PHASE I-II STUDY ON TOCILIZUMAB FOR TREATMENT OF STEROID REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE

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PROTOCOL SYNOPSIS - Phase I-II Study Using Tocilizumab for Treatment of Steroid Refractory Acute Graft-versus-Host Disease

Principal Investigator:	William Drobyski, MD
Study Design:	This is a phase I-II trial designed to evaluate the toxicity and efficacy of Tocilizumab in the treatment of steroid refractory acute GVHD.
Primary Objective:	The primary objective of the study is to determine the response rate (complete and partial) at day 56 after administration of Tocilizumab for treatment of steroid refractory GVHD
Secondary Objective:	Proportion of patients with partial, mixed or no GVHD responses; GVHD progression, primary treatment failures, GVHD flares, discontinuation of immunosuppression, incidence of chronic GVHD, overall survival, incidence of toxicities and infections. For patients with malignant disease, disease-free survival and non-relapse mortality.
Eligibility:	Patient who underwent an allogeneic hematopoietic stem cell transplantation, with biopsy proven GVHD, active acute GVHD requiring systemic immune suppressive therapy and that failed or did not respond to first line of therapy (corticosteroids ± other agent). Patients are excluded if they are intolerant or allergic to Tocilizumab, have active uncontrolled infection requiring ongoing treatment with antifungals, antibiotics or anti-viral drugs, relapsed/persistent malignancy requiring rapid immune suppression withdrawal, liver enzymes: ALT and AST > 3x upper limit of normal or presence of severe sinusoidal obstruction syndrome who in the judgement of the treating physician are not expected to have normalized bilirubin by day 56 after enrollment.
Treatment Description:	Tocilizumab will be administered intravenously at a dose of 8 mg/kg once every three weeks for three doses. After Day 56 doses may be decreased to 4mg/kg once every three weeks depending on GVHD response.
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Accrual Objective:	21 patients will be accrued.
Accrual Period:	The estimated accrual period is 3 years.
Study Duration:	Patients will be followed for 12 months following initiation of therapy.

1.0 BACKGROUND AND RATIONALE

Graft versus host disease (GVHD) is the major complication associated with allogeneic stem cell transplantation. 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.

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.[13] 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.[14,15] Furthermore, IL-6 produced by dendritic cells after activation through Toll-like receptors is able to inhibit the suppressive function of natural Tregs.[16,17] 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,[18,19] as well as those with a recipient or donor IL-6 genotype that results in increased IL-6 production,[20,21] 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.[22] 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.[23,24] 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,[25,26] amyloidosis,[27] and colitis.[28]

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. A pilot phase I/II study in patient with active Crohn's disease also suggested benefit when administered

on an every two week basis.[29] 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.[30] 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.[31] Notably, this patient had failed multiple prior therapies, including high dose steroids, infliximab, budesonide, photopheresis, sirolimus, and mycophenolate mofetil. This report provides further support for the premise that this agent may be an effective therapy for steroid resistant GVHD.

2.0 **STUDY DESIGN**

2.1 **Study Design**

This is a phase I-II trial designed to evaluate the toxicity and efficacy of tocilizumab in the treatment of steroid refractory acute GVHD.

2.2 **Primary Objective**

The primary objective of the study is to determine the response rate (complete and partial) at day 56 after administration of Tocilizumab for treatment of steroid refractory GVHD

C. Inclusion Criteria:

1. Patients age 18 and older who underwent an allogeneic hematopoietic stem cell transplantation.
2. Patients are required to have biopsy proven GVHD.
3. Patients must have active acute GVHD requiring systemic immune suppressive therapy and that failed or did not respond to first line of therapy.
    - First line therapy needs to be a minimum of corticosteroids, methylprednisolone of 1.6mg/kg/day or prednisone of 2mg/kg/day, alone or combined to other agent.
    - Failure of GVHD therapy is defined as flare of signs and symptoms of acute GVHD or progression of GVHD grade after at least 72 hours from starting therapy.
    - No response to GVHD treatment (corticosteroids ± other agent) after a minimum of 7 days of treatment.
4. Patient must be able to give informed consent.

