A pilot study to assess engraftment using CliniMACS TCR-α/β and CD19 depleted stem cell grafts from haploidentical donors for hematopoietic progenitor cell transplantation in patients with relapsed lymphoma

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Revision History

Version Date	Detail of Changes	Consent Change (Y/N)	Consent Date
01/26/2016	Expedited Change: Editorial and clarifications: --Updated protocol version date --Updated study personnel --Clarified Recipient Inclusion Criteria Section 3.1.2, current disease status and timeframe allowed between patient meeting the disease criteria and registration --Revised Donor Inclusion Criteria Section 3.3.2, age --No changes to consent documents were made	N	Recipient ICF: 10/19/2015 Donor ICF: 10/19/2015
08/15/2016	Full Change --Updated protocol version date --Updated study personnel and roles --Updated revision history --Added Recipient Inclusion Criteria Section 3.1.2.9 to include all subtypes of non-Hodgkin Lymphoma --Further defined incurable indolent histologies in background Section 1.4 --Updated Luminex Exclusion Criteria 3.4.1 --Extended window for obtaining informed consent to 42 days in Section 9 --Removed 'by RPR' from Section 9, footnote 5 --Added Section 9, Footnote 8 to clarify that the Panorex scan can be obtained through the CT sinus --Re-numbered footnotes in Section 9 --Updated Section 9, Footnote 12 to indicate WBC count required starting Day +7 Donor ICF Changes: --Are there risks? -Corrected number of days may need to donate from three to two --Are there any costs? -Removed the reference to the cell depletion being considered research	Y (donor ICF corrections only)	Recipient ICF: 10/19/2015 Donor ICF: 8/15/2016
10/23/2017	Full Change --Updated protocol version date --Updated study personnel and roles --Updated revision history --Synopsis: updated disease status inclusion criteria to restrict DLBCL patients to those who are in complete or partial remission to salvage therapy after relapse --Synopsis: updated cardiac inclusion criteria to mandate against significant heart failure symptoms or severe valvular abnormalities --Synopsis: added exclusion criteria for history of prior mediastinal radiation and reported illicit drug use --Updated DLBCL inclusion criteria 3.1.2.3 and 3.1.2.4 to restrict DLBCL patients to those who are in		

	complete or partial remission to salvage therapy after relapse --Updated cardiac inclusion criteria 3.1.5 to mandate against significant heart failure symptoms or severe valvular abnormalities --Added exclusion criteria 3.2.6 to exclude patients who have had mediastinal radiation --Added exclusion criteria 3.2.7 to exclude patients who report illicit drug use --Renumbered exclusion criteria 3.2.8 (previously was 3.2.6) --Clarified section 6.5 rituximab dosing may be adjusted per UW SOP to nearest 50mg dose as long as within 10% of original dose --Section 9 Schedule of Evaluations modified to include asking and documenting illicit drug history, added footnote 1 --Section 9 Schedule of Evaluations, subsequent footnotes re-numbered Recipient ICF Changes: --Updated version date --Unknown or Unexpected Side Effects: moved location for consistency, updated language for clarity --What if I have questions? Updated the first sentence for clarity		
04/24/2020	Full change		
	--Update to title page to remove former staff and add new staff --Updated protocol version date --Updated revision history --Updated Signature page --updated Protocol Synopsis: removed former co-investigator, Study Procedures updated to include extended monitoring --Table of Contents updated for page numbers and addition of section 7.4.4 with this Amendment --List of Abbreviations and Definition of Terms has been updated to remove DOWG and include DOT as described --Section 7.2 has been updated for extending monitoring --Section 7.4 Table 1 has been revised to describe updated reporting timelines and to reference section 7.4.4 --Section 7.4.1 was updated for change in “DOWG” to “DOT” --Section 7.4.3 updated language for clarity --Section 7.4.4 added section for Extended Safety Monitoring out to 2 years post-transplant --Section 9.0 Schedule of Evaluations updated for extended monitoring, footnote 16 -- minor spelling and grammar corrections throughout document		

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator

Signed: Date:

