Title of Trial: PROACTIVE: Phase II Single Center Open Label Study for Prevention of Acute and Chronic GVHD Using Tocilizumab in Combination with Standard GVHD Prophylaxis After Allogeneic Transplantation

Clinical Trials.gov Number: NCT03699631

Date of Protocol: September 19, 2019

CLINICAL STUDY PROTOCOL

PROACTIVE: Phase II Single Center Open Label Study for Prevention of Acute and Chronic GVHD Using Tocilizumab in Combination with Standard GVHD Prophylaxis After Allogeneic Transplantation

Saurabh Chhabra, MD

Version 4, September 19, 2019

PROTOCOL SIGNATURE PAGE

Protocol No.: 32694 **Version Date:** 09/19/2019

1. I agree to follow this protocol version as approved by the Medical College of Wisconsin (MCW) Scientific Review Committee (SRC), Institutional Review Board (IRB) and Data Safety Monitoring Committee (DSMC).
2. I will conduct the study in accordance with applicable IRB requirements, federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I have read and understand the information in the Investigator's Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP), the applicable ethical principles, the Statement of Investigator (Form FDA 1572) and with local regulatory requirements. In accordance with the FDA Modernization Act, I will ensure the registration of the trial on the www.clinicaltrials.gov website.
5. I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

MCW Principal Investigator / Study Chair

_____
Printed Name

_____ _____
Signature Date

Title: **PROACTIVE**: Phase II Single Center Open Label Study for Prevention of Acute and Chronic GVHD Using TocIlizumab in Combination with Standard GVHD Prophylaxis After Allogeneic Transplantation

MCW OnCore® No.: IIT-Chhabra-Proactive

MCW Protocol No.: 32694

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Investigational Agent(s):
Tocilizumab

REVISION HISTORY

Revision history is presented in reverse order so that the information pertaining to the most current version of the protocol is presented first in this section.

Version 4, September 19, 2019

Version 3, June 27, 2019

Version 2, November 21, 2018

Version 1, August 24, 2018

Initial submission of the protocol.

PROTOCOL SUMMARY

Title	Phase II Single Center Open Label Study for Prevention of Acute and Chronic GVHD Using Tocilizumab in Combination with Standard GVHD Prophylaxis After Allogeneic Transplantation
Protocol Number	32694
Principal Investigator	Saurabh Chhabra, MD
Study Sites	Froedtert & the Medical College of Wisconsin
Clinical Trial Phase	II
Study Disease	Hematologic malignancy
Main Eligibility Criteria	**Inclusion Criteria:** 1. Age ≥18 years. 2. Patients with any hematologic malignancy for which alloHCT is indicated. Patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) must be in complete remission at the time of alloHCT(<5% blasts in the bone marrow, normal maturation of all cellular components in the bone marrow and absence of extramedullary disease). 3. Myeloablative conditioning (MAC) regimen, based on CIBMTR criteria 4. T cell-replete peripheral blood or bone marrow graft. 5. Patients must have a matched related or unrelated donor (at least 6/6 match at HLA-A, -B and -C for related donors and at least 8/8 match at HLA-A, -B, -C and -DRB1 for unrelated donors). 6. Cardiac function: Left ventricular ejection fraction ≥45% for myeloablative conditioning. 7. Estimated creatinine clearance ≥40 mL/minute (using the Cockcroft-Gault formula and actual body weight). 8. Pulmonary function: DLCO ≥40% (adjusted for hemoglobin) and FEV1 ≥50%. 9. Liver function: total bilirubin <3 x upper limit of normal and ALT/AST <5 x upper normal limit. 10. Signed informed consent: Voluntary written consent must be given before patient registration and performance of any study related procedure not part of standard medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care.

	11. Female patient: A negative pregnancy test will be required for women of child bearing potential. Breast-feeding or lactation is not permitted. 12. Planned posttransplant maintenance therapy is allowed. **Exclusion Criteria:** 1. Prior allogeneic HCT. 2. Active CNS involvement with malignancy. 3. Patients receiving cord blood or haploidentical allograft. 4. Patients undergoing *in vivo* or *ex vivo* T cell-depleted alloHCT. 5. Karnofsky Performance Score <60%. 6. Patients with uncontrolled bacterial, viral or fungal infections (currently on treatment and with progression of infectious disease or no clinical improvement) at time of enrollment. 7. Active hepatitis B or C virus infection or known human immunodeficiency virus (HIV) positive. 8. Prior intolerance or allergy to tocilizumab 9. Use of rituximab, alemtuzumab, anti-thymocyte globulin (ATG) or other monoclonal antibody planned as part of conditioning regimen for GVHD prophylaxis. 10. History of diverticulitis, Crohn's disease or ulcerative colitis. 11. History of demyelinating disorder. 12. Any current uncontrolled cardiovascular conditions, including uncontrolled ventricular arrhythmias, NYHA class III or IV congestive heart failure, uncontrolled angina, or electrocardiographic evidence of active ischemia or active conduction system abnormalities. 13. Any serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with the completion of treatment according to this protocol.
Study Rationale	We earlier hypothesized and demonstrated that tocilizumab could attenuate the incidence of aGVHD after MAC and RIC alloHCT, using matched sibling or unrelated donor. In this study, we hypothesize that longer term IL-6 inhibition through treatment with tocilizumab by repeated dosing would mediate a beneficial effect not only on the risk of aGVHD, but also on chronic GVHD. This will be achieved by administering an additional dose of tocilizumab at Day +100 post-alloHCT, besides the pretransplant dose, as done in our previous clinical trial, thereby providing total prophylaxis against both acute and chronic GVHD.
Primary Objectives	1. Determine the probability of GVHD/relapse-free survival (GRFS) defined as survival without grade III-IV acute GVHD,

	systemic therapy-requiring chronic GVHD, relapse, or death at 12 months after matched related/unrelated donor bone marrow or peripheral blood alloHCT using myeloablative conditioning (MAC).
Secondary Objectives	1. Cumulative incidence of mild, moderate and severe chronic GVHD (by NIH criteria) and limited or extensive chronic GVHD (by conventional criteria). 2. Cumulative incidence of grade II-IV acute GVHD at Days +100 and +180. 3. Cumulative incidence of grade III-IV acute GVHD at Days +100 and +180. 4. Incidence of primary and secondary graft failure. 5. Probability of non-relapse mortality post-alloHCT. 6. Cumulative incidence of relapse/progression of the primary malignancy. 7. Probability of PFS post-HCT. 8. Probability of OS post-HCT. 9. T cell and myeloid chimerism kinetics following alloHCT at Day +28. 10. Immune reconstitution following alloHCT at Day +28, Day +100, Day +180, and Day +365. 11. Patient-reported Quality of Life assessments at baseline (enrollment), Days +28, +100, +180 and +365 post-transplant. 12a. Effect of GVHD prophylaxis on gut microbiome diversity: Difference in the level of microbiome diversity during transplant. 12b. Association of baseline gut microbiome diversity with development of aGVHD, cGVHD, and overall survival.
Endpoints	Primary endpoint: The primary endpoint of this trial is GRFS. An event for this outcome is defined as grade III-IV acute GVHD, systemic therapy requiring chronic GVHD, relapse, or death. Patients who are alive without GVHD will be censored at the last follow-up. **Secondary endpoints:** • Acute GVHD • Chronic GVHD • Hematopoietic Recovery

	• Graft Failure • Non-Relapse Mortality (NRM) • Disease Relapse or Progression • Progression-Free Survival • Overall Survival • Incidence of Infections • Donor Cell Chimerism • Immune Reconstitution • Patient-reported Quality of Life • Microbiome diversity pre-engraftment and evolution during transplant
Study Design	This is a phase II open-label trial designed to evaluate the efficacy of tocilizumab in improving GRFS after allogeneic hematopoietic cell transplantation (alloHCT) for hematologic malignancy.
Study Agent/ Intervention Description	Patients enrolled on the clinical trial will receive tacrolimus initiating at Day -1 at doses to maintain therapeutic levels per institutional preference and continued until at least Day +90 post-transplant. Methotrexate will be administered intravenously and dosed at 15 mg/m^2 Day +1 and 10 mg/m^2 Days +3, +6 and +11. Tocilizumab will be administered intravenously at a dose of 8 mg/kg on Day -1 and at day +100 (+/- 14 days).
Number of Subjects	32
Subject Participation Duration	Patients will be followed for at least 12 months following alloHCT.
Duration of Follow up	Patients will be followed indefinitely for survival following alloHCT.
Estimated Time to Complete Enrollment:	The estimated accrual period is 18 months.
Statistical Methodology:	The sample size was selected to achieve 80% power to detect an increase in GRFS at 12 months to 40% compared to a historical control value of 20% at a one-sided 5% significance level. Based on asymptotic z-test for proportions, 29 patients with a known outcome are needed (i.e., known GVHD, relapse, or death status at 12 months). Accounting for the drop out rate of 10%, a total of 32 patients will be enrolled on the study. **Analysis of primary endpoint** The GRFS will be estimated using the Kaplan-Meier estimator and plotted with a 95% confidence band. An event for this outcome is defined as grade III-IV acute GVHD, chronic GVHD requiring

	systemic therapy, relapse or death. Patients who are alive without GVHD will be censored at the last follow-up. The 12-month GRFS will be compared to the prespecified historical control value of 20% using a one-sided *z*-test. **Analysis of secondary endpoints** Demographic and other baseline data, such as disease characteristics, as well as outcome measures, will be presented overall and separately for URD and MRD patients. Categorical data, such as gender, race, etc., 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. Time-to-event outcomes with and without competing risks will be analyzed using Kaplan-Meier and Nelson-Aalen estimates, respectively, and presented with 95% confidence intervals. Binary outcomes will be analyzed using proportions with 95% confidence intervals.

STUDY CALENDAR

Study Assessments/Testing	Baseline[9]			Post-Transplant								
		Day-1	Day 0	Day +1± 2	Day+8± 2	Day+15± 2	Day+21± 2	Day+28± 7	Day+56± 7	Day+100 ± 14	Day+180 ± 14	Day+365 ± 28
History, physical exam, weight and height*	X		X	X				X	X	X	X	X
Karnofsky performance status and HCT-CI*	X											
HLA typing (donor and recipient) *	X											
Toci dose†		X								X		
Transplant data (documentation of conditioning/stem cell infusion)			X									
CBC with differential[1], comprehensive metabolic panel[2]*	X			X	X	X	X	X	X	X	X	X
Infectious disease markers[3]*	X											
LVEF*[10]	X											
DLCO and FEV1 *	X										X	X
Disease evaluation[4]*	X									X	X	X
Pregnancy test[5]*	X											

Study Assessments/Testing	Baseline[9]			Post-Transplant								
		Day-1	Day 0	Day +1± 2	Day+8± 2	Day+15± 2	Day+21± 2	Day+28± 7	Day+56± 7	Day+100 ± 14	Day+180 ± 14	Day+365 ± 28
GVHD assessments[6*]								X	X	X	X	X
AE assessments[7]	X			X				X	X	X	X	
Fasting lipid panel									X		X	
Chimerism[8*]								X				
CMV NAAT peripheral blood*								X	X	X		
Immune reconstitution*								X		X	X	X
QoL assessments (See Appendix 3)	X							X		X	X	X
Research (stool) sample collection		X[11]		X	X	X	X	X[12]				

*Indicate evaluations are currently performed as standard of care at the MCW BMT program.

[1, 2] CBC with differential, comprehensive metabolic panel will be performed as appropriate during hospitalization for alloHCT and on clinic visits and at minimum performed on days indicated on the schedule of events. CBC will be checked weekly until neutrophil and platelet recovery.

[3] Infectious disease titers include: CMV, hepatitis panel (hepA ab, hepB SAb, hepB SAg, hepB Core Ab, hepC Ab), HIV and HTLV I/II antibody.

[4] Evaluation of the malignant disease: for acute leukemia, CML and MDS this includes a bone marrow aspirate and biopsy for morphology, cytogenetics/FISH and molecular testing as appropriate. 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.

[5] Pregnancy test must be performed <30 days before the start of the transplant conditioning for females of childbearing potential and may be performed per institutional practices. (Serum or Urine)

[6] GVHD assessments performed per the standard operating procedures at the MCW BMT Program, and at minimum on Day + 28, +56, +100, +180, +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.

[7] The AE assessment will include a review of **all** toxicities including infections experienced **during the entire assessment period** and the **highest grade** for each AE during the assessment period will be recorded on the AE forms.

[8] Chimerism in whole blood fractionated as CD3 and CD33.
[9] All baseline tests/procedures must be performed <30 days before the start of the transplant conditioning unless specified otherwise.
[10] LVEF to be estimated by MUGA or transthoracic echocardiogram.
[11] First stool sample for microbiome studies will be collected after admission in the hospital pre-transplant. Subsequent samples will be collected on Day+1±2, Day+7±2, Day+15±2, Day+21±2, and until neutrophil engraftment (last stool sample will be collected after neutrophil engraftment).
[12] Stool sample will also be collected at the time of onset of acute or chronic GVHD.
† Tocililzumab will be dose at 8 mg/kg (maximum dose of 800 mg) once on the Day -1 approximately 24 hours prior to the estimated time of the hematopoietic cell infusion, and subsequently, on Day +100 (+/- 14 days, i.e., Days +86 to +114) post-alloHCT. The weight used to calculate the dose will be within 7 days of first dose of tocilizumab. Only if the patient's weight has changed >10% (over the baseline weight), then the dosage of tocilizumab (for the second dose) will be changed depending on the weight checked within 14 days prior to the second dose.