D. Exclusion Criteria:

1. Intolerance or allergy to Tocilizumab
2. Active uncontrolled infection requiring ongoing treatment with antifungals, antibiotics or anti-viral drugs.
3. Relapsed/persistent malignancy requiring rapid immune suppression withdrawal.
4. Liver enzymes: ALT and AST > 3x upper limit of normal.
5. Patients with severe sinusoidal obstruction syndrome who in the judgment of the treating physician are not expected to have normalized bilirubin by day 56 after enrollment.
6. Serum bilirubin > 2x upper limit of normal.

3.0 **TREATMENT SCHEME**

3.1 **Tocilizumab Dose**

Tocilizumab will be administered intravenously at a dose of 8 mg/kg once every three weeks. Patients with documented responses will continue to receive treatment at 8 mg/kg once every 3 weeks for at least two months (day 56). Patients that have some degree of response but without complete resolution of signs and symptoms of acute GVHD may continue to receive 8 mg/kg on a 3-week cycle until complete response is achieved or lack of further improvement. In patients who are beyond day 56 and whose GVHD has resolved, the dose of Tocilizumab will be reduced to 4 mg/kg every 3 weeks. Subsequent discontinuation of Tocilizumab will occur once patients are off other immune suppressive medications (including extracorporeal photopheresis, ECP)or are receiving sub therapeutic levels of immunosuppression (ie. FK levels< 5 ng/mL) or prednisone dose <20mg/day (or equivalent) and are free of acute GVHD signs or symptoms for at least one month.

Patients who fulfill criteria of progression of GVHD not in the setting of immunosuppressive taper, no response of GVHD or require initiation of other immune suppressive treatment for GVHD will have Tocilizumab discontinued.

3.2 **Corticosteroid Taper**

It is anticipated that all patients will be on corticosteroids at the time of initial Tocilizumab administration. Patients who are responding to Tocilizumab may have steroids tapered at the discretion of the primary physician. Similarly, patients treated with steroids but deemed not to have had a response to this agent may also be tapered if the primary physician deems that longer term steroid administration may place the patient at unacceptable risk of opportunistic infection. However, for those improving, steroid taper should not start sooner than 72 hours after administration of tocilizumab and the steroid dose must not be tapered to less than 0.25 mg/kg/day prednisone (or 0.2 mg/kg/day methylprednisolone) on Day 28.

3.3 **Acute GVHD Flare**

If acute GVHD flares during taper of prednisone, the dose of corticosteroids may be increased at the discretion of the treating physician as long as this increase is to less than 2.5 mg/kg/day of prednisone (or methylprednisolone equivalent of 2 mg/kg/day), as in this case it will be considered adding a “new agent” and the patient will be scored as a failure.

3.4 **Concurrent Immune Suppressive Medications**

Patients should remain on concurrent immune suppressive medications at the time that treatment with Tocilizumab is initiated. Patients that are deemed to be responding to Tocilizumab may have their other immune suppressive medications tapered or discontinued as clinically indicated (also see above section on corticosteroid taper).

3.5 **Discontinuation Criteria**

Tocilizumab shall be discontinued and not re-instituted if any one of the following criteria is met. The patient will be taken off study drug therapy at that point, but still followed for primary and secondary study endpoints. A response assessment will be made at the time of therapy discontinuation and at subsequent defined study endpoints. The patient will not be replaced on study. Follow-up data will be required unless consent for data collection is withdrawn:

- Additional systemic GVHD therapy is added for disease progression or non-response
- Steroid dose is escalated to ≥ 2.5 mg/kg/day of prednisone (or methylprednisolone equivalent of 2 mg/kg/day) for GVHD progression or no response
- Development of toxicity that requires withholding of study medication for more than 14 days (see Section 2.5 for details of dose modifications for toxicity)

4.0 **PHARMACEUTICAL INFORMATION**

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

Classification: Immunomodulator

4.2 **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.

4.3 **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.

4.3.1 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.

4.4 **Drug supply and storage**

Tocilizumab is commercially available. 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.

4.5 **Administration:**

Tocilizumab will be administered at the initial dose of 8 mg/kg with a maximum dosing weight of 100 kg (max dose 800 mg) and 4mg/kg for maintenance. The infusion will be administered over 60 minutes through a dedicated IV line every 3 weeks as clinically indicated. Do not administer by IV bolus.

4.5.1 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.

4.5.2 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.

Patients may remain on existing immunosuppressive regimen at the time tocilizumab is begun but no additional agents can be added unless patient is deemed to have failed treatment (see evaluation of response).

4.6 **Warnings / Precautions**

4.6.1 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.

4.6.2 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.

4.6.3 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.

