO* and FEV1 * | X | | | | | | | | | | | | | | X | | X |
| Disease evaluation[5]* | X | | | | | | | | | | | X | | | X | | X |
| Chest x-ray or chest/abdomen/pelvis CT* | X | | | | | | | | | | | | | | | | |
| Pregnancy test[6]* | X | | | | | | | | | | | | | | | | |
| GVHD assessments[7]* | | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Toxicity assessments[8] | | X | | | | X | | | | X | | X | | | X | X | X |
| Chimerism[9]* | | | | | | X | | | | | | X | | | X | | X |
| CMV NAAT peripheral blood* | | | | X | X | X | X | X | X | X | X | X | | | | | |
| Immune reconstitution[11] | | | | | | X | | | | | | X | | | X | | X |
| Proinflammatory Cytokines[10, 11] | X | | X | X | | X | | | | | | | | | | | |
| Ancillary Protocol Research (see appendix E) [11] | X | | | | | X | | | | | | X | | | X | | |

***Indicate evaluations are currently performed as standard of care at the FMLH/MCW BMT program.** [1]CBC performed three times weekly from Day 0 until ANC > 500/mcL for three days and platelet count > 20,000/mcL after nadir, while hospitalized. CBC then performed weekly through Day 63 post-transplant and every other week through Day 100 post-transplant, then at Days 180, 270 and 365 post-transplant. [2]Blood chemistries include: serum creatinine, bilirubin, alkaline phosphatase, AST and ALT. Blood chemistries performed twice weekly until hospital discharge. Blood chemistries performed weekly after hospital discharge until Day 63 post-transplant, then every other week through Day 100 post-transplant, and then at Days 180, 270 and 365 post-transplant. [3]Estimated creatinine clearance is calculated using the Cockcroft-Gault formula and actual body weight. [4]Infectious disease titers include: CMV, Hepatitis panel (HepA Ab, HepB SAb, HepB SAg, HepB Core Ab, HepC Ab), herpes simplex virus, syphilis, HIV and HTLV I/II antibody, and varicella zoster. [5]Evaluation of the malignant disease: For acute leukemia, CML and MDS this includes a bone marrow aspirate and biopsy. For lymphomas this includes imaging studies, which will be done according to institutional practices, or the same as prior to transplant, for matter of comparison. [6]Pregnancy test must be performed $\leq$ 30 days before the start of the transplant conditioning regimen. Pregnancy test is required for females of child-bearing potential, urine or blood assays are allowed per institutional standards. [7]GVHD assessments performed weekly until Day 63 post-transplant, and then at Days 100, 120, 150, 180, 270, and 365. The GVHD assessment will include a review of **all** abnormalities experienced **during the entire assessment period** and the **highest grade** for each abnormality (*whether attributed to GVHD or not*) during the assessment period will be recorded on the Acute GVHD form and/or the Follow-up GVHD form [8]The toxicity assessment will include a review of **all** toxicities experienced **during the entire assessment period** and the **highest grade** for each toxicity during the assessment period will be recorded on the Toxicity forms. [9]Chimerism in whole blood fractionated as CD3 and CD33. [10] Cytokine assessments include IL-4, IL-6, IL-10, IL-13, IL-17, interferon-gamma, tumor necrosis factor and soluble IL-6 receptor. [11]Research samples will not be collected for patients who have disease relapse, and receive further chemotherapy.

**Pre-transplant evaluations must be completed ≤ 84 days prior to patient enrollment.**

- LVEF (may be performed ≤ 84 days prior to patient enrollment).
- Pulmonary function tests, including DLCO and FEV1 (may be performed ≤ 84 days prior to patient enrollment).

**Pre-transplant evaluations must be completed ≤ 42 days prior to patient enrollment.**

- History, physical examination and weight.
- Karnofsky performance status and HCT-Specific Comorbidity Index score.
- CBC with differential and platelet count, serum creatinine, bilirubin, alkaline phosphatase, AST and ALT.
- Estimated creatinine clearance, using the Cockcroft-Gault formula and actual body weight.
- Infectious disease titers to include: CMV antibody, Hepatitis panel (HepA Ab, HepB SAb, HepB SAg, HepB Core Ab, HepC Ab), herpes simplex virus, syphilis, HIV and HTLV I/II antibody, and varicella zoster.
- Disease evaluation of the malignant disease: For acute leukemia, CML and MDS this includes a bone marrow aspirate and biopsy for pathology and cytogenetics. For lymphomas this includes imaging studies, which will be done according to institutional practices for matter of comparison post-transplant.
- Chest X-ray or chest CT.
- Pre-transplant donor and recipient samples for post-transplant chimerism studies.

**Pre-transplant evaluations performed ≤ 30 days before initiation of the transplant conditioning regimen.**

- Pre-transplant Proinflammatory cytokine panel
- Pregnancy test for females of child-bearing potential
- Assessment for toxicities

**Post-transplant evaluations**

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

- History and physical exam (per institutional standards)
- Assess GVHD weekly through Day 63 post-transplant, then at Days 100, 120, 150, 180, 270 and 365 post-transplant.
- Assessment for toxicities at Days 28, 56, 100, 180, 270 and 365 post-transplant.

- CBC with differential at least three times a week from Day 0 until ANC > 500/μL for 3 days and platelet count > 20,000/μL for 3 days (while hospitalized only) after nadir is reached. Thereafter, CBC with differential weekly until Day 63 post-transplant, then every other week through Day 100 post-transplant, and then at Days 180, 270 and 365 post-transplant.
- Serum creatinine, bilirubin, alkaline phosphatase, ALT and AST, twice a week until hospital discharge and then weekly until Day 63 post-transplant, then every other week through Day 100 post-transplant, and then at Days 180, 270 and 365 post-transplant.
- Chimerism studies will be performed at Days 28 (+/- 6 days), 100 (+/- 14 days), 180 (+/- 14 days), and 365 (+/- 14 days) post-transplant. Chimerism will be evaluated in whole blood in fractions including CD3 and CD33.
- Disease evaluation of the malignant disease at Days 100 (+/- 10 days), 180 (+/- 14 days) and 365 (+/- 14 days) post-transplant: For acute leukemia, CML and MDS this includes a bone marrow aspirate and biopsy for pathology and cytogenetics. For lymphomas this includes imaging studies, which will be done according to institutional practices and the same as prior to transplant, for matter of comparison.
- Pulmonary function tests, including DLCO and FEV1 at Days 180 and 365 (+/- 14 days) post-transplant.
- Immune reconstitution panel will be collected at approximately days 28, 100, 180 and 365.
- Proinflammatory cytokine panel will be collected on approximately days 7, 14, and 28.