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LIST OF ABBREVIATIONS

AE	adverse event
aGVHD	acute graft versus host disease
ALL	acute lymphocytic leukemia
alloHCT	allogenic hematopoietic cell transplant
ALT	alanine aminotransferase
AML	acute myeloid leukemia
ANC	absolute neutrophil count
AST	aspartate aminotransferase
AUC	area under the curve
BUN	blood urea nitrogen
CBC	complete blood cell (count)
cGVHD	chronic graft versus host disease
CR	complete response
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CMV	cytomegalovirus
DFS	disease-free survival
DLT	dose-limiting toxicity
DSM	Data and Safety Monitoring
DSMC	Data and Safety Monitoring Committee
DSMP	data and safety monitoring plan
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
GCP	good clinical practice
GRFS	graft-versus-host disease and relapse-free survival
GVHD	graft-versus-host disease

HIV	human immunodeficiency virus
HLA	human leukocyte antigen
ICH	International Conference on Harmonization
IRB	Institutional Review Board
IV	intravenous
KPS	Karnofsky Performance Score
LVEF	left ventricular ejection fraction
MAC	myeloablative conditioning
MCWCC	Medical College of Wisconsin Cancer Center
NAAT	nucleic acid amplification test
PBPC	peripheral blood progenitor cell
QoL	quality of life
SAE	serious adverse event
SD	standard deviation
SRC	Scientific Review Committee
UPIRSO	unanticipated problems involving risks to subjects or others

1 BACKGROUND

1.1 Graft-versus-Host Disease

Allogeneic hematopoietic cell transplantation (alloHCT), a curative therapy for hematologic malignancies, acts by mediating immunologic graft-versus-tumor effects against the malignancy. Unfortunately, these immune responses can also be directed toward normal host tissues, resulting in acute graft-versus-host disease (GVHD). [1] GVHD is classified as either acute or chronic GVHD, based on clinical characteristics. [2] According to the clinical definitions, classic acute GVHD (aGVHD) develops within 100 days posttransplant with distinctive clinical features in the skin, gastrointestinal (GI) tract, or liver. However, manifestations of aGVHD can reoccur, persist, or present after Day 100 posttransplant and is then classified as recurrent, persistent or late onset aGVHD. Classical chronic GVHD is not limited to a specific organ system and can present at any time after transplantation. The diagnosis of chronic GVHD is based on consensus criteria for each organ system involved. While some clinical features are diagnostic (e.g., bronchiolitis obliterans and sclerotic features of the skin), other manifestations require additional clinical and/or histological criteria to be fulfilled. Some patients may also present as an overlap syndrome with clinical features of both acute and chronic GVHD (cGVHD). Scoring systems have been developed to grade the severity of aGVHD, based on the involvement of skin, GI tract and liver: Glucksberg scale (grading aGVHD from I to IV) is most widely used [3] (see Appendix A). Importantly, aGVHD, although T-cell dependent, is known to be mediated, at least in part, by inflammatory cytokines. [4] By contrast, the mechanistic pathway in cGVHD involves Th17 differentiation [5] and germinal center B cells. [6]

The development of GVHD is a three-step process, each involving different subsets of immune cells. [7,8] The first step is activation of the innate immune system; this results in a state of systemic inflammation prior to the introduction of donor T cells. The chemotherapy and/or radiation used in the conditioning therapy invoke tissue damage with release of proinflammatory cytokines, e.g., IL-1, TNF-α, IFN-γ and IL-6. These cytokines perpetuate GVHD through direct cytotoxic effects on host tissues, [9-11] activation and/or priming of immune effector cells [12] and differentiation of proinflammatory and pathogenic alloantigen-specific T cell populations (i.e., TH1 and TH17 cells) from naïve T cell precursors. [4,13,14] Damage to the GI barrier allows increased translocation of microorganisms from the gut microbiome, with subsequent increased levels of circulating lipopolysaccharide, as well as molecules with pathogen- and damage-associated molecular patterns (PAMPs/DAMPs). [15] Consequently, antigen-presenting cells

(APC) secrete proinflammatory cytokines and present host, as well as pathogen peptides on their major histocompatibility complex (MHC) molecules. A reduction of pretransplant inflammation (e.g., using reduced intensity conditioning (RIC), reduction of gut microbiome levels) and the presence of genetic factors associated with reduced inflammatory responses are associated with a reduced risk of GVHD and transplant-related mortality (TRM). [16-18]

The second step is characterized by alloreactive T cell activation, proliferation and differentiation as a response to the presentation of host antigens by host APC in a proinflammatory context. Stimulation of specific T helper cell subsets is thought to be important for the initiation of later cytotoxic T-cell mediated tissue damage. [7,8] Th1 cells release IFN-γ and express the transcription factors STAT4 and STAT1/T-bet. [19,20] This Th1 polarization seems to be important for the development of aGVHD, especially in the GI tract. [11] Th2 cells characterized by expression of the transcription factor GATA-3 and secretion of anti-inflammatory cytokines IL-4, IL-10, and IL-13 [21] also may play a role in GVHD pathogenesis. [11,22,23] Th17 cells are characterized by the expression of the transcription factor RORγt and IL-17 secretion, [24] and are thought to be important for aGVHD severity [25] and early transplant-related severe lung injury. [26] A majority of early posttransplant circulating TCRαβ+ CD4+ and CD8+ T cells release IL-6 at relatively high levels together with classical proinflammatory cytokines, such as IFN-γ and TNFα. [27] Finally, T regulatory cells (Tregs) are a Th subset characterized by expression of the transcription factor FOXP3 and high IL-2R (CD25) expression. These cells play an important regulatory role by actively suppressing immune responses through their release of the anti-inflammatory cytokines IL-10, TGF-β and IL-35, [28] and through direct interaction with other T cell subsets. Tregs are suppressed during GVHD, and resolution of GVHD is associated with restoration of the Treg function. [29] The inflammatory environment in GVHD is promoted by the absence of an effective Treg response as both a relative and an absolute decline of Tregs in the peripheral blood and target tissues have been demonstrated in most studies. [13,30-32] The strong association between a proinflammatory milieu and the absence of an effective counter-regulatory response suggests that the inflammatory environment prevents or inhibits Treg reconstitution during GVHD.

The third step of GVHD development is characterized by local action of cytotoxic CD8+ T cells triggered by the local release of chemokines during the two first steps. These cells mediate direct cytotoxic effects upon target cell recognition, including secretion of perforin/granzyme and FAS-ligand. Other T cell subsets contribute to the organ-specific manifestations of GVHD

through polarization of macrophages toward a proinflammatory (M1) phenotype that further increases tissue damage through the release of oxidants and proinflammatory cytokines, including TNF-α and IL-6. [33,34]

A combination of a calcineurin inhibitor (tacrolimus (Tac) or cyclosporine (CSP)) and methotrexate (MTX) has been an accepted standard for prevention of GVHD. Despite prophylaxis, the incidence of grade II–IV acute GVHD (aGVHD) is 35 to 50% [35] and the rate of chronic GVHD is 40 to 60%. The occurrence of GVHD after alloHCT results in increased transplant-related mortality (TRM), which also affects overall survival (OS) [36]. The risk increases with unrelated or partially HLA-matched donors due to the greater genetic disparity between donor and recipient. Thus, new therapeutic treatment strategies are needed for prevention of acute, as well as chronic GVHD.

1.2 Interleukin-6

IL-6, a glycosylated protein with a molecular weight of 21-28 kDa,[37] is a pleiotropic cytokine that is produced by a variety of cell types, including T cells, B cells, fibroblasts, endothelial cells, macrophages, monocytes and keratinocytes. [38] Macrophages and monocytes appear to be the main sources of IL-6 during acute inflammation, while T cells seem to be a major contributor during chronic inflammation. [39] The systemic (serum/plasma) levels usually range from 1.8 to 14 pg/ml in healthy individuals. [40,41] During inflammation, a more than 105-fold increase can be observed, often correlating with disease severity. [42] IL-6 is essential for maturation, proliferation, differentiation and maintenance of B cells/plasma cells and several proinflammatory T-cell subsets.

With respect to GVHD biology, IL-6 sits at the crossroads where the fate of naïve T cells to become proinflammatory T cells or Tregs is determined. In the presence of IL-6 and transforming growth factor-β (TGF-β), naïve T cells differentiate into TH17 cells, whereas in its absence, Tregs are induced. [43,44] IL-6 produced by dendritic cells after activation through Toll-like receptors is, in addition, able to inhibit the suppressive function of Tregs. [45,46] Thus, IL-6 appears to have a pivotal role in directing the immune response toward an inflammatory phenotype and away from a regulatory response. Second, IL-6-signaling is crucial for trafficking of immune cells to inflamed tissues and lymphoid organs. This is caused both by altered expression of adhesion molecules by endothelial cells and by expression of their ligands by immunocompetent cells. Third, IL-6 has important functions in GVHD target organs, and there

may, therefore, be a risk of combined injury during GVHD (e.g., GVHD-induced immunologic damage, pharmacologic toxicity and IL-6 inhibition). Finally, IL-6, together with TNF-α released from macrophages, has been reported to directly/independently contribute to tissue damage in GVHD. [33]

The importance of IL-6 in GVHD is also supported by clinical studies showing increased incidence and severity of GVHD in patients with elevated plasma levels of IL-6, [47,48] and in those with a recipient or donor IL-6 genotype that results in increased IL-6 production. [49,50] IL-6 also is important in regulation of stem cells and tissue regeneration in several organs. [2] This has been best demonstrated for hematopoiesis, liver cells, GI mucosa and muscle cells. Impaired IL-6 function in these organs is associated with reduced regeneration after injury, and IL-6 dysregulation during chronic inflammation can contribute to organ dysfunction.

Signaling through IL-6 occurs by its binding to a low-affinity IL-6 receptor (IL-6R). [51] IL-6R exists both in a membrane-bound (mIL-6R) and a soluble form (sIL-6R). The binding of IL-6 to IL-6R induces homodimerization of gp130 cell membrane protein for intra-cellular signal transduction.[52] mIL-6R is only expressed by a limited number of cell types, including hepatocytes, hematopoietic cells (neutrophils, naïve T cells, macrophages) and a subset of intestinal epithelial cells. IL-6R signaling can be initiated in these cells through the membrane-associated complex of IL-6, IL-6R and gp130. This is called classical signaling and is often associated with tissue regeneration and anti-inflammatory effects. [2,52] IL-6R can also be shed from the membrane, generating a soluble form of the receptor, which can bind IL-6 and induce an intracellular response in any tissue expressing gp130 through a process called trans-signaling. [53,54] Thus, cells that do not express the IL-6R themselves can be IL-6 responsive and this seems important for many of the proinflammatory effects of IL-6. [37] sIL-6R is mainly formed through cleavage of mIL-6R by a disintegrin and metalloprotease (ADAM) 10 and 17 proteases. sIL-6R is constitutively released by liver and hematopoietic cells, but activation of ADAM17 during inflammation causes a rapid local increase in sIL-6R levels. The trans-signaling pathway is highly inflammatory, while classic signaling in cells directly expressing IL-6R is crucial for the differentiation of donor T cells down a Th17 pathway, which is capable of mediating GVHD. [37,55]

Gp130 is noncovalently associated with the Janus kinases (JAKs) JAK1, JAK2 and TYK2. Following receptor ligation, the JAKs are auto-phosphorylated, and they also phosphorylate gp130. This phosphorylation provides docking sites for phosphorylation of STAT1, STAT3 and

the tyrosine phosphatase SHP-2. Phosphorylated STAT3 dimerizes and is translocated to the nucleus where it acts as a transcription factor. [56] SHP-2 activates the RAS/RAF/MAPK/ERK pathway, whereas gp130 activation also leads to activation of the PI3K-AKT pathway together with the transcriptional regulator YAP1. Most of the IL-6 effects seem to be STAT3-mediated. STAT3 is controlled by a negative feedback mechanism; it induces expression of SOCS proteins and activation of the SHP-2 phosphatase. SOCS3 then binds with high affinity to the same phosphorylated binding site on gp130 as JAK1/2 and thereby inhibits further intracellular signaling. IL-6 signaling is also inhibited by internalization and degradation of the receptor complex and internalization of gp130 prevents further signaling.

1.3 IL-6 Blockade and GVHD: Preclinical Studies

The role of IL-6 in acute and chronic GVHD has been investigated in several mouse models. Givon et al. [57] examined the effect of IL-6 on bone marrow reconstitution after syngeneic and alloHCT and found that posttransplant treatment with subcutaneous recombinant IL-6 significantly supported white blood count reconstitution and improved survival in syngeneic and allogenic models transplanted with a low stem cell dose. In contrast, mice receiving IL-6 showed increases in both the severity of and mortality from GVHD. Chen et al. [58] observed increased systemic IL-6 and IL-6R levels early after both syngeneic and allogeneic transplantation. These levels returned to baseline over time in the syngeneic group, whereas IL-6 levels remained high in mice developing GVHD. Both IL-6 and IL-6R expression increased in the liver and colon and the highest IL-6R mRNA levels were observed in these two organs.

Selective IL-6 knockout in neither recipient nor donor cells were sufficient to protect from GVHD. However, GVHD treatment with anti-IL-6 resulted in significantly less weight loss, less histopathological evidence of damage to colon, liver and lungs, and significantly increased Treg levels in the spleen. The increased Treg levels were not dependent on an intact thymus; rather the IL-6 blockade increased peripheral generation of Treg cells and reduced the levels of Th1 and Th17 cells. Similar results were shown by Noguchi et al.; [59] treatment with an anti-IL-6 antibody reduced liver enzyme levels and occurrence of organ failure and was associated with reduced infiltration of Th1 and Th17 cells, increased Tregs and improved survival.