4.6.4 Hypersensitivity reactions

Anaphylaxis and infusion reactions have been reported in association with tocilizumab infusions. Appropriate medical treatment should be available for immediate use in the event of an anaphylactic reaction during administration.

4.7 **Adverse effects:**

Other adverse reactions most frequently reported in clinical trials include upper respiratory tract infections, nasopharyngitis, headache, hypertension, ALT increases, dizziness, bronchitis, rash, mouth ulceration, upper abdominal pain, and gastritis. Anti-tocilizumab antibodies have been detected in a small number (2% of patients). Hypersensitivity (allergic) reactions including a few cases of anaphylaxis have also been reported.

4.8 **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 co-administered 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.

4.9 **Supportive Care Guidelines**

4.9.1 Infectious Disease Monitoring
Patients on tocilizumab should have CMV PCR NAAT, EBV, HHV6, and Adenovirus testing by PCR in the blood prior to each dosing of tocilizumab. Viral testing may be performed more frequently if clinically indicated.

Anti-infective prophylaxis against herpes viruses, *Pneumocystis jiroveci,* bacterial and fungal infections should be followed according to standard MCW institutional practices.

4.9.2 Laboratory monitoring

Patients on Tocilizumab require weekly evaluations of liver enzymes (AST, ALT, Bilirubin) for the first two cycles of treatment. After two cycles, hepatic evaluation should be done at the discretion of the treating physician and at least once before each dose of Tocilizumab.

Fasting* lipid profile including total cholesterol, low density lipoprotein (LDL) and triglycerides should be done at baseline and before each dose of Tocilizumab for the first three doses. If no significant increases in lipid profile, they should be repeated every 6 months thereafter for monitoring. *If patient is on TPN a non-fasting lipid profile is acceptable.

Patients on calcineurin inhibitors (cyclosporine, tacrolimus) or rapamycin should have the levels checked at least twice week after the first dose of Tocilizumab for at least three weeks.

Patients who are on concurrent potentially myelosuppressive agents (e.g. gancyclovir) may need more frequent monitoring of WBC.

4.10 Toxicities and guidelines for dose reduction/withholding study drug

4.10.1 Hepatic Dysfunction

This refers to increases observed after the institution of Tocilizumab.

Liver enzyme abnormality (ALT, AST,)	**Recommendation**
> 1 to 3 x baseline	(A). For patients in whom the AST and ALT are within the normal range at the onset of enrollment into the study, the upper limit of normal for each enzyme will be used to determine whether the patient's LFTs are greater than one to three times the normal values. For persistent increases in this range for 2 consecutive weeks after the most recent dose of Tocilizumab, in the absence of any other offending hepatic toxic agents, reduce dose to 4 mg/kg. (B). For patients in whom the baseline LFTs were elevated outside of the upper limit of the normal range at the time of enrollment, these values will serve as the baseline for the calculation of whether the LFTs are >1 to 3 times normal. For persistent increases in this range, defined as AST and ALT both elevated >1 to 3X baseline for 2 consecutive weeks after the most recent dose of Tocilizumab, in the absence of any other offending hepatic toxic agents, reduce dose to 4 mg/kg.
> 3 to 5 x baseline	Hold therapy for 1 week. Discontinue any

	other offending hepatic toxic agent, if possible. Recheck labs. If AST and ALT still elevated >3 to 5 x baseline, continue to hold for additional week. Determination of baseline LFT values will be made as noted above for LFTs > 1 to 3X baseline. Resume drug when hepatic function returns to < 3 x baseline then follow recommendations for > 1 to 3 x baseline by reducing dose to 4 mg/kg. If no improvement after a total of 2 weeks of holding therapy and no other offending hepatotoxic agent can be implicated, then discontinue Tocilizumab. If there is a contributing hepatotoxic agent that cannot be discontinued and which is thought to have been responsible for increase in LFTs, then Tocilizumab may be continued at reduced dose of 4 mg/kg for one additional dose at physician's discretion. If no further increase in LFTs > 3 to 5 x baseline, continue Tocilizumab at 4 mg/kg. If LFTs increase to > 5 x baseline, discontinue therapy.