## 5.0 STATISTICAL ANALYSIS

### 5.1 **Study Design**

The study is designed as a Phase II, open label, single center trial to compare a novel GVHD prophylaxis regimen, Tac/MTX/Toc with standard Tac/MTX regimens in recipients of allogeneic transplant for malignant diseases. The primary endpoint of grades II-IV acute GVHD free survival probabilities at Day 180 post-transplant will be compared to a non-randomized contemporary Tac/MTX control group collected through the CIBMTR. The control group of patients will satisfy similar eligibility requirements as the patients enrolled in the clinical trial and their donor type will be matched to patients enrolled in the phase II trial.

#### 5.1.1 Accrual

It is estimated that 24 months of accrual will be necessary to enroll the targeted sample size. Accrual will be reported by race, ethnicity, gender, and age.

#### 5.1.2 Primary Endpoint

The primary endpoint is Day 180 Grade II-IV acute GVHD-free survival (GFS). All patients will be followed for the primary endpoint for at least 6 months.

## 5.2 **Sample Size and Power Calculation**

Sample size and power considerations are based on the comparison of the Tac/MTX/Toc arm to the CIBMTR controls. We plan to accrue 35 patients to this phase II trial. We plan to identify approximately four controls (35 X 4 = 140) for each patient enrolled in the trial, matched by donor type and limit the number of patients in the control group to 200. The probability of grade II-IV aGVHD-free survival at Day 180 with standard Tac/MTX regimen in this patient population is expected to be 40%. The intervention being studied will be considered promising if the survival probability increases by 20%. The test hypothesis being considered in this study is $H_0$: $p \leq .40$ vs. $H_1$: $p \geq 0.60$. Table 5.2.1 shows the probability of concluding Tac/MTX/Toc regimen is promising (the power) when the true Day 180 aGVHD-free survival probability with Tac/MTX/Toc regimen is 60% with a total sample size ranging from 175 to 235 using a one-sided Type I error of 0.10.

Table 5.2.1 Power to detect a 20% improvement in Day 180 Grade II-IV aGVHD-free survival

| Control group sample size | Total sample size | Power |
|---|---|---|
| 140 | 175 | 0.80 |
| 200 | 235 | 0.82 |

With 35 patients enrolled in the trial and 140 controls, there is 80% power to detect an improvement of 20% in Day 180 aGVHD-free survival and with 200 controls, there is 82% power.

## 5.3 **Guidelines for Safety Monitoring**

Monitoring of a key safety endpoint will be conducted on an ongoing basis, and if rates significantly exceed pre-set thresholds, the trial may be paused for further review. The following guidelines serve as trigger for additional review and are not formal "stopping rules" that would mandate automatic closure of study enrollment.

The key safety endpoint for this study is graft failure at Day 60 post-transplant. Graft failure will be monitored up to 60 days post-transplant. For this safety endpoint, graft failure is defined as lack of engraftment in patients who are alive and disease-free 60 days post-transplant.

The expected graft failure rate at Day 60 is 5%, graft failure rate significantly higher than 5% is considered unacceptable. . A truncated Sequential Probability Ratio Test (SPRT) based on a binomial test of proportions for graft failure will be used as described below. This sequential testing procedure conserves type I error across all of the monitoring looks for graft failure. The SPRT can be represented graphically. At each interim analysis, the number of patients enrolled in the clinical trial is plotted against the number of patients who have experienced graft failure. The continuation region of the SPRT is defined by two parallel lines. Only the upper boundary will be used for monitoring to protect against excessive graft failure. If the graph falls above the upper boundary, the SPRT rejects the null hypothesis, and concludes that there are more graft failures than predicted by the number of recipients evaluable. Otherwise, the SPRT continues until enrollment reaches the target goal.

The usual measures of performance of an SPRT are the error probabilities $\alpha$ and $\beta$ of rejecting $H_0$ when $\theta = \theta_0$ and of accepting $H_1$ when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta i)$. Note that since the test uses only the upper boundary, and is truncated by a finite sample size, the size of the test will be slightly lower than the nominal level. The test to be used in this protocol was developed from the following SPRT:

- An SPRT contrasting 5% versus 20% graft failure, with nominal type I and II errors of 7% and 22%, respectively.
- The slope of the parallel lines for monitoring graft failure is 0.110 and the intercepts are –0.925 and 1.547.

The stopping rule is summarized in Table 5.3.1

**Table 5.3.1 Stopping guidelines for graft failure at Day 60 among patients enrolled in trial**

| Number of recipients (n) | Stopping boundary (x) |
|---|---|
| 2-4 | 2 |
| 5-13 | 3 |
| 14-22 | 4 |
| 23-31 | 5 |
| 32-35 | 6 |

* Stopping guideline is triggered if ≥ x recipients out of n experience graft failure

The actual operating characteristics of the truncated test, shown in Table 5.3.2, were determined in a simulation study. The simulation assumed uniform accrual of 35 patients over a 24-month period. Graft failure will be monitored in all patients in the Tac/MTX/Toc arm. The SPRT rejects the null hypothesis in favor of the alternative 5% of the time when the true Day 60 graft failure is 5%, and 84% of the time when the true Day-60 graft failure is 20%. This corresponds to a type I error rate of $\alpha = 0.05$ and a type II error rate of $\beta = 0.16$. When the true Day 60 graft failure rate is 20%, on average, the guideline will be triggered 13.3 months after opening, when 3.4 events have been observed in 17 patients. Note that the SPRT procedure is adequately powered to distinguish between a graft failure rate of 5% and 20%.