_____ _____

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PROTOCOL SYNOPSIS

Title	A pilot study to assess engraftment using CliniMACS TCR-α/β and CD19 depleted stem cell grafts from haploidentical donors for hematopoietic progenitor cell transplantation in patients with relapsed lymphoma
Short Title	α/β depleted Haplo-HSCT for patients with relapsed lymphoma
IDE Sponsor	Peiman Hematti MD
Principal Investigator	Vaishalee Kenkre, MD
Co-Investigators	Kristin Bradley, MD, Natalie Callander, MD, Aric Hall, MD, Peiman Hematti, MD, Mark Juckett, MD
Participating Site	University of Wisconsin-Madison
Accrual Objective	14 transplant recipients 14 donors
Study Design	Single center, open label, single-arm, pilot trial
Study Duration	The estimated accrual period is two years.
Primary Objectives	To determine engraftment of neutrophils and platelets at 28 days following alpha/beta T-cell depletion using HLA haploidentical donors for stem cell transplant in relapsed lymphoma.
Secondary Objectives	Acute GVHD – The cumulative incidence of grade III – IV acute GVHD by Day +100 will be determined. The GVHD grade will be determined by the IBMTR Severity Index criteria (Appendix A). Chronic GVHD – The cumulative incidence of severe chronic GVHD by Day +180 will be recorded and defined according to the NIH consensus criteria (Appendix B). Graft failure rate – defined as < 5% donor chimerism in the CD3 and/or CD33 selected cell populations at any time in the study follow up period once initial engraftment has been achieved. Treatment-related mortality rate – defined as death from any cause other than disease progression Progression-free survival – defined as time before any progression by either PET/CT or bone marrow (Appendix C)

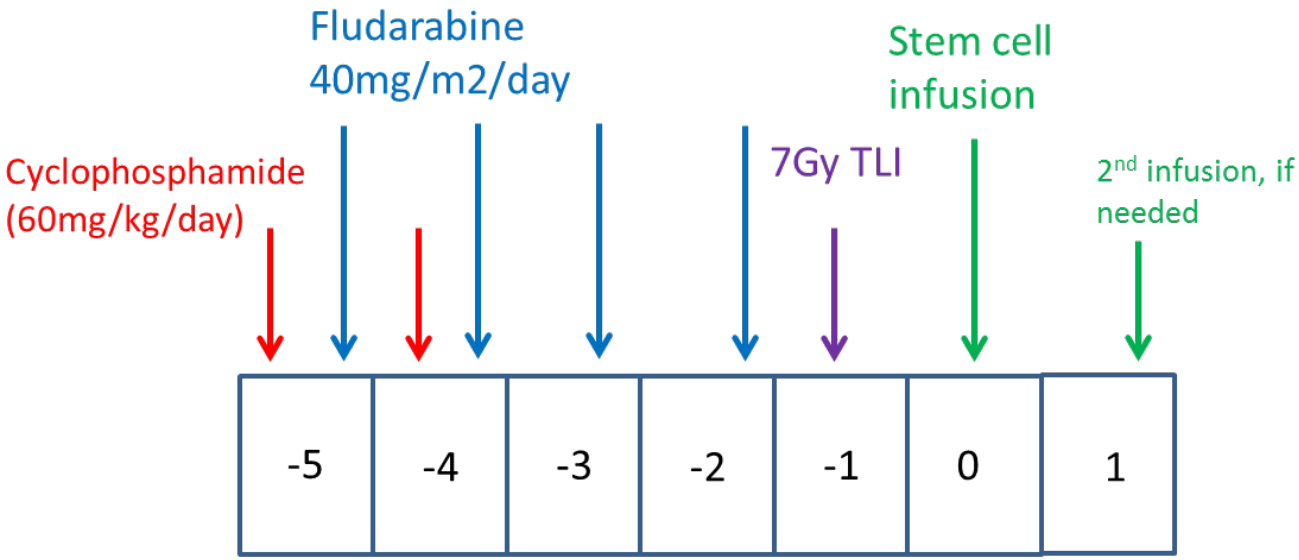
	Overall survival – after enrollment on study
Exploratory Objectives	• Evaluation of immune cell recovery
Inclusion Criteria	• Age 18 to 75 years • Patients must meet one of the following disease criteria within 24 months of registration. Salvage therapy is allowed between the patient meeting one of the below criterion and registration. Patients will be considered eligible regardless of their current disease status (i.e. complete remission, partial remission, stable disease, progressive disease) unless otherwise noted below as long as one of the below criterion has been met within the previous 24 months: o Relapsed/refractory Hodgkin lymphoma after autologous stem cell transplantation o Relapsed/refractory Hodgkin lymphoma, deemed ineligible for autologous stem cell transplantation due to refractory disease o Relapsed/refractory diffuse large B cell lymphoma after autologous stem cell transplantation (history of transformed lymphoma is acceptable). Disease must be in at least complete remission or partial remission with the use of salvage therapy before study treatment commences. o Relapsed/refractory diffuse large B cell lymphoma, deemed ineligible for autologous stem cell transplantation due to refractory disease (history of transformed lymphoma is acceptable). Disease must be in at least complete remission or partial remission with the use of salvage therapy before study treatment commences. o Relapsed/refractory T cell lymphoma relapsed after at least 1 prior line of therapy o Relapsed/refractory follicular lymphoma relapsed after at least 1 prior line of therapy o Relapsed/refractory mantle cell lymphoma relapsed after at least 1 prior line of therapy