4.10.2 **Neutropenia**

ANC	**Recommendation**
<1000 intervening period between doses	If ANC <1000, then may give G-CSF to increase WBC at physician discretion. Remove other offending agents that might lower ANC if possible. If ANC >1000 at time of next dose and patient does not require sustained G-CSF administration to maintain ANC>1000, then reduce Tocilizumab to 4 mg/kg for this and subsequent doses. If patient is still requiring daily G-CSF administration then physician may at his /her discretion elect to hold for an additional week to determine whether ANC>1000 can be achieved without requirement of sustained G-CSF administration.
< 1000at time of next dose,	If ANC remains <1000 at time of next dose hold dose for 1 week, administer G-CSF if not previously done, and re-check. If ANC

	remains <1000, hold 1 additional week, and continue G-CSF. If ANC not >1000 after second week of holding dose,discontinue Tocilizumab.

4.10.3 Infection
Patients with severe or refractory infections may have Tocilizumab held at the attending physician's discretion. Antibody can be reinstituted when the infection episode is controlled and hemodynamic stability restored.

4.10.4 Other Unexpected Toxicities:
Study drug may be held for other CTCAE v.4 Grade 3-4 toxicities that are considered probably related to Tocilizumab at the discretion of the treating physician.

5.0 GUIDELINE FOR SERIOUS ADVERSE EVENT REPORTING

5.1 Definitions

Adverse Event - 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 adverse event 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.

Life-threatening Adverse Event – Any adverse event that places the patient or subject, in view of the investigator, 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.)

Unexpected Adverse Event – An adverse event that was not described in the study protocol or informed consent.

• **Serious Adverse Event (SAE)** – Any adverse event occurring that results in any of the following outcomes:
• Death – days 0-200, regardless of cause.
• life-threatening adverse event (see above).
• persistent or significant disability/incapacity.
• congenital anomaly.
• requires intervention to prevent permanent impairment or damage.

Attribution - The designation for the determination of whether an adverse event is related to a medical product, treatment or procedure will be as follows:
• Related – includes adverse events that are definitely, probably, or possibly related to the medical treatment or procedure.
• Not Related – includes adverse events are doubtfully related or clearly not related to the medical treatment or procedure.

The MCW Serious Adverse Event (SAE) Report Form should be completed for all adverse events that meet the expedited reporting requirements

Serious adverse events that do not meet the requirement for expedited reporting (not related to study treatment or expected) will be reported to the IRB as part of the annual renewal of the protocol.

5.2 Monitoring the Progress of Trial and the Safety of Participants

This Phase I/II clinical trial will be monitored by the Medical College of Wisconsin Cancer Center Data Safety Monitoring Committee (DSMC) and the principal investigator (PI), William R Drobyski MD. The PI will review the outcome of the data for each individual patient on an ongoing basis. The PI of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the Institutional Review Board. The PI will ensure that the

monitoring plan is followed and that all data required for oversight of monitoring are accurately reported, that all adverse events are reported according to the protocol guidelines, and that any adverse reactions reflecting patient safety concerns are appropriately reported. Study toxicities are graded using the adapted NCI Common Terminology Criteria for Adverse Event (CTCAE) v 4.0 (where appropriate, use the criteria for transplant patients.)

5.3 Reporting of Adverse Events

The adverse event reporting in this clinical trial will follow an adapted version of the MCW Guidelines for SAE reporting. These guidelines detail the expedited reporting requirements, definitions of particular events. All severe adverse events (SAEs), which are defined as an event that occurred during or following the administration of Tocilizumab to a subject during the course of the study that resulted in death, considered life threatening, required inpatient hospitalization or prolonged existing hospitalization, resulted in persistent disability, congenital anomaly or events judged as medically important, meeting the MCW expedited reporting criteria will be reported to the MCW Institutional Review Board (IRB) within 3 days by the PI or research coordinator upon learning of the event. For patients being cared for at Froedtert Hospital, health care providers communicate with the PI or research coordinator as events occur triggering subsequent reporting. For patients not being cared for at Froedtert Hospital, the outside facilities communicate with the PI, or research coordinator for these reporting purposes. Toxicities contributing to and meeting the study stopping rule criteria will be reported to the IRB within 3 days of study staff awareness. All other SAEs and deaths, not meeting the expedited reporting criteria, will be submitted to the IRB as part of the annual continuation review report to the IRB.

5.4 Expedited Reporting Requirements

All unexpected and serious adverse events meeting MCW expedited reporting criteria which may be due to study procedures or intervention must be reported to the MCW IRB as soon as possible but within at least 3 calendar days of the investigator learning of the event.