**Table 5.3.2 Operating Characteristics of Sequential Testing Procedure for 60-Day Graft Failure from a Simulation Study with 10,000 Replications**

| Graft Failure | | | | |
|---|---|---|---|---|
| True 60-day rate | 5% | 10% | 15% | 20% |
| Probability reject the null hypothesis | 0.050 | 0.289 | 0.600 | 0.836 |
| Mean month stopped | 25.2 | 22.0 | 17.6 | 13.3 |
| Mean # endpoints | 1.7 | 2.9 | 3.5 | 3.4 |
| Mean recipients with 60 days follow-up | 33.9 | 29.4 | 23.1 | 17.0 |

## 5.4 Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, race/ethnicity, performance status, primary disease, disease specific risk categories, hematopoietic cell transplant comorbidity index (HCT CI), donor type and HLA matching, donor/recipient CMV status, donor/recipient sex match, donor/recipient ABO match, and conditioning regimen. Comparisons between groups will be performed for continuous variables via a Kruskal-Wallis test and for categorical variables, via the chi-square test.

## 5.5 Analysis of the Primary Endpoint

The primary outcome of the trial is grade 2 – 4 aGVHD-free survival (GFS) at 180 days after transplant. Day 180 GFS probabilities with 90% confidence intervals will be estimated for the treatment group as well as the control using the Kaplan-Meier estimator. The primary null hypothesis of the study is that there is no difference in GFS between the treatment arms at 180 days post-transplant. The primary analysis will be performed using the difference in Kaplan-Meier estimates for GFS at 180 days. A 90% confidence interval for the difference in GFS at 180 days will also be constructed.

## 5.6 Analysis of Secondary endpoints

### 5.6.1 Acute GVHD

Incidence of grades 2-4 and 3-4 acute GVHD at Day 100 and Day 180 will be estimated for each group using the cumulative incidence function treating death prior to grade 3-4 acute GVHD as the competing risk. Cumulative incidence of grade 3 – 4 acute GVHD at Day 100 and Day 180 will be compared between groups.

### 5.6.2 Chronic GVHD

Incidence of chronic GVHD at 6 and 12 months will be estimated for each group using the cumulative incidence function treating death prior to chronic GVHD as the competing risk. Cumulative incidence of chronic GVHD will be compared between groups using Gray's test.

### 5.6.3 Hematologic Recovery

Probabilities of achieving an absolute neutrophil count $\geq 500/mm^3$, and transfusion independent platelet count $>20,000/mm^3$ and $>50,000/mm^3$ will be estimated using the cumulative incidence function treating death without the event as the competing risk. Incidence of neutrophil and platelet engraftment will be compared between groups.

### 5.6.4 Treatment-related mortality (TRM)

The event for this endpoint is death due to any cause other than relapse of the underlying malignancy. The time to this event is time from transplant to death, relapse, or the last follow-up whichever occurs first. TRM at 6 and 12 months for each treatment group will be estimated

using the cumulative incidence function treating relapse as the competing risk. TRM will be compared between the two groups using Gray's test.

5.6.5 Incidence of disease relapse/progression

The event for this endpoint is relapse of the underlying malignancy. The time to this event is the time from transplant to first evidence of laboratory recurrence or progression of primary disease according to standard criteria, death, or the last follow-up whichever occurs first. Incidence of relapse/progression at 6 and 12 months for each treatment group will be estimated using the cumulative incidence function treating TRM as the competing risk. Incidence of relapse/progression will be compared between the two groups using Gray's test.

5.6.6 Disease-free Survival (DFS)

The event for this endpoint is death or relapse/progression. The time to this event is from the time of transplant to death, relapse/progression, or the last follow-up whichever occurs first. The DFS probabilities at 6 and 12 months will be estimated for each group using the Kaplan-Meier estimator. DFS will be compared between the two treatment groups using the log-rank test.

5.6.7 Overall Survival (OS)

The event is death from any cause. The time to this event is from the time of transplant to death or the last follow-up whichever occurs first. The overall survival probabilities at Day 180 will be estimated for each group using the Kaplan-Meier estimator. OS will be compared between the two treatment groups using the log-rank test.

5.6.8 End points only applicable for patients receiving Tac/Mtx/Toc

The endpoints below will be assessed only in patients who are receiving the intervention (Tac/MTX/Toc) and will not be compared with controls.

5.6.9 Donor Cell Chimerism

The percentage of donor cell chimerism at day 100 post transplantation will be summarized.

5.6.10 Incidence of Toxicities

Incidence of serious adverse events and grade 3-5 CTACAE v4 will be summarized.

5.6.11 Incidence of Infections

The incidence of definite and probable viral, fungal and bacterial infections will be tabulated.

# APPENDIX A
# GVHD ASSESSMENT

## GVHD GRADING, STUDY DEFINITIONS AND ENDPOINTS

### 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 |

## Diagnostic Assessment of Chronic GVHD

| | 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**<br>*Clinical features:*<br>☐ Maculopapular rash<br>☐ Lichen planus-like features<br>☐ Papulosquamous lesions or ichthyosis<br>☐ Hyperpigmentation<br>☐ Hypopigmentation<br>☐ Keratosis pilaris<br>☐ Erythema<br>☐ Erythroderma<br>☐ Poikiloderma<br>☐ Sclerotic features<br>☐ Pruritus<br>☐ Hair involvement<br>☐ Nail involvement<br>**% 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 "hidebound" (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**<br>Mean tear test (mm):<br>☐ >10<br>☐ 6-10<br>☐ ≤5<br>☐ Not done | ☐ No symptoms | ☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca | ☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal 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 dysphagia, anorexia, nausea, vomiting, abdominal pain or diarrhea without significant weight loss (<5%) | ☐ Symptoms associated with mild to moderate weight loss (5-15%) | ☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation |
| **LIVER** | ☐ Normal LFT | ☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN | ☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN | ☐ Bilirubin or enzymes > 5 x ULN |

| | SCORE 0 | SCORE 1 | SCORE 2 | SCORE 3 |
|---|---|---|---|---|
| **LUNGS**† **FEV1** ☐ **DLCO** ☐ | ☐ No symptoms<br>☐ FEV1 > 80% **OR** LFS=2 | ☐ Mild symptoms (shortness of breath after climbing one flight of steps)<br>☐ FEV1 60-79% **OR** LFS 3-5 | ☐ Moderate symptoms (shortness of breath after walking on flat ground)<br>☐ FEV1 40-59% **OR** LFS 6-9 | ☐ Severe symptoms (shortness of breath at rest; requiring $0_2$)<br>☐ FEV1 ≤39% **OR** LFS 10-12 |
| **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, moderate 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 and assign a score to its severity (0-3) based on its functional impact where applicable (none – 0,mild -1, moderate -2, severe – 3)**

| | | |
|---|---|---|
| Esophageal stricture or web___ | Pericardial Effusion___ | Pleural Effusion(s)___ |
| Ascites (serositis)___ | Nephrotic syndrome___ | Peripheral Neuropathy___ |
| M yasthenia Gravis___ | Cardiomyopathy___ | Eosinophilia > 500µl___ |
| Polymyositis___ | Cardiac conduction defects___ | Coronary artery involvement___ |
| Platelets <100,000/µl ___ | Progressive onset___ | |