Tawara et al. [60] investigated the effect of IL-6 derived from donor T cells. Selective IL-6 knockout in donor/graft T cells was associated with less severe GVHD and prolonged survival. Pretransplant anti-IL-6 treatment also significantly improved survival and clinical as well as

histopathologic severity of GVHD, but the Tregs were not altered. Importantly, the GVT effect was maintained despite the reduction in GVHD. The systemic cytokine levels and the levels of circulating cells were not altered. Selective ablation of IL-6 in recipient bone marrow cells did not reduce the incidence or severity of GVHD.

Organ-specific effects of IL-6 in GVHD have been investigated in mouse models. Varelias et al. [26] examined the role of IL-6 in idiopathic pulmonary syndrome (IPS) after alloHCT and demonstrated that local IL-6 secretion induced Th17 cell differentiation that was necessary for disease development. IPS could be prevented by IL-17 knockout or using anti-IL-17 antibody. Le Huu et al. [61] investigated the role of IL-6 in a sclerodermatous cGVHD model and observed increased IL-6 during disease progression. Treatment with anti-IL-6 antibody prior to manifestations of scleroderma significantly decreased the severity, while no reduction was observed when IL-6 blockade was started after the onset of cGVHD. Treatment with anti-IL-6 was associated with a significant increase in the number of splenic Treg cells, whereas the expression of IFN-γ, TNFα, IL-6, IL-18, TGF-β1, CCL2, CCL3 and CCL5 in affected skin was significantly reduced.

The role of STAT3 in the regulation of activation and differentiation of Tregs and Th17 cells after alloHCT has been investigated in several mouse models (i.e., STAT3-knockout mice) and *in vitro* models. Emerging evidence also suggests that activation of STATs in B cells is also important for GVHD. [62] Several important observations suggest that targeting of IL-6/JAK2/STAT3 signaling may be effective in preventing GVHD. [63-67] As has been demonstrated in our previous clinical trial, IL-6 blockade using tocilizumab is an effective GVHD preventative strategy since IL-6 and STAT3 activation are closely linked to the development of both Th17 and Treg cells, and since early STAT3 phosphorylation posttransplant seems to precede development of GVHD. [66] Currently available clinical data indicate that IL-6 blockade is most effective when used as GVHD prophylaxis, whereas manifest GVHD is likely less susceptible to the effects of IL-6 blockade.

1.4 Tocilizumab

Tocilizumab (Actemra™) is a humanized anti-IL-6R 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 Castleman's disease. [68,69] A pilot phase I/II

study in patient with active Crohn's disease also suggested benefit when administered on every two-week basis. [70] Tocilizumab is generally well tolerated in patients with rheumatoid arthritis; the most commonly reported side effects being dyslipidemia (21–25%), increased liver transaminases (5–6%) and transient decreases in neutrophil counts. [71-73] IL-6 blockade inhibits the acute phase response and may thereby mask signs of acute severe infections. Although some studies in rheumatoid arthritis have shown a higher rate of infections in patients treated with tocilizumab, [71,72] this could not be confirmed in a large multicenter study. [73]

1.4.1 Tocilizumab and Treatment of GVHD

Murine studies of GVHD have shown that treatment with an anti-IL-6R antibody is able to significantly reduce GVHD-associated mortality and pathologic damage. [58] In addition to a few case reports and abstracts, [2] two published case series report the effects of tocilizumab in the treatment of steroid-refractory aGVHD. [74,75]

Drobyski et al. [75] published a series of eight patients who received tocilizumab for steroid-refractory aGVHD. Responses were observed in four of six patients with severe aGVHD that had failed to respond to first, and in most cases, second-line therapies. Five patients had grade IV GVHD (four GI and one skin), while one patient had grade II GI and one had grade III liver GVHD. One of these patients died early after tocilizumab administration and was not evaluable, one patient did not respond, three patients were classified as partial responders, and two patients were considered complete responders. In one of the patients with partial response, tocilizumab was discontinued for the possibility of causing worsening of preexisting hyperbilirubinemia. Infections were responsible for the major adverse events associated with tocilizumab, with a total of 13 documented infections. Roddy et al. [74] reported the effect of tocilizumab in patients with steroid-refractory aGVHD, including seven patients with grade IV and two with grade III. Two patients were classified as complete responders, and two patients had mixed response with persistence of severe aGVHD in one organ but resolution in other organs, and four patients did not respond. In this series, four patients had infectious events with two deaths being reported, but no liver toxicity was observed.

1.4.2 Tocilizumab and GVHD prophylaxis

A phase I/II study conducted by Kennedy et al. assessed if tocilizumab could attenuate the incidence of acute GVHD [76] when given as adjunct to the GVHD prophylactic regimen. Eligible patients were 18 to 65 years old and underwent T-replete HLA-matched alloHCT with either

total body irradiation (TBI)-based myeloablative (MAC) or RIC using unrelated or sibling donors. One intravenous (IV) dose of tocilizumab (8 mg/kg, capped at 800 mg, over 60 mins' infusion) was given the day before alloHCT along with standard GVHD prophylaxis (cyclosporine [5 mg/kg per day on Days –1 to +1, then 3 mg/kg per day to maintain therapeutic levels (trough levels of 140–300 ng/mL) for 100 days plus methotrexate [15 mg/m² on Day 1, then 10 mg/m² on Days 3, 6 and 11]). The primary endpoint was incidence of grade 2–4 aGVHD at Day 100, assessed and graded, as per the Seattle criteria. Immunological profiles were compared with a non-randomized group of patients receiving alloHCT, but not treated with tocilizumab. A total of 48 patients receiving CSP+MTX as GVHD prophylaxis were enrolled into the study. The incidence of grade II–IV aGVHD in patients treated with tocilizumab at Day 100 was 12% (95CI 5–24), and the incidence of grade III–IV aGVHD was 4% (1–13). Grade II–IV aGVHD involving the skin developed in five (10%) patients of 48 treated with tocilizumab, involving GI tract in four (8%) patients; there were no reported cases involving the liver. Low incidences of grade II–IV aGVHD were noted in patients receiving both TBI-based MAC (12% [95%CI 2–34) and fludarabine and melphalan RIC (12% [4–27]). There were no reports of graft failure and immune reconstitution was preserved in tocilizumab recipients, but with suppression of known pathogenic STAT3-dependent pathways. The addition of tocilizumab appeared generally safe with no increase in graft rejections, time to neutrophil engraftment, chimerism posttransplant, or early relapse compared with historical controls. Only three patients experienced severe liver toxicity during the first month after transplantation. This study showed that inhibition of IL-6 superimposed on the calcineurin inhibitor-based GVHD prophylaxis is a potential strategy to reduce the incidence of acute GVHD without compromising immune reconstitution. [76]

A phase II study of tocilizumab was conducted by Drobyski *et al.* in alloHCT patients using matched related/unrelated donor and receiving Tac/MTX for GVHD prophylaxis. [77] Tocilizumab was administered IV as a single dose of 8 mg/kg the day before alloHCT. A total of 35 patients were enrolled in the study and all received busulfan-based conditioning (Flu/BU4, Flu/BU2 or BU/CY). The majority of patients (83%) received peripheral blood grafts. The primary endpoint is grade II–IV GVHD during the first 180 days posttransplant. The median follow-up of surviving patients was 12 months (range 5–16). The incidence of grades II–IV and III–IV aGVHD at Day 100 was 14% (95CI 5–30%) and 3% (95CI 0–11%), respectively. In the first 100 days post-alloHCT, there were no cases of acute GVHD of lower GI tract and only one patient developed grade IV aGVHD of skin. Five patients died from relapse between 140 to 270 days posttransplant, while five patients died as a result of TRM (GVHD, n=2; infection, n=2; IPS

(n=1). TRM was 9% (95CI 2–20%) and relapse was 17% (95CI 7–31%) at six months. In addition, we performed a matched (1:4) case-control analysis with contemporary controls (n=130) from the Center for International Blood and Marrow Transplant Research (CIBMTR) from 2000 to 2014 that were identified according to the same eligibility criteria for the trial except for the use of Tac/MTX as GVHD prophylaxis and were matched for age, Karnofsky Performance Score (KPS), disease and donor type. The incidence of grades II–IV aGVHD at Day +180 was significantly lower in the tocilizumab/Tac/MTX cohort when compared with the CIBMTR Tac/MTX control population (17% vs. 45%, P=0.001). Corresponding probabilities of grade II–IV aGVHD-free survival at six months, which was the primary objective of the study, were 69% and 42% (P=0.001 by stratified log rank test). There were no differences in relapse, TRM, DFS or OS at six months between the two groups. These two prospective studies thus show that tocilizumab has promising activity in preventing aGVHD, particularly lower GI tract GVHD and late onset aGVHD.

1.5 Success of allogeneic transplant: relapse versus non-relapse mortality

AlloHCT used for the treatment of hematologic malignancies is associated with two main risk factors for poor outcomes: transplantation-related mortality (also referred to as NRM) and mortality from disease relapse (relapse-related mortality). Efforts to mitigate one cause of mortality have often compromised the other. For example, efforts to reduce GVHD risk by T-cell depletion of the allograft can lower transplantation-related morbidity/mortality, but can also increase relapse risk. [78,79] Similarly, efforts at reducing relapse with intensified pre-transplant conditioning regimen can lead to increased mortality from organ dysfunction, infections, or GVHD. [80] Therefore, clinical trials are needed that evaluate the post-transplant outcomes by focusing on both NRM and relapse risk concurrently, rather than having only one as the primary objective. In addition, both endpoints NRM and relapse mortality do not reflect non-lethal morbidity. Because of these concerns, a composite endpoint such as GRFS would be ideal to assess all significant and relevant endpoints in a study evaluating a new clinical strategy with the potential to impact outcomes after alloHCT. [81] The events for GRFS include grade III-IV acute GVHD, systemic therapy-requiring chronic GVHD, relapse, or death in the first year post-HCT. GRFS, therefore, represents ideal recovery from HCT (at one year) and a measure of cure without ongoing morbidity.

Using a cohort of 628 adult patients treated with tacrolimus and methotrexate as GVHD prophylaxis between 2006 and 2009, data from the Center for International Blood and Marrow

Transplant Research determined that the 1-year probability of GRFS was 23% (95% confidence interval [CI] 20-26). [81] In other words, only approximately one-quarter of patients transplanted for hematologic malignancy survived without at least one of these major complications during the first 12 months after alloHCT.

We earlier hypothesized and demonstrated that tocilizumab could attenuate the incidence of aGVHD after MAC and RIC alloHCT, using matched sibling or unrelated donor. [77] In this study, we hypothesize that longer term IL-6 inhibition through treatment with tocilizumab by repeated dosing would mediate a beneficial effect not only on the risk of aGVHD, but also on chronic GVHD. This will be achieved by administering an additional dose of tocilizumab at Day +100 post-alloHCT, besides the pretransplant dose, as done in our previous clinical trial, thereby providing total prophylaxis against both acute and chronic GVHD.

1.6 Studies of the Microbiome in Transplant Patients

The microbiome, consisting of a varied community of microbes (bacteria, viruses, fungi, microeukaryotes, and sometimes multicellular parasites), exists in niches across the human body. The skin, lung, nares, vagina, and gastrointestinal tract are among the most heavily colonized, with the largest number of microorganisms inhabiting the colonic lumen. While the majority of the over trillion organisms that live within a healthy human colon are nonpathogenic members of the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria, alterations in the balance of these microorganisms have been associated with adverse outcomes ranging from GVHD and infection to relapse post-HCT. [82] This clinical association between intestinal microorganisms and HCT outcomes has been investigated for decades – and has informed the still controversial practices of infection prophylaxis, gut decontamination, the “neutropenic diet,” and isolation of patients in laminar air flow rooms. Single-institution studies have demonstrated that low microbial diversity in the stool after allogeneic HCT is associated with poor survival. [83,84] Additionally, specific alterations have been associated with increased risk of acute GVHD, infectious outcomes and most recently, relapse. [85-87] While these findings are compelling, the generalizability of these proposed microbial biomarkers is unclear. This is particularly important as there is known geographic variation of the intestinal microbiome and practice variability in antibiotic use for prophylaxis and treatment from institution to institution, in part due to different antibiograms.

Novel methods in microbiome research are likely to facilitate translational breakthroughs – these methods allow (1) detailed taxonomic classification of microorganisms at the strain level, (2) metabolic characterization of the small molecules and proteins that a microbial community makes, (3) measurement of microbial genomic evolution in clinical time courses, and (4) culturing of previously fastidious organisms from the microbiome for *in vitro* investigation and cultivation as potential therapeutic live bacterial clinical interventions. Given recent reports suggesting that alterations in the microbiome can impact the efficacy of immunologic therapies, it is imperative that the link between the microbiome and transplant outcomes is investigated.[88,89]

1.6.1 Microbiome and Outcomes after Allogeneic Transplantation using Tocilizumab as GVHD prophylaxis

The study will test the secondary hypothesis that the use of IL-6 blockage (tocilizumab) which has been shown to lower gastrointestinal GVHD rates preserves the gut microbiome diversity (determined by 16s rRNA sequencing analysis of the sequential stool sample collected pre- and post-transplant until neutrophil engraftment). Additional analyses on patient samples will be conducted to answer the key question concerning the impact of the gut microbiome on transplant outcome. Aliquots preserved additionally will establish a cohort of stool samples collected prospectively for future sequencing by shotgun metagenomic sequencing and metabolomic analysis.

2. HYPOTHESIS AND OBJECTIVES

We earlier hypothesized and demonstrated that tocilizumab could attenuate the incidence of aGVHD after MAC and RIC alloHCT, using a matched sibling or an unrelated donor. [90] In this study, we hypothesize that longer term IL-6 inhibition through treatment with tocilizumab by repeated dosing would mediate a beneficial effect not only on the risk of aGVHD, but also on chronic GVHD. This will be achieved by administering an additional dose of tocilizumab at Day +100 post-alloHCT, besides the pretransplant dose, as done in our previous clinical trial, thereby providing total prophylaxis against both acute and chronic GVHD.