Any deaths that occur within 60 days of treatment will be immediately reported to the DSMC and MCW IRB.

All Grade 4 (life-threatening) toxicities occurring between days 0-200 after the administration of the first dose of Tocilizumab that meet MCW expedited reporting requirements must be reported as soon as possible but within at least 3 calendar days of the investigator learning of the event.

6.0 **GVHD GRADING, STUDY DEFINITIONS AND ENDPOINTS**

6.1 **Staging and Grading of Acute GVHD**

Staging*

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

Grading Index of Acute GVHD*

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

6.2 **Response Definitions**

Complete response is defined as a CIBMTR score of 0 for the GVHD grading in all evaluable organs. For a response to be scored as a CR at day 56 or later the participant must still be in CR on that day and have had no interfering additional therapy for an earlier progression, PR or NR.

Partial response is defined as improvement in one or more organs involved with GVHD symptoms without progression in others. For a response to be scored as PR at day 56 or later, the participant must still be in PR on that day and have had no interfering additional therapy for an earlier progression, PR or NR

Mixed response is defined as improvement in one or more organs with deterioration in another organ manifesting symptoms of GVHD or development of symptoms of GVHD in a new organ.

Progression is defined as deterioration in at least one organ without any improvement in others, or development of signs and symptoms of chronic GVHD..

No response is defined as follows. Patients receiving additional immune suppressive therapy will be classified as nonresponders. If acute GVHD progresses (new organ involvement or increased organ specific symptoms sufficient to increase the organ stage by one or more) after at

least 7 days of study drug administration, or if there is no response (no reduction in any GVHD organ staging) after 7 days of study drug administration, administration of the study drug may be discontinued, and the patient may be treated with alternative secondary GVHD therapy at the discretion of the treating physician.

6.3 **Primary Endpoint**

The primary endpoint is proportion of CR/PR at Day 56 of therapy. **CR is defined as resolution of all signs and symptoms of GVHD in all evaluable organs in comparison to Day 1 scoring.** For a response to be scored as CR at Day 56, the participant must still be in CR on that day and have had no intervening salvage therapy for an earlier progression, PR or NR.

6.4 **Secondary Endpoints**

6.4.1 **Proportion of Partial Response (PR), Mixed Response (MR), No Response (NR) and Progression:**

Proportion of Complete Response (CR), Partial Response (PR), Mixed Response (MR), No Response (NR) and Progression among surviving patients at Day 56: Scoring of CR, PR, MR, NR and progression are in comparison to the participant's acute GVHD status (score) on Day 0 of the study.

6.4.2 Proportion of Primary Treatment Failures

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

6.4.3 **GVHD Flares**

Flares are defined as any progression of acute GVHD after an initial response (i.e., earlier CR or PR) that requires re-escalation of steroid dosing, or initiation of additional topical or systemic therapy. While all "flares" will be captured, only flares that require additional systemic therapy (that is additional drugs) or re-escalation of steroids to $\geq$ 2.5 mg/kg/day of prednisone [or methylprednisolone equivalent of 2 mg/kg/day] will be counted as failure for the primary endpoint. The rate of all flares before Day 90 will be examined as a secondary endpoint.

6.4.4 **Immunosuppression Discontinuation**

Discontinuation of immunosuppression will be assessed by Day 56, Day 180 and Day 360 after enrollment. The date of discontinuation of corticosteroids will be recorded. In addition, dates for discontinuation of all other systemic immunosuppressive medications (where applicable), including cyclosporine or tacrolimus, sirolimus, etc. for treatment or prevention of acute GVHD will be captured.

6.4.5 Chronic GVHD

The incidence of chronic GVHD at 6 months after enrollment will be examined.

6.4.6 Overall Survival

Overall survival at Day 180 will be computed.

6.4.7 Incidence of Toxicities

Incidence of serious adverse events and grade 3-5 CTACAE v4 at day 56 after initiation of Tocilizumab will be summarized.

6.4.8 Incidence of Infections

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

6.4.9 Disease-free Survival at 6 and 12 Months Post-randomization

Disease-free survival at 6 months will be computed. The events for disease-free survival are death and relapse of the underlying malignancy.

6.4.10 Non-relapse Mortality at 6 and 12 Months Post-randomization

Non-relapse mortality at 6 months will be computed. The events for non-relapse mortality are death due to any cause other than relapse of the underlying malignancy.