**OTHERS:** Specify:_____

Mild chronic GVHD involves only 1 or 2 organs or sites (except the lung: see below), with no clinically significant functional impairment (maximum of score 1 in all affected organs or sites). Moderate chronic GVHD 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). A lung score of 1 will also be considered moderate chronic GVHD. Severe chronic GVHD indicates major disability caused by chronic GVHD (score of 3 in any organ or site). A lung score of 2 or greater will also be considered severe chronic GVHD.

# APPENDIX B
# Laboratory Correlatives

## Laboratory Correlatives

Laboratory correlative for this clinical trials include evaluation of proinflammatory cytokines and immune reconstitution. The schedule of evaluations and blood volume required are listed below:

| | Baseline | **7** | **14** | **21** | **28** | **100** | **180** | **365** |
|---|---|---|---|---|---|---|---|---|
| Immune reconstitution | | | | | 30mL | 30mL | 30mL | 30mL |
| Proinflammatory Cytokines | 10mL | 10mL | 10mL | | 10mL | | | |

**Proinflammatory Cytokines:**
Tocilizumab blocks the activity of IL-6 and may interfere with further downstream pro inflammatory cascade. The clinical trial will evaluate levels of IL-4, IL-6, IL-10, IL-13, IL-17, interferon-gamma, tumor necrosis factor and soluble IL-6 receptor early in the study period.

## Post Transplant Immune Reconstitution

The laboratory studies proposed to measure patient response to Tocilizumab treatment are based on the known activity of IL-6 in promoting inflammation and autoimmunity in both T and B cells. While the effects of Tocilizumab on specific immune cell subsets has been described in patients with autoimmune disease, GVHD and its treatment have profound effects on the immune system. The additional effect of blocking the action of IL-6 in GVHD is not well described. We will examine a number of immune cell populations to determine whether there is any correlation between clinical response and immune parameters in this study. Testing will be performed at approximately the following time points: day 28, day 100, day 180 and day 365. The specific immune cell populations to be examined are as follows:

1. **B cell** effects by assessment of antigen inexperienced B cells, and, transitional B cells, memory B cells, and plasma cells. Specifically we will use multi-parameter flow cytometry measuring the level of expression of CD27, CD38, CD5, IgD, and IgM on the surface of CD19+ B cells. Using these antibodies the following subsets can be defined:

**Table 1. CD19+ B Cells**

| CD19 | CD27 | IgD | IgM | CD38 | Subset |
|---|---|---|---|---|---|
| + | - | - | low | high | Pre-B cells |
| + | - | - | Int | -/low | Transitional |
| + | - | High | High | -/low | Naïve mature |
| + | -/low | Int/low | Int/low | -/low | Activated |
| + | High | low | | | In Vivo Activated |
| + | Int | -/Low | +/- | Low/int | Memory/Post-GC |
| int | High | - | - | high | Plasamablast/Plasma Pre-GC |
| + | - | + | ? | high | Transitional-GVH |
| + | + | + | | Low | IgD+ memory B |

2. **Regulatory T cells (Tregs)-** Regulatory T cells defined as CD3+ CD4+ CD25+ CD127- FoxP3+ are known to inhibit the manifestation of GVHD. Given that IL-6 can inhibit the expansion of Tregs, blockage of this effect may result in increased numbers in patients responding to Tociizumab therapy. This population will be expressed as a percentage of all CD4+ cells and as absolute numbers at the various time points tested.

**Table 2. Regulatory T cells**

| CD3 | CD4 | CD25 | CD127 | FoxP3 | Subset |
|---|---|---|---|---|---|
| + | + | Bright | Neg | + | Tregs |
| + | + | + | + | +/- | Activated CD4 effector cells |

3. **TH17, TH1, TH2, TC1, TC2 Cell Populations-** Elevated levels of TH-17 T cells are associated with GVHD and are increased in the presence of IL-6. Therefore, this subset might be reduced in recipients of Tocilizumab responding to treatment. TH-17 cells can be identified by the production of IL-17 when activated and can be found in both CD4+ and CD8+ T cells subsets. CD4+ T helper and CD8+ T cytotoxic cells can likewise be distinguished by the expression of cell surface markers and cytokine production upon activation. We will use antibodies to CD3, CD4. CD8, CD294, IL17 and IFN-gamma to distinguish the following subsets.

**Table 3. TH17, TH1, TH2, TC1, & TC2 T cells**

| CD3 | CD4 | CD8 | CD294 | IFN-□ | IL-17 | Subset |
|---|---|---|---|---|---|---|
| + | + | - | | | + | CD4+ TH17 |
| + | - | + | | | + | CD8+ TH17 |
| + | + | - | | + | + | CD4+ Transitional TH17 |
| + | - | + | | + | + | CD8+ Transitional TH17 |
| + | + | - | - | + | | TH1 |
| + | + | - | + | - | | TH2 |
| + | - | + | - | + | | TC1 |
| + | - | + | + | - | | TC2 |

4. **Activated T Cells-** Both GVHD and autoimmune disease result in T cell activation. Response to Tocilizumab in patients treated for SLE was associated with fewer circulating activated T cells, and the presence of a unique subset of TCR alpha/beta positive T cells lacking expression of both CD4 and CD8 that are activated and found in increased numbers in patients with autoimmune disease. To detect activated T cells and TCR αβ CD4-CD8- cells we will use antibodies to CD3, TCR αβ, CD4, CD8, CD25, HLA-DR and CD69 as shown in table 4.