The study will test the secondary hypothesis that the use of IL-6 blockage (tocilizumab) which has been shown to lower gastrointestinal GVHD rates preserves the gut microbiome diversity (determined by 16s rRNA sequencing analysis of the sequential stool sample collected pre- and post-transplant until neutrophil engraftment). Additional analyses on patient samples will be conducted to answer the key question concerning the impact of the gut microbiome on

transplant outcome. Aliquots preserved additionally will establish a cohort of stool samples collected prospectively for future sequencing by shotgun metagenomic sequencing and metabolomic analysis.

2.1 Primary Objectives

Determine the probability of GRFS defined as survival without grade III–IV acute GVHD, systemic therapy-requiring chronic GVHD, relapse, or death at 12 months after matched related/unrelated donor bone marrow or peripheral blood alloHCT, using myeloablative conditioning (MAC).

2.2 Secondary Objectives

- Cumulative incidence of mild, moderate and severe chronic GVHD (by NIH criteria) and limited or extensive chronic GVHD (by conventional criteria).
- Cumulative incidence of grade II–IV acute GVHD at Days +100 and +180.
- Cumulative incidence of grade III–IV acute GVHD at Days +100 and +180.
- Incidence of primary and secondary graft failure.
- Probability of non-relapse mortality post-HCT.
- Cumulative incidence of relapse/progression of the primary malignancy.
- Probability of PFS post-HCT.
- Probability of OS post-HCT.
- T cell and myeloid chimerism kinetics following alloHCT at Day +28.
- Immune reconstitution following alloHCT at Day +28, Day +100, Day +180, and Day +365.
- Patient-reported Quality of Life assessments at baseline, Day+28, Day+100, +180 and +365 post-transplant
- Effect of GVHD prophylaxis on gut microbiome diversity: Difference in the level of microbiome diversity during transplant.
- Association of baseline gut microbiome diversity with development of aGVHD, cGVHD, and overall survival.

3 STUDY DESIGN

3.1 General Description

This is a phase II, open-label, single-arm trial designed to evaluate the efficacy of tocilizumab in improving the GVHD and relapse-free survival.

3.1.1 Number of Subjects

Thirty-two (32) patients.

3.2 Primary Endpoint(s)

The primary endpoint of this trial is GRFS. An event for this outcome is defined as grade III–IV acute GVHD, systemic therapy requiring chronic GVHD, relapse or death. Patients who are alive without GVHD will be censored at the last follow-up.

3.3 Secondary Endpoint(s)

3.3.1 Acute GVHD

Cumulative incidences of grades II–IV and III–IV acute GVHD will be determined at Day +100 and Day +180 post-HCT. Acute GVHD will be graded according to Appendix 1. The time of onset of grade II–IV acute GVHD and time to development of the highest grade until Day +180 will be recorded. Development of acute GVHD will be considered an event for this endpoint. Death but not disease relapse will be considered a competing event.

3.3.2 Chronic GVHD

The cumulative incidence of chronic GVHD by NIH Consensus criteria will be determined. Development of chronic GVHD or death will be considered events for this endpoint. The highest grade of chronic GVHD will be recorded. In addition, the time of onset of chronic GVHD and the time to highest chronic GVHD grade will be recorded. Death but not disease relapse will be considered a competing event.

3.3.3 Hematopoietic Recovery

Hematopoietic recovery will be assessed according to neutrophil and platelet counts recovery after HCT. Neutrophil recovery or engraftment is defined as achieving an absolute neutrophil count (ANC) ≥500/mm^3 for three consecutive measurements on three different days. The first of the three days will be designated the day of neutrophil engraftment. The competing event is

death without engraftment. Platelet recovery is defined by either the first day of a sustained platelet count >20,000/mm^3 for three days 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.

3.3.4 Graft Failure

Graft failure will be assessed as a secondary endpoint, including primary and secondary graft failure. Primary graft failure is defined as no neutrophil recovery to > 500 cells/μL by Day 28 post-HCT. Secondary graft failure will be assessed according to neutrophil count after initial hematologic recovery. Secondary graft failure is defined as initial neutrophil engraftment followed by subsequent decline in absolute neutrophil counts <500 cells/μL, unresponsive to growth factor therapy, but cannot be explained by disease relapse or drugs.

3.3.5 Nonrelapse Mortality (NRM)

NRM is defined as death after alloHCT without relapse. The cumulative incidence of NRM will be estimated at Day +100 and one year after alloHCT. An event for this endpoint is death without evidence of disease progression or relapse. Disease progression or relapse will be considered a competing event.

3.3.6 Disease Relapse or Progression

Relapse is defined by either morphological, cytogenetic or radiologic evidence of the pretransplant hematologic malignancy. 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. NRM will be considered a competing event.

3.3.7 Progression-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 last follow-up.

3.3.8 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. Survivors

will be censored at last follow-up.

3.3.9 Incidence of Infections

The incidence of grade≥3 (CTCAE v5) viral, fungal and bacterial infections until day+180 will be determined. The cumulative incidence of CMV viremia post-alloHCT will be described.

3.3.10 Donor Cell Chimerism

Chimerism will be evaluated using sorted whole blood in CD3+ and CD33+ fractions. Mixed chimerism is defined as the presence of donor cells, as a proportion of total cells to be >5% and <95%. Full donor chimerism is defined as ≥95% of donor cells. Donor cells of ≤5% will be considered as graft rejection. Donor cell chimerism will be assessed for all patients at Day +28 , and Day +100 for both CD3+ and CD33+ fractions. The proportion of patients with each level of chimerism listed above will be described. CD3+ donor cell chimerism will be used to define the donor/recipient chimerism status.

3.3.11 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.

3.3.12 Patient-Reported Quality of Life (PR-QoL) Endpoints

The QoL endpoints of this study are depressive symptoms, anxiety, fatigue, sleep, and pain at baseline, Day +28, Day +100, Day +180, and Day +365 post-transplant.

- Depression: 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).[18]
- 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).[18]
- 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.[19, 20] 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).[20] 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.[21, 22]
- Pain: The BPI assesses pain intensity as well as pain-related interference in function.[23, 24] 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.

3.3.13 Microbiome

The gut microbiome taxonomic diversity will be evaluated using 16S ribosomal RNA amplicon sequencing. Data will be generated per standard protocols [91]. Resultant sequencing data will be quality filtered, adapter sequences will be trimmed, and sequences will be analyzed using the QIIME pipeline [92]. Shannon diversity will be calculated [93].

Guidelines for reporting of Depression and/or Suicidal Ideation:

Should the patient endorse any thoughts of suicidality or self harm per the IDAS, the study co-investigator for this portion of the ancillary testing (Dr. Jennifer Knight) will contact them by phone and standard of care referrals will be made. 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.

3.4 Primary Completion

The study will reach primary completion 18 months from the time the study opens to accrual.

3.5 Study Completion

The study will reach study completion 30 months from the time the study opens to accrual.

4 PATIENT SELECTION

4.1 Eligibility Criteria

Patients must have baseline evaluations performed prior to the first study drug dose and must meet all inclusion and exclusion criteria. In addition, the patient must be thoroughly informed

about all study aspects, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to enrollment. The following criteria apply to all patients enrolled onto the study, unless otherwise specified.

4.2 Inclusion Criteria

1. Age ≥18 years.
2. Patients with any hematologic malignancy for which alloHCT is indicated. Patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) must be in complete remission at the time of alloHCT (<5% blasts in the bone marrow, normal maturation of all cellular components in the bone marrow and absence of extramedullary disease).
3. Myeloablative conditioning (MAC) regimen, based on CIBMTR criteria [94]
4. T cell-replete peripheral blood or bone marrow graft.
5. Patients must have a matched related or unrelated donor (at least 6/6 match at HLA-A, -B and -C for related donors and at least 8/8 match at HLA-A, -B, -C and -DRB1 for unrelated donors).
6. Cardiac function: Left ventricular ejection fraction ≥45% for myeloablative conditioning.
7. Estimated creatinine clearance ≥40 mL/minute (using the Cockcroft-Gault formula and actual body weight).
8. Pulmonary function: DLCO ≥40% (adjusted for hemoglobin) and FEV1 ≥50%.
9. Liver function: total bilirubin <3 x upper limit of normal and ALT/AST <5 x upper normal limit.
10. Signed informed consent: Voluntary written consent must be given before patient registration and performance of any study-related procedure not part of standard medical care, with the understanding that consent may be withdrawn by the patient at any time without prejudice to future medical care.

11. Female patient: A negative pregnancy test will be required for women of childbearing potential. Breast-feeding or lactation is not permitted.
12. Planned posttransplant maintenance therapy is allowed.

4.3 Exclusion Criteria

1. Prior allogeneic HCT.
2. Active CNS involvement with malignancy.
3. Patients receiving cord blood or haploidentical allograft.
4. Patients undergoing *in vivo* or *ex vivo* T cell-depleted alloHCT.
5. Karnofsky Performance Score <60%.
6. Patients with uncontrolled bacterial, viral or fungal infections (currently on treatment and with progression of infectious disease or no clinical improvement) at time of enrollment.
7. Active hepatitis B or C virus infection or known human immunodeficiency virus (HIV) positive.
8. Prior intolerance or allergy to tocilizumab.
9. Use of rituximab, alemtuzumab, anti-thymocyte globulin (ATG) or other monoclonal antibody planned as part of conditioning regimen for GVHD prophylaxis.
10. History of diverticulitis, Crohn's disease or ulcerative colitis.
11. History of demyelinating disorder.
12. Any current uncontrolled cardiovascular conditions, including uncontrolled ventricular arrhythmias, NYHA class III or IV congestive heart failure, uncontrolled angina, or electrocardiographic evidence of active ischemia or active conduction system abnormalities.
13. Any serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with the completion of treatment according to this protocol.

5 STUDY ENTRY AND WITHDRAWAL; STUDY PROCEDURES

5.1 Study Entry Procedures

5.1.1 Required Preregistration Screening Tests and Procedures

The study-specific assessments are detailed in this section and outlined in the Study Calendar. A written, signed informed consent form (ICF) must be obtained before any study-specific assessments are initiated. A signed ICF copy will be given to the subject and a copy will be filed in the medical record. The original will be kept on file with the study records.
All patients who are consented will be registered in OnCore®, the MCW Cancer Center Clinical Trial Management System. The system is password protected and meets HIPAA requirements.

5.1.2 Registration Process

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. All source documents that support eligibility include a signed informed consent/HIPAA and signed eligibility checklist. These must be available for review and verification.
At the point of registration, the study staff will register the patient in the electronic database (where applicable), including demographic, consent and on-study information. The patient will be assigned a unique sequence number for the study.
Patients will be enrolled on this trial within 30 days preceding the conditioning regimen for alloHCT.

5.1.3 Pretreatment Period

Pretransplant Evaluations

The following observations must be completed within 30 days before the initiation of the conditioning regimen unless otherwise specified.

- History, physical examination, height and weight.
- Karnofsky performance status and HCT-Specific Comorbidity Index score.
- CBC with differential and comprehensive metabolic panel.
- Fasting lipid panel.
- Infectious disease markers: CMV antibody, Hepatitis panel (HepA Ab, HepB SAb, HepB SAg, HepB Core Ab, HepC Ab), HIV and HTLV I/II antibody.

- LVEF (may be performed >30 days prior to patient enrollment, ECHO or MUGA).
- Pulmonary function tests, including DLCO and FEV1 (may be performed >30 days prior to patient enrollment).
- 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 (may be performed >30 days prior to patient enrollment).
- Pregnancy test per institutional practices for females of childbearing potential. NOTE: pregnancy test must be performed <30 days before initiation of the conditioning regimen. May be serum or urine.
- Research (stool) sample will be collected pre-transplant after admission to the hospital during the (pre-transplant) conditioning period. Research samples will be stored temporarily at MCW (Silvia Munoz-Price lab) and shipped in batches to Memorial Sloan Kettering Cancer Center, New York, NY (Marcel van den Brink lab) for additional downstream transcriptome and volatile metabolite sampling, which requires rapid processing.

5.2 Study Procedures during Treatment

Patients must meet eligibility criteria eligible on Day -1 to be treated.

5.3 Posttransplant

5.3.1 Posttransplant evaluations

Study Visit	Target Day Posttransplant
Baseline*	≤30 days from conditioning
<4 weeks	Day+1; Day+8, Day+15, Day+21
4 weeks	28 ± 7 days (21–35 days)
8 weeks	56 ± 7 days (49–63 days)
100 days	100 ± 14 days (86–114 days)
6 months	180 ± 14 days (166–194 days)

Study Visit	Target Day Posttransplant
12 months	365 ± 28 days (337–393 days)

*Unless specified otherwise

The following observations will be made:

- History and physical exam will be conducted on Days +28, +56, +100, +180, +365 post-HCT.
- History and physical exam and laboratory studies of complete blood count and comprehensive metabolic panel will be conducted, starting day+1, at least three time a week until neutrophil and platelet recovery.
- Assessment for AEs on Days +28, +56, +100, +180 post-transplant.
- Comprehensive metabolic panel on Days +28, +56, +100, +180, +365 post-transplant.
- Fasting lipid panel on Days +56 and +180 post-transplant.
- Chimerism studies performed at Days +28posttransplant. Chimerism will be evaluated in whole blood in fractions including CD3+ and CD33+.
- Disease evaluation of the malignant disease at Days +100, +180 and +365 post-transplant.
- Pulmonary function tests, including DLCO and FEV1 at Days +180 and +365 post-transplant.
- Immune reconstitution panel will be collected at Days +28, +100, +180, and +365.
- Research (stool) samples will be collected post-transplant on a weekly basis until after engraftment and subsequently at the time of onset of acute or chronic GVHD (as shown in study calendar). Research samples will be stored temporarily at MCW (Silvia Munoz-Price lab) and shipped in batches to Memorial Sloan Kettering Cancer Center (Marcel van den Brink lab) for additional downstream transcriptome and volatile metabolite sampling, which requires rapid processing.