7.0 **PATIENT REGISTRATION AND EVALUATIONS**

7.1 **Patient Registration**
Patients who meet eligibility criteria and signed informed consent will be registered in the study.

7.2 **Patient Treatment Schedule**
After registration patients may receive Tocilizumab once every three weeks until criteria in 3.1 is met. The schedule below outlines the target days for infusion.

Table 7.2: Schedule Evaluations

Tocilizumab Dose	Target Day
1	1
2	22± 3
3	43 ± 3
4	64± 7
5	85± 7
6	106± 7
7	127± 7
8	148± 7
9	169± 7
10	190± 7
11	221± 7
12	242± 7
13	262± 7
14	283± 7
15	304± 7
16	325± 7
17	346± 7
18	365± 7

*Beyond the third dose the table outlines the proposed scheduled of infusions. Patients who start maintenance dose should continue with the same schedule until drug discontinuation criteria are met. Patients may continue on tocilizumab beyond one year and the schedule to continue should be every three weeks.

7.3 **Patient Evaluations**
7.3.1 Pre-Tocilizumab evaluations
Evaluations prior to study entry should occur within 14 days from initiation of Tocilizumab. These evaluations are:

- History and physical examination
- Karnofsky performance score or ECOG evaluation
- CBC with differential, platelet count, creatinine, total bilirubin, LDH, alkaline phosphatase, AST, ALT, sodium, magnesium, potassium, chloride, and CO2.
- Baseline NAAT for CMV, EBV, HHV-6 and Adenovirus
- Blood and urine culture for bacteria and fungus if clinically indicated
- Assessment of acute GVHD (Appendix A)

7.3.2 Evaluations after initiation of Tocilizumab

The following required observations are summarized in Table 7.3.2:

- History and physical exam to assess GVHD and other morbidity. GVHD assessments should be completed on days of tocilizumab drug administration.
- CBC with differential, platelet count, creatinine, LDH, liver functions (Bilirubin, LDH, alkaline phosphatase, AST, and ALT), sodium, magnesium, potassium, chloride, and CO2.
- PCR NAAT for CMV, EBV, HHV-6 and Adenovirus, prior to each Tocilizumab dose.
- Surveillance blood culture for bacteria and fungus, if clinically indicated

Table 7.3.2: Schedule Evaluations

			Day (±3 days)								Day (±7 days)									
	Study entry	**1**	**8**	**15**	**22**	**29**	**36**	**43**	**50**	**57**	**64**	**85**	**106**	**127**	**148**	**169**	**190**	**221**	**242***	**Every 3 weeks until day 365**
Tocilizumab[1]		**X**			**X**			**X**			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
H&P[2]	**X**				**X**			**X**			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
GVHD[3]	**X**				**X**			**X**			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
H/O Panel [4]	**X**	**X**	**X**	**X**	**X**			**X**			**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Fasting* lipid profile[5]	**X[5]**	**X[5]**			**X[5]**			**X**								**X[5]**				**X[5]**
Calcineurin inhibitor levels[5]			**X (2x/ wk)**	**X (2x/ wk)**	**X (2x/ wk)**															
CMV[6]	**X**				**X**			**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
EBV, HHV-6, ADENO[7,8]	**X**				**X**			**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Urine culture for bacteria and fungus[9]	**X**																			
Blood Cultures[10]	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**

***Continue every three week schedule until week 52 from start of treatment.**

[1]Tocilizumab infusions will start at day 1 of the study and continue every three weeks at a dose of 8mg/kg. After day 56 (week 8) depending on GVHD responses, patients will have their dose reduced to 4mg/kg every three weeks. If patients fulfill criteria to stop tocilizumab subsequent evaluations are not required.

[2]History and physical to assess for other comorbidities.

[3]Acute GVHD assessments according to Appendix A. Also if patients develop signs or symptoms of chronic GVHD while on trial.

[4] Hematology/Oncology panel that includes: CBC with differential, sodium, potassium, CO2, blood urea nitrogen, creatinine, lactate dehydrogenase (LDH), AST, ALT, total bilirubin, alkaline phosphatase, glucose.

[5] Fasting lipid profile including total cholesterol, low density lipoprotein (LDL) and triglycerides should be done at baseline and before each dose of Tocilizumab for the first three doses. If no significant increases in lipid profile, they should be repeated every 6 months thereafter for monitoring. Lipid panel need not be repeated if baseline lipid panel has been done within 7 days of starting first dose of Tocilizumab. *If patient is on TPN a non-fasting lipid profile is acceptable.