**Table 4. Activated T cells**

| CD3 | CD4 | CD8 | TCRαβ | CD25 | HLA-DR | CD69 | Subset |
|---|---|---|---|---|---|---|---|
| + | + | - | + | + | + | + | Recently activated CD4 |
| + | + | - | + | - | + | - | Chronically activated CD4 |
| + | - | + | + | + | + | + | Recently activated CD8 |
| + | - | + | + | - | + | - | Chronically activated CD8 |
| + | - or + | - or + | + | - | - | - | Unactivated CD4 or CD8 |
| + | - | - | + | | | | TCRαβ+ double neg |
| + | - | - | - | | | | TCRγδ+ T cells |

5. **Naive, central memory, and effector CD4 and CD8 subsets, terminal effector CD8+ T cells, NK cells and NK-T cells-** Similar to B cells, the presence of naïve CD4 or CD8 T cell subsets is a marker of immune reconstitution in recipients of HSCT. GVHD and its therapy hinder immune reconstitution, and in SLE patients receiving Tocilizumab the presence of naïve T cells correlated with therapy response. In contrast an increased number of effector T cells may be an indication that the disease process has not subsided. In addition while NK cells are not typically considered to be a causative agent of GVHD, nor are they likely to be affected by Tocilizumab, a subset of NK cells that express CD3 do display suppressor activity and may be elevated in patients responsive to Tocilizumab in our study. To identify these subsets the following antibodies in combination are required: CD3, CD4, CD8, CCR7, CD45RA, CD45RO, CD56/CD16.

**Table 5. Naïve, central memory, and effector T cells, NK and NK-T cells**

| CD3 | CD4 | CD8 | CCR7 | CD45 RA | CD45 RO | CD56+ CD16 | Subset |
|---|---|---|---|---|---|---|---|
| + | + | - | + | + | - | - | Naïve CD4 |
| + | + | - | + | - | + | - | Central memory CD4 |
| + | + | - | - | - | + | - | Effector memory CD4 |
| + | - | + | + | + | - | - | Naïve CD8 |
| + | - | + | + | - | + | - | Central memory CD8 |
| + | - | + | - | - | + | - | Effector memory CD8 |
| + | - | + | - | + | - | - | Terminal Effector memory CD8 |
| - | | | | | | + | NK Cells |
| + | - | +/- | | | | + | NK-T Cells |

# APPENDIX C
# ADVERSE EVENT REPORTING

## Introduction

Clinical trials that assess interventions in the setting of hematopoietic cell transplantation is challenging as the number of expected AEs is high. The Blood and Marrow Transplant Clinical Trial Network (BMT CTN) establish a capture an AE capture approach specific for transplant trials. This approach has the objective of optimizing the capture of AEs, by reducing the noise-signal ratio and identifies AEs that are not expected after transplant or overlaps with manifestations of common complications, such as GVHD or graft failure.

## Definitions

**Adverse Event (AE)** - Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this treatment. An AE can considered therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, medical treatment or procedure whether or not related to the medicinal product.

**Expectedness**: An adverse event can be Expected or Unexpected

- **Expected adverse events** are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.
- **Unexpected adverse events** are those that vary in nature, intensity or frequency from information in the current adverse event list, the Investigator's Brochure, the package insert, or when it is not included in the informed consent document as a potential risk.

**Life-threatening Adverse Event** – Any adverse event that places the patient or subject, in view of the investigator or treating physician, at immediate risk of death from the reaction. Study toxicities are graded using the adapted NCI Common Toxicity Criteria (where appropriate use the criteria for transplant patients.)

• **Serious Adverse Event (SAE)** – Any adverse event occurring that results in any of the following outcomes:
• Death – regardless of cause.
• life-threatening adverse event (see above).
• persistent or significant disability/incapacity.
• congenital anomaly.
• requires intervention to prevent permanent impairment or damage.

**AE Grading:** All AEs that occur in this protocol will be graded according to the NCI Common Terminology Criteria for Adverse Event (CTCAE) v 4.0.

**AE Reporting:** Depending on the type, severity and whether it is expected, each AE will need to be reported in an appropriate timeline to MCW IRB, Data Safety and Monitoring Committee and FDA (if applicable for studies under an Investigational New Drug [IND] protocol).

**Adverse Events Reported to the DSMB**
All adverse events that are classified as grades 3 to 5 will be reported to the DSMC. The differences are the timing and amount of information associated with each.

The two-tier approach collects adverse events at specific time points during the trial (calendar-driven) and event-driven upon knowledge of an event deemed serious (grades 3 to 5) or unexpected to be seen in a transplant setting.

**Grades 3-5 Adverse Events**
For the calendar-driven collection of toxicity, the protocol mandates collection of toxicity on a form that captures most of the most important toxicities observed post transplant. The forms would capture all the AEs that occur in a preceding period, for example day 28 toxicity form, collects all the AEs that occurred from enrollment to day 28 and the grade of each AE. This first period includes all non-hematologic toxicity, since it is expected significant hematologic toxicity will occur immediately post transplant as an effect of the transplantation. Hematologic toxicity will be capture when it occurs beyond day 28 post transplant.

**Unexpected Grades 3-5**
The threshold for collection of adverse events outside the calendar forms is related to the grade (grades 3-5) and expectedness related to the transplant procedure or possibly related to the study drug. These events are required to be reported to the DSMC in a more immediate time frame and also require more information. For any adverse event that meets these criteria, the report would require a narrative of the event, associated laboratory and imaging information, associated medications and any relevant source documents. These reports are uploaded into Oncore and we will notify the DSMC chair and MCW IRB within 24 hours or 3 to 5 calendar days depending on the severity of the event. Both the PI and the CTO BMT Section leader will be responsible to review and determine if an event meet criteria prior to uploading it in Oncore.

Reporting timelines:

1. **Fatal (grade 5) or Life Threatening** events must be reported within 24 hours but not later than 3 calendar day of the investigator's observation or awareness of the event.
2. **All other events grades 3-4 unexpected** events (non-fatal/non life-threatening) must be reported within 3 to 5 calendar days of the investigator's observation or awareness of the event.