The post-transplant investigations will be performed within a window ranging from a week to four weeks before and after the target date, as mentioned in the table above (Section 5.3.1).

5.3.2 GVHD Assessment

Patients will be monitored for development of acute and chronic GVHD, per standard operating procedures at the MCW BMT Program, but at minimum on Days +28 (+/-7), +56 (+/-7), +100 (+/-14), +180 (+/-14), +365 (+/-28) post-HCT. Diagnosis of acute GVHD may not necessitate biopsy confirmation in at least one involved organ, but would be preferred. When more than one organ is involved, biopsy confirmation of all involved organs is recommended but not necessary.

With liver-only GVHD, biopsy confirmation is strongly recommended. Acute GVHD will be assessed by consensus criteria (Appendix 2) [95] and chronic GVHD diagnosis and grading will be according to NIH Criteria [96,97]. Please see Appendix 2.
Independent GVHD adjudication is mandated in the study. Acute and chronic GVHD will be adjudicated by an independent Faculty Research Committee (FRC)-appointed review panel. Protocol PIs will be blinded to the adjudication panel and will not be permitted to grade or modify GVHD assessments. GVHD case report forms (CRFs) will be completed by treating MDs/NPs/APPs in real time, as indicated in study calendar. FRC adjudication panel will grade GVHD, using a calendar-driven approach (Days +100 and +180 for aGVHD and Days +180 and +365 for chronic GvHD).

5.3.3 Clinical Grading of Chronic GVHD (Appendix)

- None.
- Mild chronic GVHD involves only one or two 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 one organ or site with clinically significant but no major disability (maximum score of 2 in any affected organ or site) or (2) three 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.
- Presence of limited or extensive chronic GVHD will be also be recorded.

5.4 Study Withdrawal Procedures

5.4.1 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue for or until:

- Disease progression.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the investigator's judgment.
- Inter-current illness that prevents further treatment administration.

- Patient decides to withdraw from the study.
- Significant patient noncompliance with protocol.
- Unacceptable adverse event(s).

See specifics in 5.5.3.

5.5.2 Patient-Initiated Withdrawal: A patient may decide to withdraw from the study at any time.

5.5.3 Investigator-Initiated Withdrawal: The investigator will withdraw a patient whenever continued participation is no longer in the patient's best interests. Reasons for withdrawing a patient include, but are not limited to, disease progression, the occurrence of an adverse event or a concurrent illness, a patient's request to end participation, a patient's noncompliance as determined by the investigator or simply significant uncertainty on the part of the investigator that continued participation is prudent. There may also be administrative reasons to terminate participation, such as concern about a patient's compliance with the prescribed treatment regimen.

5.5.4 Sponsor-Initiated Withdrawal: Sponsor's decision to discontinue the study.

5.5.5 Withdrawal Documentation Procedure: The reason for study withdrawal and the date the patient was removed from the study must be documented in the case report form.

6 TREATMENT PLAN

6.1 Conditioning Regimens

Eligible patients will receive myeloablative conditioning at the discretion of the treating physician.

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) Formulas:

Males IBW = 50 kg + 2.3 kg/inch over 5 feet.

Females IBW = 45.5 kg + 2.3 kg/inch over 5 feet.

Adjusted Ideal Body Weight Formula:

AIBW = IBW + [(0.40) x (ABW - IBW)]

6.2 Hematopoietic Cell Transplantation

Bone marrow or mobilized peripheral blood graft will be the graft source in this study.

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.

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.

6.2.2 PBPC Infusion

PBPC grafts will be infused 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.

6.3 Tacrolimus/Methotrexate/Tocilizumab

6.3.1 Tacrolimus

Tacrolimus will be given per standard operating procedures from the MCW BMT program, The dose should be adjusted accordingly to maintain a suggested level of 5–10 ng/mL. The dose of tacrolimus may be switched to oral at a 1:3 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.

6.3.2 Methotrexate

Methotrexate will be administered, per institutional practices.. 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.

6.3.3 Tocilizumab

Tocilizumab will be administered intravenously (IV) at a dose of 8 mg/kg (maximum dose of 800 mg) once on the Day -1 approximately 24 hours prior to the estimated time of the hematopoietic cell infusion, and subsequently, on Day +100 (+/- 14 days, i.e., Days +86 to +114) post-alloHCT. The weight used to calculate the dose will be within 7 days of first dose of tocilizumab. Only if the weight has changed >10% (over the baseline weight), then the dosage of tocilizumab (for the second dose) will be changed depending on the weight checked within 14 days prior to the second dose. The infusion will be administered over 60 minutes through a dedicated IV line and must not be administered by IV bolus. Commercially available tocilizumab will be utilized for the study.

6.3.3.1 Contraindications to Tocilizumab dosing on Days +100 (range, Day +86 to +114):

1) Grade 4 (according to CTCAE version 5 hematologic or nonhematologic events (which have life-threatening consequences and require urgent treatment). Dosing will be allowed upon recovery to grade ≤3. Transfusion to increase platelet and hemoglobin is allowed. Grade 3 febrile neutropenia and grade 3 increased AST/ALT/bilirubin are also contraindications to dosing of tocilizumab; recovery to grade 2 or lower would allow for tocilizumab dosing within the time frame of Day +86 through Day +114.
2) Disease (relapse or progression of the primary malignancy) for which treatment is indicated.
3) Donor leukocyte infusion (DLI) prior to planned second dose of tocilizumab for any indication: infection, disease control or to address mixed chimerism.
4) Grade III–IV acute GVHD. Patients with grade I–II acute GVHD and on topical or systemic corticosteroids therapy (equivalent to prednisone ≤20 mg/d) can receive a second dose of tocilizumab.
5) Active chronic GVHD requiring systemic therapy.
6) Acute GVHD treated with tocilizumab before Day +100.

6.4 Supportive Care

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

6.4.1 Growth Factors

The use of G-CSF after alloHCT is allowed, per physician discretion

6.4.2 Prophylaxis against Infections

Patients will receive infection prophylaxis according to standard operating procedures of the MCW BMT program.

6.5 Participant Risks

See section 7.2, known AEs.

6.6. Quality of Life (QoL) Assessments

6.6.1. Instruments

Participants will complete a battery of self-report surveys at the following time points: baseline, Day +28, Day +100, Day +180 and Day +365 post-transplant (total of 5 time points). The 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). QoL measures will be administered to English-speaking patients. All QoL assessments will be completed by patients on a single day, during the routine clinic visit associated with the corresponding study time point. QoL at each time point will be summarized using simple descriptive statistics (mean, SD). Change in patient-reported outcomes from enrollment to Day 100, 6 and 12 months will be calculated. The primary objective is to compare alteration in QoL symptoms between this cohort receiving two doses of tocilizumab and the prior tocilizumab cohort receiving only one dose of tocilizumab. PR-QoL outcomes among survivors at each time point will be compared in an initial analysis using two sample t-statistics. The missing data pattern of the QoL measurements will be examined using graphical techniques and logistic regression models conditional on survival.

Should patients endorse any thoughts of suicidality or self-harm per the IDAS, study Co-I and Psycho-Oncology Medical Director, 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 Hospital Cancer Center.

7 ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS

7.1 Definitions

7.1.1 Adverse Event (AE) and Serious Adverse Events (SAE)

An AE is defined as any abnormal laboratory value(s) or test result(s), even when they do not induce clinical signs or symptoms or require therapy', as per 21 CFR 312.32(a). The investigator and his or her team will follow the Medical College of Wisconsin policies related to adverse event reporting. This information may be found on the Human Research Protection Program website.

Serious AE (SAE) means any untoward medical occurrence that at any dose:

- **Death.** Results in death.
- **Life threatening**. Is life threatening (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- **Hospitalization**. Requires inpatient hospitalization or prolongation of an existing hospitalization.
- **Disability/incapacity**. Results in persistent or significant disability or incapacity. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- **Medically important event**. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent one of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (e.g., prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term severe is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as serious, which is based on patient/event outcome or action

criteria described above and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm3 to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

7.1.2 Unanticipated Problem Involving Risk to Subject or Other (UPIRSO)

The investigator and his or her team will follow the Medical College of Wisconsin policies related to unanticipated problems involving risks to subjects or others. This information may be found on the Human Research Protection Program website.

7.1.3 AE Attribution and Grading

Adverse Event Grading

Grade	Description
0	No AE (or within normal limits).
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local or noninvasive intervention (e.g., packing cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL).
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
4	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE

Adverse Event Attribution

Attribution is an assessment of the relationship between the AE and the medical intervention.

Relationship	Attribution	Description
Unrelated to investigational agent/intervention	Unrelated	The AE *is clearly **NOT** related* to the intervention
	Unlikely	The AE *is **doubtfully related*** to the intervention
Related to investigational agent/intervention	Possible	The AE ***may be related*** to the intervention
	Probable	The AE ***is likely related*** to the intervention
	Definite	The AE ***is clearly related*** to the intervention

Relationship Assessment: In-Depth Definitions

For all collected AEs, the clinician who examines and evaluates the subject will determine the adverse event's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below:

Definitely Related: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Probably Related: There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time sequence to administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

Possibly Related: There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse drug event may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

Unlikely: A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other

drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).

Unrelated: The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

7.2 Known AEs List

7.2.1 Tacrolimus

Tacrolimus side effects include:

- Cardiovascular: hypertension
- Neurologic: confusion, dizziness, insomnia, seizures, tremors, changes in how clearly one can think
- Gastrointestinal: nausea, vomiting
- Hematologic: microangiopathic hemolytic anemia, thrombocytopenia
- Endocrine and metabolic: hypomagnesemia, hypokalemia, hypocalcemia, hyperlipidemia
- Miscellaneous: unwanted hair growth, changes in vision, liver problems, reversible renal insufficiency, infections and posttransplant lymphoproliferative disorders

7.2.2 Methotrexate

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

- Neurologic: fever, dizziness, chills, undue fatigue
- Gastrointestinal: ulcerative stomatitis, nausea, abdominal distress, diarrhea
- Hematologic: leucopenia, anemia and suppressed hematopoiesis (leading to infection)
- Miscellaneous: abnormal liver tests, kidney failure and pulmonary complications after transplantation

7.2.3 Tocilizumab

Tocilizumab side effects include:

- Hypersensitivity reactions

- Respiratory: upper respiratory tract infections, nasopharyngitis, bronchitis
- Gastrointestinal: mouth ulceration, upper abdominal pain, gastritis, gastrointestinal perforations, pancreatitis.
- Hepatic: transaminases elevation
- Neurologic: headache, dizziness
- Cardiovascular: hypertension
- Dermatologic: skin rash
- Serious infections (See Section 8.1.5)

7.3 Time Period and Grade of AE Capture

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

7.4 Monitoring and Recording an Adverse Event

Definition. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE.

Reporting source. AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination or other diagnostic procedures.

Prior to the trial. Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned).

Pretreatment events following signed informed consent. For serious pretreatment events, the investigator must determine both the intensity of the event and the relationship of the event to study procedures.

Treatment events. For serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

Not serious AEs. For non-serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

Follow-up of Adverse Events

All adverse events will be followed with appropriate medical management until Day+180 or until they are resolved, if they are related to the study treatment.

7.4.1 Procedure for Reporting Drug Exposure during Pregnancy and Birth Events

If a woman becomes pregnant, or suspects that she is pregnant, while participating in this study, she must inform the investigator immediately and permanently discontinue the study drug. The sponsor-investigator must notify the DSMC by email. The pregnancy must be followed for the final pregnancy outcome.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the sponsor-investigator must also immediately notify the DSMC by email. Every effort should be made to follow the pregnancy for the final pregnancy outcome.

7.4.2 Subject Complaints

If a complaint is received by anyone on the study staff, it will be discussed with the study staff and will be addressed on a case-by-case basis. The PI will be notified of any complaints. Complaints will be reported to the IRB if indicated.

If the subject has questions about his or her rights as a study subject, wants to report any problems or complaints, obtain information about the study or offer input, the subject can call the Medical College of Wisconsin/Froedtert Hospital research subject advocate at 414-955-8844. This information is provided to the subject in their consent.

A product complaint is a verbal, written or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality or stability of a drug product. Study staff, who identify a potential product complaint situation should immediately contact the sponsor and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a sponsor representative. Product complaints in and of themselves are not Reportable Events. If a product complaint results in an SAE, an SAE form should be completed.

7.4.3 Routine Reporting Procedures for AEs

Expedited Reporting Procedures for SAEs, SARs, UPIRSOs and DLTs.

Since this is an investigator-initiated study, the principal investigator, also referred to as the sponsor-investigator, is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the sponsor-investigator's IRB.

Signs or symptoms reported as adverse events will be graded and recorded by the investigator, according to the CTCAE. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

The investigator will assess all adverse events and determine reporting requirements to the Medical College of Wisconsin Cancer Center (MCWCC) DSMC and MCW IRB.

Only grade 1 and 2 adverse events related to the study drug and all grade 3, 4 and 5 adverse events regardless of attribution will be entered into OnCore®.

Reporting to the Data and Safety Monitoring Committee (DSMC)

Regardless of the causality, all unexpected grade 3, and all grade 4, and 5 SAEs, as well as any others requiring expedited reporting as defined in this protocol must be reported to the MCWCC DSMC within five calendar days of study staff's knowledge.