[5] Patients on calcineurin inhibitors (cyclosporine, tacrolimus) or rapamycin should have the levels checked at least twice a week after the first dose of Tocilizumab for at least three weeks.

[6, 7] PCR NAAT (nucleic acid amplification test) for cytomegalovirus, Epstein virus, HHV-6 and adenovirus.

[8] Can be performed more frequently if clinically indicated.

[9] If clinically indicated

[10] Surveillance blood cultures for bacteria and fungus, if clinically indicated

8.0 **STATISTICAL CONSIDERATIONS**

This study is a single arm phase I/II study designed to assess the safety and efficacy of tocilizumab for treatment of steroid resistant acute GVHD. Historical data indicates that approximately 25% of steroid resistant patients respond to second or third line therapy. The study is designed as a two-stage trial according to the Simon optimal design with 80% power to detect an increase in the response rate at day 56 from 25% to 50%, using a 10% one-sided type I error rate. This design will require a total sample size of 21 patients, with 8 patients enrolled in the first stage. If 2 or fewer of the first 8 patients respond, the study will be stopped for futility. Otherwise, if 8 or more of the 21 total patients respond, the treatment will be considered sufficiently promising. This study design has a 68% chance of stopping after the first 8 patients, if the response rate is 25%, and an 80% chance of considering the treatment promising if the response rate is 50%.

Stopping Rules

Deaths in the first 28 days will be monitored. A mortality probability of >20% in the first month is unexpected and the protocol team would want to review the protocol should this rate be exceeded. Entry of patients into the trial will stop while the protocol and deaths are reviewed if there is significant evidence that the day 28 overall mortality rate is more than 20% based on the exact binomial test. The stopping rule is summarized in the following table.

Number of patients in the trial	Stop if death occurs in:
3-4	3
5-7	4
8-11	5
12-14	6
18-21	7

The actual operating characteristics of this stopping rule, shown in the table below, were determined in a simulation study that assumed uniform accrual of 21 individuals over a three-year time period.

TABLE 1: OPERATING CHARACTERISTICS OF STOPPING RULE FOR OVERALL MORTALITY WITHIN 28 DAYS FROM A SIMULATION STUDY WITH 10,000 REPLICATIONS

True 28 day Rate	20%	35%	40%	45%
Probability Reject Null	0.103	0.564	0.726	0.852
Mean Month Stopped	34.0	25.8	22.3	18.9
Mean # Endpoints in 28 Days	4.15	5.40	5.32	5.02
Mean # Patients Enrolled	19.8	15.0	13.0	11.0

The testing procedure for overall mortality within 28 days rejects the null hypothesis in favor of the alternative 10% of the time when the true 28 day mortality is 20%, and 85% of the time when the rate is 45%. This corresponds to a type I error rate of $\alpha = 0.10$ and a type II error rate of $\beta = 0.15$. When the true 28 day mortality rate is 45%, on average, the stopping rule will be triggered 19 months after opening, when 5 events have been observed in 11 patients.

1.1. Analysis of Secondary Endpoints

1.1.1. Proportion of CR, PR, MR, NR and Progression

Proportions of Complete Response (CR), Partial Response (PR), Mixed Response (MR), No Response (NR) and Progression at Days 28 and 56 will be compared between the treatment groups using the chi-square test.

1.1.2. Proportion of Primary Treatment Failures among Surviving Patients

Proportion of primary treatment failures among surviving patients at Days 28 and 56 will be compared using the Z test for comparing binomial proportions.

1.1.3. GVHD Flares Requiring Additional Therapy

The proportion of patients experiencing a flare after an initial response will be evaluated at Day 90 and compared between treatment groups using the Z test for comparing binomial proportions. This will be done both for all flares as well as flares that require additional systemic therapy.

1.1.4. Immunosuppression Discontinuation

The incidence of discontinuation of immunosuppression will be computed separately in each treatment group using the cumulative incidence curve, with death prior to discontinuation as the competing risk. Pointwise 95% confidence intervals will be provided at Day 56, Day 180, and Day 360. Comparison of the cumulative incidence curves between treatment will be done using Gray's test.

1.1.5. Cumulative Steroid Dose at Day 56 after treatment

The median and range of cumulative steroid doses at each time point will be provided separately for each treatment group. The median cumulative steroid dose will be compared between treatment groups using the Wilcoxon rank sum test.