The proposed method outlined here follows the same requirements from MCW IRB for prompt reporting of an event or **Unanticipated Problem Involving Risks to Subjects or Others (UPIRSO)**: which is defined as any incident, experience, or outcome that meets all of the following criteria:

1. Unanticipated (in terms of nature, severity , or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, instructions for Use/Device Manual and or Investigator's Brochure; and (b) the characteristics of the subject population being studies;
2. Related or possibly related to participation in the research (in this guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research ) or test article; and
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

**DSMC Reporting**

The table below summarizes the reporting practices from the study to the DSMC. For the ongoing review of an opened trial, the DSMC will receive a report that includes in a tabular form all the grades 3 to 5 that occurred in the trial, summarized according to reporting periods: for example 0-28d, 28-56d, 56 to 100d, 100-180d, 180-270d, 270 to 365d and 0-365d. Additionally the report will include all the unexpected grades 3-5 that occurred in trial participants since activation of the trial and lastly the number of patients who met the graft failure stopping guidelines.

For the unexpected grades 3 to 5, upon knowledge of the event, this will be discussed with the study PI and the CTO BMT section leader. If this fulfills criteria for expedited reporting to the DSMC and IRB, a narrative will be uploaded in Oncore and the DSMC will be notified, either through its secretary or directly to the Chair.

| | All Grades 3-5 | Unexpected Grades 3-5 |
|---|---|---|
| Type of collection | Calendar-driven | Event-driven |
| Reporting format | Tabular or graphical format | Narrative with supporting documents |
| Timing of reporting | Biennial basis - cumulative | Within 3 to 5 calendar days from knowledge of the event and cumulative in a biennial basis. |

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# APPENDIX E
# Ancillary Study

# TOCILIZUMAB FOR THE PREVENTION OF DEPRESSION AND COGNITIVE CHANGES AMONG ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION RECIPIENTS

An addendum to parent study "PHASE II OPEN LABEL OF TACROLIMUS/METHOTREXATE AND TOCILIZUMAB FOR THE PREVENTION OF ACUTE GRAFT VERSUS HOST DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION"
Principal Investigator: William R. Drobyski, MD

**PRINCIPAL INVESTIGATOR: Jennifer M. Knight, MD**
**Co-Investigators: PI and Co-I's of parent study**

## 1.0 BACKGROUND AND RATIONALE

### 1.1 Depression, cancer, and inflammation

35-45% of cancer patients have emotional distress,[1] with depression being a significant predictor of increased mortality among cancer and hematopoietic stem cell transplant (HCT) recipients.[2, 3] Depression is associated with an elevation in pro-inflammatory cytokines, as is graft-versus-host disease (GVHD), a significant source of morbidity and mortality for allogeneic HCT recipients.[4, 5] It is unknown whether blocking inflammation associated with GVHD would also alleviate depressive symptoms in HCT patients.

Pro-inflammatory cytokines associated with infection or other diseases involving inflammation (i.e. GVHD) act on the brain to cause sickness behavior, including depression.[6] Given the cytokine dysregulation involved in GVHD,[5] it is mechanistically plausible that there may be onset or exacerbation of depressive symptoms in association with this disease, and in fact significant deficits in quality of life (QOL) are associated with GVHD.[7]

Specifically, IL-6 is consistently identified as a key cytokine implicated in inflammation-associated mood dysregulation,[8-10] including among cancer patients.[11, 12] Studies demonstrate that blocking other pro-inflammatory cytokine receptors associated with depression (i.e. TNF alpha) reduces depressive symptoms in individuals with high levels of inflammation.[13] Despite accumulating evidence that inflammation and IL-6 play a role in mood alteration and depressive symptomatology, tocilizumab, an IL-6 receptor antagonist, has never been evaluated for its possible role in improving mood or preventing deterioration of mood in association with pro-inflammatory states. This represents a novel approach for targeting depressive symptoms in an immunologically compromised population with a high degree of psychiatric morbidity. Though less robustly examined in the literature than depression, there is also indication that anxiety,[14, 15] fatigue,[16, 17] sleep,[17] and pain[18] are associated with alterations in IL-6. Therefore, these additional cognitive components may also be affected by the administration of tocilizumab.

### 1.2 Gene expression and inflammation in transplant

Research suggests that ß-adrenergic signaling regulates many cellular processes contributing to the initiation and progression of cancer,[19] with the downstream effects of this signaling pathway reflected in the expression of a discrete grouping of genes comprising the conserved transcriptional response to adversity (CTRA) profile. This CTRA profile (53 inflammatory, interferon-related, and antibody-related genes) has been identified as overexpressed in other populations under conditions of chronic stress or adversity and is consistent with the physiology of stress-associated illness.[20] We recently identified that unrelated donor HCT recipients exposed to the chronic stress of low socioeconomic status display significantly increased expression of ß-adrenergic signaling pathways as well as increased expression of the CTRA gene expression profile (manuscript under review). This increased expression was significantly associated with adverse clinical outcomes including increased relapse and decreased overall leukemia-free survival (manuscript under review). Given the iterative and feedback dynamics involved in processes affecting expression of these ß-adrenergically-mediated gene expression profiles, affecting peripheral inflammatory stimulation as occurs with tocilizumab may alter associated gene expression.

### 1.3 Rap1 prenylation

Rap1, a small GTPase, plays an important role in cell function by regulating cell adhesion.[21-26] In this way, Rap1 suppresses metastasis by localizing at the plasma membrane of tumor cells through the process of prenylation, the post-translational attachment of a lipid tail to Rap1. When Rap1 is localized at the plasma membrane of tumor cells, Rap1 promotes adhesion of the tumor cells and inhibits their metastatic spread. In contrast, when Rap1 prenylation is inhibited, Rap1 cannot anchor at tumor cell membranes, resulting in loss of cell-cell adhesion and increased metastasis of tumor cells. Recently, it was discovered that prenylation of Rap1 is regulated by novel signaling pathways involving cAMP.[27, 28] Both cAMP [29-31] and Rap1[32, 33] have been found to participate in cellular responses to IL-6. These findings support the hypothesis that IL-6-mediated signaling may affect the prenylation of Rap1 and alter the localization of prenylated Rap1 at the cellular plasma membrane. Therefore, tocilizumab may alter Rap1 prenylation and membrane localization, thereby suppressing the metastatic potential of tumor cells.