Report Method: The investigator will use email to report SAEs and applicable AE to the DSMC. The SAE report must include event term(s), serious criteria, and the sponsor-investigator's or sub-investigator's determination of both the intensity of the event(s) and the relationship of the event(s) to study drug administration. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE v5 as a guideline whenever possible.

The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

Reporting to MCW Institutional Review Board (IRB)

The principal investigator must report events to the MCW IRB within five business days of his awareness of the event.

[Guidance on Adverse Event Reporting to the IRB is available online at MCW IRB Policies and Procedures.]

Event Type	Report Recipients					
	PI/Study Chair/ Coordinating Center	Institutional Review Board	DSMC	FDA (if applicable)	CTO Regulatory Office	Other
Serious Adverse Event	ASAP	5 days (or annual CPR)[1]	5 days	7 or 15 days[2]	ASAP	
Unanticipated Problems Involving Risks to Subjects of Others	ASAP	5 days (or annual CPR)[1]	5 days	7 or 15 days[2]	ASAP	
Evidence of Causal Relationship between Drug and AE	ASAP	5 days (or annual CPR)[1]	5 days	7 or 15 days[2]	ASAP	
Dose-Limiting Toxicity	ASAP	5 days (or annual CPR)[1]	5 days	7 or 15 days[2]	ASAP	

Contacts

Role	Name	Entity/Department	Institution	Telephone	Email
Sponsor-Investigator	Saurabh Chhabra, MD	Hematology/Oncology	MCW	414-805-0578	schhabra@mcw.edu

Footnotes

[1] Consult MCW IRB Policies (contact your regulatory representative)

[2] FDA guidelines: Suspected adverse reaction, Unexpected and Serious = 7 Days; If not = 15 days

8 PHARMACEUTICAL INFORMATION

8.1 Actemra® (Tocilizumab)

8.1.1 Product Description

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

Classification: Immunomodulatory.

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.

Pharmacokinetics:

Pharmacokinetic studies indicate that tocilizumab undergoes biphasic elimination from the circulation. In rheumatoid arthritis patients treated with 4 and 8 mg/kg every four 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 an 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 nonlinear 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 four weeks at steady state.

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.

Contraindications:

Side Effects: Complete and updated adverse event information is available in the Investigational Drug Brochure and/or product package insert.

Tocilizumab side effects include:

- Hypersensitivity reactions
- 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
- Serious infections (See Section 8.1.5)

8.1.2 Solution 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.

8.1.3 Route of Administration

The infusion will be administered over 60 minutes through a dedicated IV line and must not be administered by IV bolus.

8.1.4 Storage Requirements

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. Do not use beyond the expiration date on the container, package or prefilled syringe.
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.

8.1.5 Warnings and Precautions

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. If a serious infection develops, tocilizumab should be withheld until the infection is controlled.

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.

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.

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 CYPs 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 two weeks of starting therapy and persist for several weeks after stopping therapy.

8.1.6 Availability

This drug is commercially available, but will be paid for by the study.

8.1.7 Investigator and Site Responsibility for Drug Accountability.

Accountability for the study drug is the responsibility of the principal investigator/designee. The investigator will ensure that the drug is used only in accordance with this protocol. Drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each patient, and disposal of the drug (if applicable) will be maintained by the clinical site. Accountability records will include dates, quantities, lot numbers, expiration dates (if applicable), and patient numbers.

9 STATISTICAL CONSIDERATIONS

9.0 Study Populations

The Full analysis set (FAS) will consist of all eligible patients enrolled in the study. This population will be used for the efficacy analyses.

The Safety set (SS) will consist of all eligible patients enrolled in the study who received at least one dose of the study medication.

9.1 Study Endpoints

Analysis of Primary Endpoint

The primary analysis will be performed on the FAS population using the intention-to-treat principle including all patients enrolled in the trial. The GRFS will be estimated, using the Kaplan-Meier estimator and plotted with a 95% confidence band. An event for this outcome is defined as grade III–IV acute GVHD, chronic GVHD requiring systemic therapy, relapse or death. Patients who are alive without GVHD will be censored at the last follow-up. The 12-month GRFS will be compared to the prespecified historical control value of 20%, using a one-sided z-test.

Analysis of Secondary Endpoints

Demographic and other baseline data, such as disease characteristics, as well as outcome measures, will be presented overall and separately for URD and MRD patients. Categorical data, such as gender, race, etc., 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.
Time-to-event outcomes with and without competing risks will be analyzed, using Kaplan-Meier and Nelson-Aalen estimates, respectively, and presented with 95% confidence intervals. Binary outcomes will be analyzed, using proportions with 95% confidence intervals.

Effect of GVHD prophylaxis on microbiome diversity: Difference in the level of microbiome diversity detected at pre- and post-transplant. Microbiome diversity will be compared at each time point using Mann-Whitney tests. Association of pre-transplant microbiome diversity with development of aGVHD, cGVHD, and overall survival (corrected for GVHD prophylaxis): This analysis will use the pre-transplant stool microbiome cohort; diversity will be grouped by tertiles or if a more appropriate classification is identified in the analysis, that may be used instead. Acute GVHD (grade 2-4 or 3-4) and chronic GVHD (any or moderate-severe) will be described in each group using cumulative incidence with death or relapse as a competing event, and compared between groups using Gray's test. Overall survival will be described using the Kaplan-Meier estimator and compared between groups using the log-rank test. Multivariate models will be constructed in a similar manner as for the primary endpoint using Fine and Gray models for aGVHD and cGVHD, and using Cox models for OS.

Missing data

Patients who are lost to follow-up will be censored at the day of the last contact with known status as appropriate for each outcome (for example, last day known alive for overall survival, and last day known alive without relapse or GVHD for GRFS).

9.2 Study Design

The study is designed as a phase II, open-label, single-center trial to evaluate a novel GVHD prophylaxis regimen, Tac/MTX/tocilizumab. The primary endpoint of the study is GRFS, GVHD/ relapse-free survival at 12 months posttransplant.

9.3 Accrual Rate

It is estimated that 18 months of accrual will be adequate to enroll the targeted sample size.

9.4 Sample Size Justification

The sample size was selected to achieve 80% power to detect an increase in GRFS at 12 months to 40% compared with an historical control value of 20% at a one-sided 5% significance level. Based on asymptotic z-test for proportions, 29 patients with a known outcome are needed (i.e., known GVHD, relapse or death status at 12 months). We will recruit 32 subjects to allow for approximately 10% loss to follow-up by 1 year. Patients who have received at least one dose of tocilizumab would still be followed if they are withdrawn from the study.

9.5 Stopping Rules

Formal statistical toxicity monitoring will be in place for 100-day overall mortality. Based on program experience, a 100-day mortality rate <10% is expected. The monitoring boundary was selected to provide a 10% probability of declaring excessive mortality, if the true probability is 10%. The following table shows the number of 100-day mortality events that would trigger a safety review depending on the number of evaluable patients. For the purposes of the safety monitoring patients in the safety set who are alive with at least 100 days of follow-up or have died within 100 days are considered evaluable.

Number of Evaluable Patients	Boundary for excessive 100-day mortality
1-2	-
3-10	3+
11-15	4+
16-20	5+
21-25	6+
26-29	7+

The following table shows the probability of finding excessive 100-day mortality during the study for several underlying true values of 100-day mortality rate.

100-day mortality rate	Probability of crossing boundary
5%	1.5%
10%	11%
20%	55%
25%	76%
30%	89%

10 DATA AND SAFETY MONITORING PLAN (DSMP)

Data and Safety Management Overview

The MCWCC DSMC and the MCW IRB will approve protocol-specific DSM plans. A local, investigator-initiated trial will be required to be continuously monitored by the principal investigator of the study with safety and progress reports submitted to the DSMC.
The DSMP for this study will involve the following entities:

10.1 Study Team

The study team minimally consists of the principal investigator, the clinical research coordinator, regulatory specialist and the study biostatistician. While subjects are on treatment, the principal investigator will meet regularly with the research coordinator and the study biostatistician to review study status. This review will include but not be limited to reportable SAEs and UPIRSOs and an update of the ongoing study summary that describes study progress in terms of the study schema. The appropriateness of further subject enrollment and the specific intervention for a next subject enrollment is addressed. All meetings including attendance are documented.

10.2 Quality Assurance

The MCWCC Clinical Trials Office (MCWCC CTO) provides ongoing quality assurance audits. Quality Assurance: This protocol was classified as intermediate risk and will be reviewed internally by the MCW Cancer Center Clinical Trials Office Quality Assurance Staff according to the current version SOP, 6.5.2 Internal Quality Assurance Reviews.

10.3 Clinical Trials Office

The MCWCC CTO provides administrative assistance and support to the DSMC.

10.4 DSMC

The MCWCC places the highest priority on ensuring the safety of patients participating in clinical trials. Every cancer interventional trial conducted at MCW includes a plan for safety and data monitoring.
More information can be found related to the MCWCC Data and Safety Monitoring Plan at the MCWCC website (Data and Safety Monitoring Plan).
This study will be reviewed by the MCWCC DSMC. A summary of the MCWCC DSMC activities are as follows:

- Review the clinical trial for data integrity and safety
- Review all unexpected grade 3, and all grade 4, and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol (Grades 4 and 5 events must be reported to the DSMC within five calendar days of study staff's knowledge.)
  Grade ≤4 hematological AEs occurring within the first 28 days post-transplant will be routine reported to the DSMC.
- 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 MCWCC 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.

11 REGULATORY COMPLIANCE, ETHICS AND STUDY MANAGEMENT

11.1 Ethical Standard

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements.

11.2 Regulatory Compliance

This study will be conducted in compliance with:

- The protocol
- Federal regulations, as applicable, including: 21 CFR 50 (Protection of Human Subjects/Informed Consent); 21 CFR 56 (Institutional Review Boards) and §312 (Investigational New Drug Application; and 45 CFR 46 Subparts A (Common Rule), B (Pregnant Women, Human Fetuses and Neonates), C

(Prisoners), and D (Children), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

11.3 Prestudy Documentation

Prior to implementing this protocol at MCWCC, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the MCW IRB.

11.4 Institutional Review Board

The protocol, the proposed informed consent form and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the MCW Institutional Review Board. Prior to obtaining approval, the protocol must be approved by the Medical College of Wisconsin Cancer Center Scientific Review Committee. The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the study interventions/products, study procedures and risks are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study product.

Consent forms will be IRB approved and the subject (and Legally Authorized Representative, if necessary) will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. In accordance with 46 CR 46.111, the subject will sign and date the informed consent document prior to any procedures being done specifically for the study.

A witness should only sign when required, per FH/MCW IRB policy. If a witness signs the document when not required, the study staff should document in the legal medical record (or note to file) the relationship to the patient and why a witness signed. (i.e., "Although not required, the subject's spouse was present during the consenting process and signed as the witness." Or "Although not required, hospital staff was present for consenting process and signed as a witness.")

The subjects will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the trial.

A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. If there are changes to the consent form, all revisions will be reviewed with study subject at the next appropriate opportunity. Patients that require reconsenting will be defined in the IRB approved amendment submission. The process for obtaining informed

consent will again be performed. Study subjects will not be reconsented for continuing reviews. The MCWCC CTO will follow the MCW/FH IRB's policy for subjects who demonstrate limited English proficiency or limited literacy.

After the subject's visit in which the consent is signed, it is documented in the clinic chart that the consent has been signed and that all questions have been answered to the subject's satisfaction after adequate time for review of the consent. It is also documented that a copy of the consent is given to the subject. The original consent is kept with the subject's study file, and a copy of the consent is sent to the OCRICC office, which will then submit to HIM a copy of the signed consent to be scanned into EPIC, the legal medical record.

11.5 Subject Confidentiality and Access to Source Documents/Data

Subject confidentiality is strictly held in trust by the sponsor-investigator, participating investigators, and any staff, [and the sponsor(s) and their agents]. This confidentiality includes the clinical information relating to participating subjects, as well as any genetic or biological testing.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the principal investigator.

The conditions for maintaining confidentiality of the subjects' records are required for the life of the data. These rules apply equally to any and all MCWCC projects.

One risk of taking part in a research study is that more people will handle the personal health information collected for this study. The study team will make every effort to protect the information and keep it confidential, but it is possible that an unauthorized person might see it. Depending on the kind of information being collected, it might be used in a way that could embarrass the subject or affect his/her ability to get insurance.

While data are being collected and after all data have been collected but are still in the process of being analyzed, the subject's data/PHI are stored in the locked Clinical Research office in the Clinical Trials Office. Databases in which the study subject information is stored and accessed are password protected, allowing for limited access by authorized personnel only. Data/PHI kept in the Case Report Forms contain the study identifiers, subject initials, date of birth and date of service.

The principal investigator will allow access to all source data and documents for the purposes of monitoring, audits, IRB review and regulatory inspections.

The study monitor or other authorized representatives of the principal investigator may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

11.6 Protection of Human Subjects

11.6.1 Protection from Unnecessary Harm

Each clinical site is responsible for protecting all subjects involved in human experimentation. This is accomplished through the IRB mechanism and the informed consent process. The IRB reviews all proposed studies involving human experimentation and ensures that the subject's rights and welfare are protected and that the potential benefits and/or the importance of the knowledge to be gained outweigh the risks to the individual. The IRB also reviews the informed consent document associated with each study in order to ensure that the consent document accurately and clearly communicates the nature of the research to be done and its associated risks and benefits.

11.6.2 Protection of Privacy

As noted, patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign informed consent documents. The original signed document will become part of the patient's medical records, and each patient will receive a copy of the signed document.

11.7 Changes in the Protocol

Once the protocol has been approved by the MCW IRB, any changes to the protocol must be documented in the form of an amendment. The amendment must be signed by the investigator and approved by IRB prior to implementation.