1.1.6. Chronic GVHD

The incidence of chronic GVHD will be computed using the cumulative incidence estimator, treating death prior to chronic GVHD as a competing risk. Pointwise confidence intervals will be provided at 6 and 12 months, and the cumulative incidence curves will be compared using Gray's test.

1.1.7. **Overall and GVHD-free Survival**

Overall survival will be computed using the Kaplan-Meier estimator. Pointwise confidence intervals will be provided at Days 180 and 360. Survival curves will be compared between treatment groups using the log-rank test. GVHD-free survival will be estimated using simple proportions of patients alive and without GVHD if there is no censoring prior to 180 or 360 days. If there is censoring, the proportion of patients alive and without GVHD will be estimated in each treatment using multi-state model techniques.[i]

1.1.8. **Disease-free Survival at 6 Months Post-treatment**

Disease-free survival will be estimated using the Kaplan-Meier method, treating death or relapse of malignancy as events in patients with malignancies. This outcome will be compared between the two treatment groups using the log-rank test.

1.1.9. **Non-relapse Mortality at 6 Months Post- treatment**

Non-relapse mortality will be estimated using the cumulative incidence method, treating relapse as the competing risk. The cumulative incidence curves for non-relapse mortality will be compared between the treatments using Gray's test.

APPENDIX A
Acute GVHD assessment

Today's Date _____ Patient ID/Name _____ Karnofsky/Lansky _____

	0	1	2	3	4	5		GVHD	Drug Rxn	Cond Reg	TPN	Infect	VOD	Other
Skin	☐	☐	☐	☐	☐		% body rash:_____	☐	☐	☐		☐		
Lower GI	☐	☐	☐	☐	☐	☐	Vol: _____	☐	☐	☐	☐	☐		
Upper GI	☐	☐						☐	☐	☐	☐	☐		
Liver	☐	☐	☐	☐	☐		Current bili: _____	☐	☐	☐	☐	☐	☐	

Codes Differential Diagnosis

Systemic Agents:	☐ CSA	☐ Tacrolimus	☐ Infliximab	☐ Dacluzimab	
	☐ Pentostatin ☐ Prednisone	☐ Sirolimus ☐ Methylprednisolone	☐ Etanercept ☐ Study Drug	☐ MMF ☐ Ontak	☐ Other _____

Topical Agents: Skin topical steroids ☐ YES ☐ NO; Non-absorbed oral steroids (e.g., Budesonide, Entocort) ☐ YES ☐ NO

Is the patient eating equivalent of one meal/day? ☐ YES ☐ NO Is the patient having formed stools? ☐ YES ☐ NO

Does the patient have evidence of chronic GVHD? ☐ YES ☐ NO

Did patient receive study drug at this visit? ☐ YES ☐ NO What dose of the study drug did the patient receive?: _____mg/kg

Current steroid dose: Prednisone _____ mg/day or _____ mg/kg/day; Methylprednisolone _____ mg/day or _____mg/kg/day

Has the steroid dose been increased to ≥ 2.5mg/kg/day of prednisone (or 2 mg/kg/day methylpred)? ☐ YES ☐ NO; if yes, date dose increased _____

If yes, reason for dose increase: ☐ flare for GVHD; ☐ Idiopathic Pneumonia Syndrome (IPS); ☐ other (please specify) _____

Code Definitions:

Skin:	Lower GI (Diarrhea):	Upper GI:	Liver (Bilirubin):
0 No rash 1 Maculopapular rash, < 25% of body surface 2 Maculopapular rash, 25-50% of body surface 3 Generalized erythroderma 4 Generalized erythroderma with bullous formation and desquamation	0 No diarrhea 1 ≤ 500 mL/day or < 10 mL/kg/day 2 501-1000 mL/day or 10-19.9 mL/kg/day 3 1001-1500 mL/day or 20-30 mL/kg/day 4 > 1500 mL/day or > 30 mL/kg/day 5 Severe abdominal pain with or without ileus, or stool with frank blood or melena	0 No protracted nausea and vomiting 1 Persistent nausea, vomiting or anorexia	0 < 2.0 mg/dL 1 2.1-3.0 mg/dL 2 3.1-6.0 mg/dL 3 6.1-1.0 mg/dL 4 > 15.0 mg/dL

Signature _____ Date _____

APPENDIX B

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