### 1.4 Rationale

Given that IL-6 is implicated in depressive symptomatology, the current trial aim is to assess whether the immunomodulator and IL-6 antagonist tocilizumab may be effective in reducing depressive symptoms among individuals undergoing allogeneic stem cell transplantation. Given additional evidence that anxiety, fatigue, sleep, and pain may also be associated with IL-6, we will examine the effect of tocilizumab on these symptoms as well. Further, given that IL-6 is associated with ß-adrenergic signaling and Rap1 prenylation, we will explore whether there are changes in gene expression patterns and Rap1 prenylation in response to tocilizumab. These additional psychosocial, genetic, and

biochemical assessments will leverage findings from the parent study "Phase II open label of tacrolimus/methotrexate and tocilizumab for the prevention of acute graft versus host disease after allogeneic hematopoietic stem cell transplantation".

## 2.0 STUDY DESIGN

This is a non-randomized trial comparing the efficacy of tocilizumab to decrease depressive symptoms among a cohort of allogeneic HCT recipients. The intervention group is as described in the parent protocol "Phase II open label of tacrolimus/methotrexate and tocilizumab for the prevention of acute graft versus host disease after allogeneic hematopoietic stem cell transplantation". Administration of tocilizumab will occur through the Medical College of Wisconsin HCT Program. Individuals participating in a longitudinal study evaluating the biobehavioral implications of allogeneic HCT at the University of Wisconsin-Madison (UW) will serve as controls for the psychosocial data. 165 allogeneic transplant participants with follow up QOL outcomes have been enrolled to date and none of them have received tocilizumab.

Participants will complete a battery of self-report surveys at the following time points: baseline, day 28, day 100, and day 180 post-transplant in conjunction with scheduled data collection as part of the parent protocol (Table 1). These additional self-report surveys to be completed include: Inventory of Depression and Anxiety Symptoms (IDAS; depression and anxiety), Fatigue Symptom Inventory (FSI; fatigue), Pittsburgh Sleep Quality Index (PSQI; sleep), and Brief Pain Inventory (BPI; pain). Control participants from UW have also completed these surveys at the time points as described above. Blood samples will be collected at the above time points. Two tubes of blood will be drawn at each time point: a PAXGene RNA tube and an 8.0 mL BD Vacutainer CPT tubes (Table 1). These tubes will be used to describe RNA expression[34] and prenylation[27], respectively.

### 2.1 Primary Objective

The primary objective of this ancillary study is to compare depressive symptoms among allogeneic HCT recipients receiving tocilizumab to a control group of allogeneic HCT recipients who did not receive tocilizumab.

### 2.2 Secondary Objectives

Secondary objectives of this ancillary study are:

1) To compare levels of anxiety, fatigue, sleep, and pain between allogeneic HCT recipients who received prophylactic tocilizumab vs. allogeneic HCT recipients who did not receive tocilizumab (UW control cohort).
2) To describe gene expression and Rap1 prenylation among allogeneic HCT recipients receiving prophylactic tocilizumab.

## 2.3 Patient Eligibility

2.3.1-2.3.2 Inclusion and exclusion criteria same as parent study.

2.3.3 Eligiblity for the Control Arm

Consistent with eligibility criteria for the control arm study being done at UW, eligible participants are adult allogeneic transplant recipients who have been followed for at least 6 months post-transplant.

2.4 – 2.8 Donor Selection, Treatment Plan, Supportive Care, Participant Risk, Study Drug Information same as parent study.

## 3.0 STUDY ENDPOINTS
(In addition to those of parent study)

## 3.1 Primary endpoint

The primary endpoint of this ancillary study is to compare depressive symptoms at day 28 post-transplant for a group of allogeneic HCT recipients who receive tocilizumab as compared to a control group of allogeneic recipients not receiving tocilizumab. This will be addressed through the General Depression subscale of the IDAS and includes 20 questions with a scoring range of 20-100 (mean in community dwelling adult of 44.99 and standard deviation of 14.75).[35]

## 3.2 Secondary endpoints

The secondary endpoints of this ancillary study are 1) to compare depressive symptoms at day 100 and day 180 as well as anxiety, fatigue, sleep, and pain at day 28, day 100, and day 180 post-transplant for a group of allogeneic HCT recipients who receive tocilizumab as compared to a control group of allogeneic recipients not receiving tocilizumab; and 2) to describe gene expression and Rap1 prenylation among allogeneic HCT recipients receiving prophylactic tocilizumab at baseline, day 28, day 100, and day 180.

QOL outcomes:

- Depression: As described in section 3.1.
- Anxiety: Anxiety will be assessed using two subscale items of the IDAS including panic (health population mean = 12.58, SD = 5.26) and traumatic intrusions (healthy population mean = 7.60, SD = 4.20).[35]
- Fatigue: The FSI will be utilized to assess fatigue; a score of 3 or greater on items assessing fatigue in the past week (average of items 1-3; FSI Composite) indicates clinically meaningful fatigue.[36, 37] The FSI can also be evaluated using the average rating of the degree to which fatigue interfered with some general activities (0-10; FSI Interference); participants' ratings of the number of days in the past week they felt fatigued (0-7; FSI Days); and participants' rating of what percent of each day in the past week, on average, they felt fatigued (0-100; FSI Percent).[37]

Individuals scoring at or above the cutoff also report significantly greater scores on these other subscales.

- Sleep: Sleep will be assessed using the PSQI, with a score of >5 considered disturbed sleep as adjusted for cancer populations.[38, 39]
- Pain: The BPI assesses pain intensity as well as pain-related interference in function[40, 41] BPI Pain Severity score ranges from 0-40 (first four items), and the BPI Pain Interference score is a mean of the last 7 items (5a-5g) with a range of 0-10.

Biological outcomes:

- Gene expression: Description of transcription factor binding motifs and CTRA gene expression levels will be described at all study time points.
- Rap1 prenylation: Western blotting on the cytosolic and membrane fractions of isolated PBMCs will determine the distribution of Rap1 in the different fractions as well as the status of Rap1 prenylation.