If it becomes necessary to alter the protocol to eliminate an immediate hazard to patients, an amendment may be implemented prior to IRB approval. In this circumstance, however, the Investigator must then notify the IRB in writing within five working days after implementation.

The IRB may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the IRB. The investigator will submit all protocol modifications to the sponsor and the regulatory authority(ies) in accordance with the governing regulations.

Changes to the protocol may require approval from the sponsor.

Any departures from the protocol must be fully documented in the source documents.

11.8 Investigator Compliance

The investigator will conduct the study in compliance with the protocol given approval/favorable opinion by the IRB and the appropriate regulatory authority(ies).

<u>Onsite Audits</u>

Auditing is essential to ensure that research conducted at the Medical College of Wisconsin (MCW) Cancer Center is of the highest quality and meets MCW and regulatory agency standards.

Regulatory authorities, the IRB and/or sponsor may request access to all source documents, data capture records and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

12 DATA HANDLING AND RECORD KEEPING

12.1 Overview

Every effort is made to uphold the integrity of the project, the research, the institution, and the researchers involved. Data collection guidelines and methodologies are carefully developed before the research begins. Investigators focus on the following to ensure data integrity: well-trained data collectors/recorders to ensure consistency and quality, well-designed data collection protocols and ongoing monitoring. In this way, study rigor and validity are maintained. Data is protected from physical damage as well as from tampering, loss or theft.

12.2 Data Management Responsibilities

This project's data management is a multidisciplinary activity that includes investigators, research coordinators and nurses, data mangers, support personnel, biostatisticians and database programmers. Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

12.2.1 Principal Investigator

The principal investigator oversees the management of patient records/case report forms and ensures that a) complete and accurate data will be obtained and provided to the sponsor; b) patient records are maintained to include history, prescribed medication and investigational product(s), measurements, exams, evaluations and adverse events; c) corrections are applied to clinical research data according to principles of good research practice (i.e., single-line delete, date and initial). He or she will ensure that there is correlation between the case report forms and the source documents**.**

12.2.2 Research Coordinator

A research coordinator creates, collects, and organizes clinical trial documentation. He or she ensures that source documentation and data abstraction and entry are being done at protocol specified time points.

12.2.3 Research Nurse/Medical Staff

The research nurse and medical staff documents protocol-required care or assessment of the subject's outcomes, adverse events and compliance to study procedures.

12.2.4 Biostatistician

The biostatistician may assist in CRF development (content and design), dataset specifications (annotation of CRFs and record layout) and validation.

12.3 Handling and Documentation of Clinical Supplies

The MCWCC principal investigator will maintain complete records showing the receipt, dispensation, return, or other disposition of all investigational drugs. The date, quantity and batch or code number of the drug, and the identification of patients to whom study drug has been dispensed by patient number and initials will be included. The sponsor-investigator will maintain written records of any disposition of the study drug.
The principal investigator shall not make the investigational drug available to any individuals other than to qualified study patients. Furthermore, the principal investigator will not allow the investigational drug to be used in any manner other than that specified in this protocol.

12.4 Source Documents

Source documents for clinical information (patient history, diagnosis, clinical and diagnostic test reports, etc.) are maintained in the patient's clinical file.

All source documents will be written following ALCOA standards:

ALCOA Attribute	Definition
Attributable	Clear who has documented the data.
Legible	Readable and signatures identifiable.
Contemporaneous	Documented in the correct time frame along with the flow of events. If a clinical observation cannot be entered when made, chronology should be recorded. Acceptable amount of delay should be defined and justified.
Original	Original, if not original should be exact copy; the first record made by the appropriate person. The investigator should have the original source document.
Accurate	Accurate, consistent and real representation of facts.
Enduring	Long-lasting and durable.
Available and accessible	Easily available for review by treating physicians and during audits/inspections. The documents should be retrievable in reasonable time.
Complete	Complete until that point in time.
Consistent	Demonstrate the required attributes consistently.
Credible	Based on real and reliable facts.
Corroborated	Data should be backed up by evidence.

12.5 Case Report Forms

The principal investigator and/or his/her designee will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study-specific Case Report Forms (CRFs) will document safety and treatment outcomes for safety monitoring and data analysis. All study data will be entered into OnCore® via standardized CRFs, in accordance with the study calendar, using single data entry with a secure access account. The Clinical Research Coordinator will complete the CRFs as soon as possible upon completion of the study visit; the investigator will review and approve the completed CRFs.

The information collected on CRFs shall be identical to that appearing in original source documents. Source documents will be found in the patient's medical records maintained by MCWCC personnel. All source documentation should be kept in separate research folders for each patient.

In accordance with federal regulations, the Investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered onto CRFs. The principal investigator will approve all completed CRFs to attest that the information contained on the CRFs is true and accurate.

All source documentation and data will be available for review/monitoring by the MCWCC DSMC and regulatory agencies.

12.6 Study Record Retention

The principal investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity and use by subjects, as well as written records of the disposition of the drug when the study ends.

The principal investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

Study documentation includes all CRFs, data correction forms or queries, source documents, sponsor-investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

In accordance with FDA regulations, the investigator shall retain records for a period of two years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until two years after the investigation is discontinued and FDA is notified.

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APPENDIX 1. *PERFORMANCE STATUS CRITERIA*

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity Fully active, able to carry on all pre-disease performance without restriction	100	Normal, no complaints, no evidence of disease
		90	Able to carry on normal activity; minor signs or symptoms of disease
1	Symptoms, but ambulatory Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work)	80	Normal activity with effort; some signs or symptoms of disease
		70	Cares for self, unable to carry on normal activity or to do active work
2	In bed <50% of the time Ambulatory and capable of all self-care, but unable to carry out any work activities Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs
		50	Requires considerable assistance and frequent medical care
3	In bed >50% of the time Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	40	Disabled, requires special care and assistance
		30	Severely disabled, hospitalization indicated Death not imminent
4	100% bedridden Completely disabled Cannot carry on any self-care Totally confined to bed or chair	20	Very sick, hospitalization indicated Death not imminent
		10	Moribund, fatal processes progressing rapidly
5	Dead	0	Dead

APPENDIX 2. *GVHD ASSESSMENT*

ACUTE GVHD ASSESSMENT

Clinical Acute GVHD Assessment

Date _____ Patient ID _____ Karnofsky/Lansky _____

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

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

Code Definitions:

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

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

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

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

Signature _____

STAGING AND GRADING OF ACUTE GVHD

Staging

Stage	Skin	GI	Liver
1	< 25% rash	Diarrhea > 500ml/d or persistent nausea	Bilirubin 2-3mg/dl
2	25-50%	> 1000 ml/d	Bilirubin 3-6 mg/dl
3	> 50%	> 1500 ml/d	Bilirubin 6-15 mg/dl
4	Generalized erythroderma with bullae	Large volume diarrhea and severe abdominal pain ± ileus	Bilirubin > 15 mg/dl

Grading of Acute GVHD

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

Grading of Chronic GVHD (NIH Criteria)

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capabl of self-care, >50% of waking hours ou 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† **SCORE % BSA** ☐ *GVHD features to be scored by BSA:* **Check all that apply:** ☐ Maculopapular rash/erythema ☐ Lichen planus-like features ☐ Sclerotic features ☐ Papulosquamous lesions or ichthyosis ☐ Keratosis pilaris-like GVHD	☐ No BSA involved	☐ 1-18% BSA	☐ 19-50% BSA	☐ >50% BSA
SKIN FEATURES SCORE:	☐ No sclerotic features		☐ Superficial sclerotic features "not hidebound" (able to pinch)	**Check all that apply:** ☐ Deep sclerotic features ☐ "Hidebound" (unable to pinch) ☐ Impaired mobility ☐ Ulceration

Other skin GVHD features (NOT scored by BSA)
Check all that apply:
☐ Hyperpigmentation
☐ Hypopigmentation
☐ Poikiloderma
☐ Severe or generalized pruritus
☐ Hair involvement
☐ Nail involvement
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____

MOUTH *Lichen planus-like features present:* ☐ **Yes** ☐ **No**	☐ 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

☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist:* ☐ **Yes** ☐ **No** ☐ **Not examined**	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	☐ Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
GI Tract ***Check all that apply:*** ☐ Esophageal web/ proximal stricture or ring ☐ Dysphagia ☐ Anorexia ☐ Nausea ☐ Vomiting ☐ Diarrhea ☐ Weight loss ≥5%* ☐ Failure to thrive	☐ No symptoms	☐ Symptoms without significant weight loss* (<5%)	☐ Symptoms associated with mild to moderate weight loss* (5-15%) **OR** moderate diarrhea without significant interference with daily living	☐ Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
LIVER	☐ Normal total bilirubin and ALT or AP < 3 x ULN	☐ Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥ 3 x ULN	☐ Elevated total bilirubin but ≤3 mg/dL or ALT > 5 ULN	☐ Elevated total bilirubin > 3 mg/dL
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
LUNGS**				
Symptom score:	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring 0_2)
Lung score: **%** FEV1 ☐	☐ FEV1≥80%	☐ FEV1 60-79%	☐ FEV1 40-59%	☐ FEV1 ≤39%
Pulmonary function tests ☐ Not performed				
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	☐ 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 (*See Supplemental figure*‡) ☐ Not examined *Currently sexually active* ☐ Yes ☐ No	☐ No signs	☐ Mild signs‡ and females with or without discomfort on exam	☐ Moderate signs‡ and may have symptoms with discomfort on exam	☐ Severe signs‡ with or without symptoms

☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____

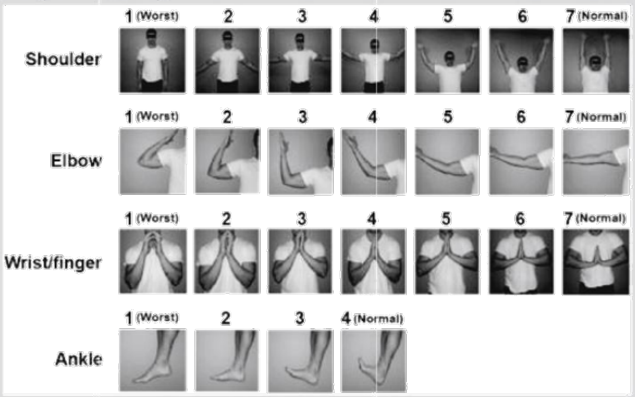
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____

Other indicators, clinical features or complications related to chronic GVHD (check all that apply and assign a score to severity (0-3) based on functional impact where applicable none – 0,mild -1, moderate -2, severe – 3)

☐ Ascites (serositis)___	☐ Myasthenia Gravis___	
☐ Pericardial Effusion___	☐ Peripheral Neuropathy___	☐ Eosinophilia > 500/µl___
☐ Pleural Effusion(s)___	☐ Polymyositis___	☐ Platelets <100,000/µl ___
☐ Nephrotic syndrome___	☐ Weight loss>5%* without GI symptoms ___	☐ Others (specify):_____

Overall GVHD Severity *(Opinion of the evaluator)* ☐ **No GVHD** ☐ **Mild** ☐ **Moderate** ☐ **Severe**

Photographic Range of Motion (P-ROM)



APPENDIX 3*: QOL MEASURES*

QOL MEASURES

Participant # _____ Date: _____

IDAS

Instructions: Below is a list of feelings, sensations, problems, and experiences that people sometimes have. Read each item to determine how well it describes your recent feelings and experiences. Then select the option that best describes **how much** you have felt or experienced things this way **during the past two weeks, including today**.

	Not at all	A little bit	Moderately	Quite a bit	Extremely
1. I was proud of myself……..	1	2	3	4	5
2. I felt exhausted…….	1	2	3	4	5
3. I felt depressed…….	1	2	3	4	5
4. I felt inadequate……	1	2	3	4	5
5. I slept less than usual……	1	2	3	4	5
6. I felt fidgety, restless……	1	2	3	4	5
7. I had thoughts of suicide …….....	1	2	3	4	5
8. I slept more than usual…….	1	2	3	4	5
9. I hurt myself purposely …….	1	2	3	4	5
10. I slept very poorly……..	1	2	3	4	5
11. I blamed myself for things	1	2	3	4	5
12. I had trouble falling asleep	1	2	3	4	5
13. I felt discouraged about things……	1	2	3	4	5
14. I thought about my own death	1	2	3	4	5
15. I thought about hurting myself	1	2	3	4	5
16. I did not have much of an appetite	1	2	3	4	5

17. I felt like eating less than usual	1	2	3	4	5
19. I did not feel much like eating……	1	2	3	4	5
21. I felt optimistic……	1	2	3	4	5
23. I felt that I had accomplished a lot	1	2	3	4	5
24. I looked forward to things with enjoyment	1	2	3	4	5
25. I was furious……	1	2	3	4	5
26. I felt hopeful about the future	1	2	3	4	5
27. I felt that I had a lot to look forward to	1	2	3	4	5
28. I felt like breaking things	1	2	3	4	5
29. I had disturbing thoughts of something bad that happened to me	1	2	3	4	5
30. Little things made me mad	1	2	3	4	5
31. I felt enraged……	1	2	3	4	5
32. I had nightmares that reminded me of something bad that happened	1	2	3	4	5
33. I lost my temper and yelled at people…….	1	2	3	4	5
34. I felt like I had a lot of interesting things to do.	1	2	3	4	5
35. I felt like I had a lot of energy	1	2	3	4	5
36. I had memories of something scary that happened	1	2	3	4	5
37. I felt self-conscious knowing that others were watching me	1	2	3	4	5
38. I felt a pain in my chest …….	1	2	3	4	5
39. I was worried about embarrassing myself socially	1	2	3	4	5
40. I felt dizzy or light headed	1	2	3	4	5
41. I cut or burned myself on purpose	1	2	3	4	5

42. I had little interest in my usual hobbies or activities	1	2	3	4	5
43. I thought that the world would be better off without me…….	1	2	3	4	5
44. I felt much worse in the morning than later in the day	1	2	3	4	5
45. I felt drowsy, sleepy……	1	2	3	4	5
46. I woke up early and could not get back to sleep…….	1	2	3	4	5
47. I had trouble concentrating	1	2	3	4	5
48. I had trouble making up my mind	1	2	3	4	5
49. I talked more slowly than usual	1	2	3	4	5
50. I had trouble waking up in the morning…….	1	2	3	4	5
51. I found myself worrying all the time	1	2	3	4	5
52. I woke up frequently during the night	1	2	3	4	5
53. It took a lot of effort for me to get going	1	2	3	4	5
54. I woke up much earlier than usual	1	2	3	4	5
55. I was trembling or shaking……..	1	2	3	4	5
56. I became anxious in a crowded public setting	1	2	3	4	5
57. I felt faint…….	1	2	3	4	5
58. I found it difficult to make eye contact with people	1	2	3	4	5
59. My heart was racing or pounding	1	2	3	4	5
60. I got upset thinking about something bad that happened	1	2	3	4	5
61. I found it difficult to talk with people I did not know well	1	2	3	4	5
62. I had a very dry mouth…….....	1	2	3	4	5
63. I was short of breath……	1	2	3	4	5

64. I felt like I was choking…….. 1 2 3 4 5

FSI

Instructions: The following questions ask about fatigue during the **past week**. For each of the following, circle the one number that best indicates how that item applies to you.