## 4.0 ENROLLMENT PROCEDURES

**4.1 Patient enrollment** same as parent study.

**4.2 Guidelines for serious adverse event reporting** same as the parent study. Additionally, should patients endorse any thoughts of suicidality or self harm per the IDAS, the study PI for this ancillary protocol (Dr. Jennifer Knight) will contact them by phone. Should completion of the study surveys prompt participants to want treatment for any of the other symptoms, participants will be offered a referral for appropriate care through the Quality of Life Center at the Froedtert Cancer Center.

**4.3 Study monitoring** same as parent study. Psychosocial data and two additional tubes of blood will be collected as described above.

### 4.4 Specimen collection

A clinical research coordinator will collect blood to be stored in two different tubes, a PAXGene RNA tube and a 8.0 mL BD Vacutainer CPT tube, at baseline, day 28, day 100, and day 180 post-transplant. The PAXGene RNA tube will be stored at -80C in the Neuroscience Research Center until they are batched and shipped to UCLA for gene expression analysis. The 8.0 mL BD Vacutainer CPT tube will be sent to Dr. Carol Williams' laboratory for storage at -80C until Western blot analysis is done to assess Rap1 prenylation.

### 4.5 Psychosocial assessments

Participants will complete assessments to evaluate depression, anxiety, fatigue, sleep and pain. The primary endpoint of this ancillary study is to compare depression at baseline and day 28, day 100, and day 180 and the secondary endpoints are to compare anxiety,

fatigue, sleep, and pain at these same time points. These self-report surveys include: Inventory of Depression and Anxiety Symptoms (IDAS; depression and anxiety), Fatigue Symptom Inventory (FSI; fatigue), Pittsburgh Sleep Quality Index (PSQI; sleep), and Brief Pain Inventory (BPI; pain). Participants will also provide information about income and education level at their baseline visit.

### 4.6 Gene expression profiling

The UCLA Social Genomics Core (Directed by Dr. Steve Cole, Professor, Hematology/Oncology, UCLA) will conduct all gene expression profiling on obtained blood samples by using Illumina HT-12 human gene expression bead arrays [42]. Total RNA will be extracted from the whole blood samples stored at MCW, subjected to quality assurance assays to test suitable mass (by spectroscopy) and integrity (by Agilent Bioanalyzer RNA Integrity Score) for analysis, and subjected to microarray target synthesis and hybridization in collaboration with the UCLA Neuroscience Genomics Core Laboratory using standard Illumina assay equipment and protocols. The output of these analyses is quantification of whole genome RNA production.

### 4.7 Rap1 prenylation

PBMCs will be isolated from the whole blood samples collected from patients at all time points. Members of Dr. Carol Williams' laboratory (Professor, Pharmacology and Toxicology, MCW) will conduct Western blotting on the cytosolic and membrane fractions of isolated PBMCs to determine the distribution of Rap1 in the different fractions, and the status of Rap1 prenylation in the cells.

## 5.0 STATISTICAL ANALYSIS

**Sample size justification**
The sample sizes of 35 patients treated with tocilizumab and 165 control patients are determined by the parent studies. With this sample size, our study will have 80% power to detect a 0.53 standard deviation (SD) difference between the two groups at a two-sided 5% significance level. For the primary outcome, assuming a standard deviation of 11 (based on preliminary analysis of the data from the UW cohort), differences of 5.8 points or more on the day 28 General Depression subscale of the IDAS will be detectable. This difference is almost half of the 11 point difference seen between population controls and psychiatric clinic patients.[35]

**Descriptive analyses**
Demographic characteristics to be described for each group will include age, race, gender, Karnofsky Performance Score (KPS), body mass index (BMI), comorbidity index (HCT-CI), and socioeconomic status (as defined by income and education level). Additional medical characteristics to be described for each group include disease, disease status, CMV status, conditioning regimen intensity, donor and HLA match, GVHD prophylaxis, and graft type. Categorical data will be presented by frequencies and

percentages. Descriptive summary statistics (e.g. frequency, mean, median, range and standard deviation) will be used to present numeric data.

### 5.1 Analysis of primary endpoint

SAS 9.3 or newer (SAS Institute, Cary, NC) will be used to analyze study data. We will conduct a one-way analyses of covariance (ANCOVA) including data from both control and intervention conditions while controlling for baseline depressive symptoms and development or presence of aGVHD to compare scores on the depressive scale of IDAS (primary objective).

### 5.2 Analysis of secondary endpoints

#### 5.2.1 QOL outcomes

We will compare anxiety, fatigue, sleep, and pain (secondary objectives) at all time points, as well as depressive symptoms at the two additional time-points via repeated measures linear model using all time points simultaneously while controlling for baseline status on each factor. A mixed effects model with a random subject effect will be used, and the inference will focus on contrasts describing within-timepoint differences. Additional exploratory regression analyses adjusting for covariates that differ between the two groups will be performed. If the groups differ on more than 1-2 covariates, propensity-score based adjustment will be used to reduce the number of variables in the regression models for the outcome. The same covariates, demographic, and medical characteristics as described in Section 5.0 will be used. No adjustment for multiple testing will be performed in these exploratory analyses.

#### 5.2.2 Gene expression profiling

Levels of ß-adrenergic mediated gene expression, transcription factor binding motifs, and CTRA gene expression profiles will be described for all study participants; baseline levels may be compared to later time points.

#### 5.2.3 Rap1 prenylation

Western blotting on the cytosolic and membrane fractions of isolated PBMCs will determine the distribution of Rap1 in the different fractions as well as the status of Rap1 prenylation in collected cells. Baseline Rap1 prenylation will be assessed by the first blood draw and may be compared to later time points.

# TABLE 1. PATIENT CLINICAL ASSESSMENTS

| Study Assessments | Baseline | | | |
|---|---|---|---|---|
| | | **28** | **100** | **180** |
| Income and education level | X | | | |
| Inventory of Depression and Anxiety Symptoms | X | X | X | X |
| Fatigue Symptoms Inventory | X | X | X | X |
| Pittsburgh Sleep Quality Index | X | X | X | X |
| Brief Pain Inventory | X | X | X | X |
| Blood draw (2 tubes) | X | X | X | X |

*Assessments are in addition to those being collected as part of parent protocol

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