1. Rate your level of fatigue on the day you felt **most** fatigued during the past week.

0	1	2	3	4	5	6	7	8	9	10
Not at all fatigued										As fatigued as I could be

2. Rate your level of fatigue on the day you felt **least** fatigued during the past week.

0	1	2	3	4	5	6	7	8	9	10
Not at all fatigued										As fatigued as I could be

3. Rate your level of fatigue on the **average** in the last week.

0	1	2	3	4	5	6	7	8	9	10
Not at all fatigued										As fatigued as I could be

4. Rate your level of fatigue **right now**.

0	1	2	3	4	5	6	7	8	9	10
Not at all fatigued										As fatigued as I could be

5. Rate how much, **in the past week**, fatigue interfered with:

	No interference										Extreme interference
a. Your general level of activity.....	0	1	2	3	4	5	6	7	8	9	10
b. Your ability to bathe and dress yourself.....	0	1	2	3	4	5	6	7	8	9	10
c. Your normal work activity (includes both work outside the home and housework)	0	1	2	3	4	5	6	7	8	9	10
d. Your ability to concentrate	0	1	2	3	4	5	6	7	8	9	10
e. Your relations with other people.....	0	1	2	3	4	5	6	7	8	9	10
f. Your enjoyment of life	0	1	2	3	4	5	6	7	8	9	10
g. Your mood.....	0	1	2	3	4	5	6	7	8	9	10

6. Indicate **how many days**, in the past week, you felt fatigued for any part of the day:

0	1	2	3	4	5	6	7

7. Rate **how much of the day**, on average, you felt fatigued in the past week

0	1	2	3	4	5	6	7	8	9	10
None of the day										The entire day

PSQI

Instructions: The following questions relate to your usual sleep habits during the **past month only**. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the **past month**, what time have you usually gone to bed at night?

BED TIME _____

2. During the **past month,** how long (in minutes) has it usually taken you to fall asleep each night?

NUMBER OF MINUTES _____

3. During the **past month**, what time have you usually gotten up in the morning?

GETTING UP TIME _____

4. During the **past month**, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.)

HOURS OF SLEEP PER NIGHT _____

For each of the remaining questions, check the one best response. Please answer all questions.

5. During the **past month**, how often have you had trouble sleeping because you . . .

a) Cannot get to sleep within 30 minutes

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

b) Wake up in the middle of the night or early morning

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

c) Have to get up to use the bathroom

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

d) Cannot breathe comfortably

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

e) Cough or snore loudly

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

f) Feel too cold

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

g) Feel too hot

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

h) Had bad dreams

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

i) Have pain

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

j) Other reason(s), please describe_____
_____

How often during the past month have you had trouble sleeping because of this?

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

6. During the **past month**, how would you rate your sleep quality overall?

Very good	_____
Fairly good	_____
Fairly bad	_____
Very bad	_____

7. During the **past month**, how often have you taken medicine to help you sleep (prescribed or "over the counter")?

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

8. During the **past month**, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

Not during the past month _____	Less than once a week _____	Once or twice a week _____	Three or more times a week _____

9. During the **past month**, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

No problem at all	_____
Only a very slight problem	_____
Somewhat of a problem	_____
A very big problem	_____

BPI

Instructions: The following questions ask about pain during the **past week**. For each of the following, circle the one number that best indicates how that item applies to you.

1. Please rate your pain by circling the number that best describes your pain at its **worst** in the last week.

0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

2. Please rate your pain by circling the number that best describes your pain at its **least** in the last week.

0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

3. Please rate your pain by circling the number that best describes your pain on the **average** in the last week.

0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

4. Please rate your pain by circling the number that best describes your pain **right now**.

0	1	2	3	4	5	6	7	8	9	10
No pain										Pain as bad as you can imagine

5. Circle the number that describes how, **during the past week**, pain has interfered with your:

	Does not interfere									Completely interferes
a. General activity	0	1	2	3	4	5	6	7	8	9	10
b. Mood	0	1	2	3	4	5	6	7	8	9	10
c. Walking ability	0	1	2	3	4	5	6	7	8	9	10
d. Normal work (includes both work outside the home and housework)	0	1	2	3	4	5	6	7	8	9	10
e. Relations with other people	0	1	2	3	4	5	6	7	8	9	10
f. Sleep	0	1	2	3	4	5	6	7	8	9	10
g. Enjoyment of life	0	1	2	3	4	5	6	7	8	9	10

THESE QUESTIONS NEED ONLY BE ANSWERED ONCE; NO NEED TO ANSWER AT SUBSEQUENT STUDY FOLLOW-UP VISITS:

Please indicate below what best represents your household income:

_____ <$10,000
_____ $10,001-$25,000
_____ $25,001-$40,000
_____ $40,001-$55,000
_____ $55,001-$70,000
_____ $70,001-$85,000
_____ $85,001-$100,000
_____ > $100,000

Please indicate which education level best describes you:

_____ Less than 12 years
_____ High School
_____ Trade School
_____ Some College
_____ College Graduate
_____ Post-Graduate Degree

APPENDIX 4. *GUT MICROBIOME STUDY: STOOL SAMPLE COLLECTION AND PROCESSING*

Sample Information Management for Microbiota Studies

Each stool sample will be given a unique identifier.

For example:

Patient 0001 and has sample A and B. Patient 0002 has sample A.

Sample IDs: MCW_0001-A, MCW_0001-B, MCW_0002-A

As we acquire, aliquot, and store samples, we will not to try to squeeze additional information (metadata), such as date or day of collection or patient initials into the sample ID. Such additional information becomes very difficult to deal with later and statistical software programs can't deal with them well. Please don't name samples things like "Pt 002 Week 3" or "Pt 002 pre-transplant" or "Pt JFK (Day -2)". If a patient has more than one transplant, day and week designations are problematic. Another problem with this sort of a sample-naming scheme leads to discrepancies between the target time point (e.g. day 30) and the actual timepoint collected (e.g. day 32)

The following approach is recommended:

Keep the names of samples simple and then keep a separate table of sample metadata, as below:

Sample ID*	Patient MRN (not to be shared across centers	Unique Patient ID (okay to share across centers)	Date Collected	Freezer Location
MCW_0001-A	7654321	MCW-pt-0001	2018-09-30	Box3
MCW_0001-B	7654321	MCW-pt-0001	2019-01-01	Box3
MCW_0002-A	1234567	MCW-pt-0002	2019-07-01	Box1

*sample ID refers to what is written on the tube

Instructions For Stool Specimen Collection

Please place the two gel packs in your freezer in the morning 3 days before your clinic visit. Specimen can be collected within 2 days of your clinic visit.

STEP 1. Raise the toilet seat. Place the stool collection frame on the back of the toilet bowl (see Figure 1A). All four corners of the collection frame should be supported by the toilet bowl. Remove the lid from the collection bowl, place collection bowl in frame, and place toilet seat down (Figure 1B).

Figure 1A

Figure 1B

STEP 2. Deposit your stool directly into the collection container. Try not to urinate into the collection container.

STEP 3. After collecting your specimen, remove the container from the frame (Figure 2A). Place the container on a flat surface and firmly press the lid closed (Figure 2B).

Figure 2A

Figure 2B

STEP 4. Place the closed container into the zip-lock bag provided (see Figure 3) and seal the bag.

Figure 3

STEP 5. Discard collection frame in trash.

STEP 6. Package your specimen immediately, following the instructions below. Write the date and time of collection on the label of the zip-lock bag provided. There is also a unique ID number pre-printed on the label.

STEP 7. Place the zip-lock bag containing the specimen container inside the styrofoam box (Figure 4A), along with the two cold packs arranged on the side and on top (Figures 4B and 4C).

Figure 4A

Figure 4B

Figure 4C

STEP 8. Place the lid on the box and place the rubber band provided around the box (Figure 5). You can now place the styrofoam box in the paper bag provided to bring in to clinic.

Stool Sample Collection, Processing, and Shipping

Sample Collection

Suggested collection days:

Inpatient: twice weekly (e.g. Mondays and Thursdays) – once pre-transplant and weekly (+/- 2 days) post-transplant until after neutrophil engraftment.

Tube Preparation

Please aliquot into sturdy tubes that can withstand cold temperatures[2]. Standard urine- or sputum-collection cups crack at freezing temperatures and should not be used.

If labeling tubes by hand, please use an alcohol-proof marker[3]. Sharpie, etc. will dissolve in the ethanol used during DNA extraction.

If using printed labels, please ensure that they will not peel, crack, or smear at freezing temperatures[4, 5].

Aliquoting

Aliquoting should be done as soon as possible after sample is received.

For solid or semi-solid samples, use a clean disposable spatula[6] to transfer stool into cryovials[2].

- Prepare 5 aliquots of ~0.5 mL each.
- For more liquid samples, can transfer up to 1 mL material.

For liquid samples, use transfer pipette[7] to prepare 1.5 mL aliquots of material.

All aliquots should be stored at -80 until shipping.

Shipping Address:

Attn: Annie Slingerland Z-1419
Memorial Sloan-Kettering Cancer Center
408 East 69th Street New York, NY 10021 United States
646-888-2304

Shipping

Samples should be sent overnight, with enough dry ice to last 2-3 days in the event that delivery is delayed.

Please ship only on Monday, Tuesday, or Wednesday to ensure that sample does not sit on loading dock over the weekend.

After Shipping

Please email Annie (slingera@mskcc.org) with tracking number and a shipping manifest (.xlsx or .csv) detailing

for each tube:

- text of written or printed label
- unique sample ID (discussed in “Microbiota Data Collection and Management”)
- unique ID for associated patient (discussed in “Microbiota Data Collection and Management”)
- date of collection (or day of transplant/conditioning)
- preservative added, if any
- # of aliquots made of sample and how many are included in shipment (e.g. 1 of 5; 2 shipped)
- If sending more than 20 tubes, please include a map of each tube’s location within the grid of the box

Questions?

van den Brink lab tel: 646-888-2317 (ask for Tsoni, Annie, or Melissa)

Dr. van den Brink's office & assistants: 646-888-2304.

Suggested Materials:

1. Outpatient kit:

   commode specimen collection system (www.fishersci.com, 02-544-208, $2.75/ea, 60/cs)

   (specimen container will need a unique specimen number label)

14 x 9 x 9” corrugated box (www.uline.com, S-4985, $0.66 to $0.94/box, multiples of 25/cs)

8 x 6 x 4.25” foam container (www.uline.com, S-18312, $4.70/ea, 20/cs)

(2) cold bricks - 7.5 oz (www.uline.com, S-14294, $0.824/ea, 42/cs)

12 x 15 biohazard zip lock bag (www.ourshippingsupplies.com A-LN4099, $0.18/ea, 1000/cs)

14 x 10 x 15.5” paper shopping bag (www.uline.com, S-9661, $0.345 to $0.38/bag, multiples of 200/cs)

outpatient collection directions

2. *Microtubes*, 2ML, w/ screw cap (www.fishersci.com, NC0445483, or www.sarstedt.com 72.694.006,$0.32/ea, 1000/cs)

3. *VWR lab markers*, black ink, fine point (www.vwr.com, 52877-310, $6.19/ea, 10/pack)

4. *Cryo-Clear clear labels,* for 0.5 ml tubes, 0.94" L x 0.5" W (http://www.usascientific.com/labelsonsheets.aspx,

9125-0238, $3.15/sheet of 119 stickers, 20 sheets/box)

5. *Tough-Spots*, small, colors, for 0.5 ml tubes, 3/8" diameter (http://www.usascientific.com/labelsonsheets.aspx,

9185-1008, $2.85/sheet of 192 stickers, 20 sheets/box)

6. *Sterile Disposable Spatula*, Double-Ended, with Spoon & Scoop (https://us.vwr.com/store/product/4531734/vwr-

disposable-polypropylene-spatulas, 89097-816, $1.39/ea, 100/pack)

7. *Disposable Graduated Transfer Pipettes*, individually wrapped (https://www.fishersci.com/shop/products/fisherbrand

-disposable-graduated-transfer-pipettes-5/p-163764, 13-711-20, $0.21/ea, 500/pack)

8. *Feces tube*, 101x16.5mm, sterile (https://www.fishersci.com/us/en/healthcare-products.html, NC0729971, $0.47/ea, 500/case).