	○ Relapsed/refractory small lymphocytic lymphoma/chronic lymphocytic leukemia relapsed after at least 1 prior line of therapy ○ Relapsed/refractory non-Hodgkin lymphoma, if not specified above, relapsed after at least 1 prior line of therapy • Karnofsky score of 60% or better ("Requires occasional assistance, but is able to care for most of his/her needs"). • Pulmonary: DLCO (corrected for hemoglobin) > 40%; and FEV1 > 50% • Cardiac: EF ≥ 50%. No uncontrolled angina or active cardiac symptoms consistent with congestive heart failure (class III or IV), by the New York Heart Association criteria. No symptomatic ventricular arrhythmias or ECG evidence of active ischemia. No evidence by echocardiography of severe valvular stenosis or regurgitation. • Renal: estimated GFR by MDRD formula > 40 mL/min/1.73m^2 • Women of child bearing potential must have a negative serum or urine pregnancy test within 14 days prior to study registration and agree to use adequate birth control during study treatment. A female of childbearing potential (FCBP) is a sexually mature female who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months). • Voluntary written consent
Exclusion Criteria	• Active CNS lymphoma within two weeks of registration. Patients with a history of CNS involvement must have adequate treatment as defined by at least two negative spinal fluid assessments separated by at least one week. (Otherwise LP is not required if no clinical suspicion or evidence of CNS involvement.) Patients who have received cranial radiation therapy must still be eligible to receive total lymphoid irradiation to 7 Gy. • New or active infection as determined by fever, unexplained pulmonary infiltrate or sinusitis on radiographic assessment.

	Infections diagnosed within 4 weeks of registration must be determined to be controlled or resolving prior to treatment. • Presence of HIV, or active hepatitis A, B, or C infection • Allergy or hypersensitivity to agents used within the treatment protocol. • For an indolent lymphoma histology (follicular lymphoma, SLL/CLL) or mantle cell lymphoma, the patient should not have an HLA-matched sibling, who would be an eligible donor, available. • History of prior mediastinal radiation • Reported illicit drug use • Vulnerable population groups, i.e., prisoners, those lacking consent capacity, non-English speaking, illiterate, pregnant females.
Treatment Description	Patients will undergo a conditioning regimen prior to Peripheral Blood Stem Cell graft from a haploidentical donor depleted of TCR α/β+ and CD19+ cells.
Study Procedures	• Following screening and enrollment, the haploidentical family donor will receive stem cell mobilization therapy with subcutaneous G-CSF prior to PBSC collection by leukapheresis. • Patients will undergo a conditioning regimen consisting of Cyclophosphamide 60 mg/kg IV daily Days -5 and -4, Fludarabine 40 mg/m^2 IV daily, Days -5 to -2, Total lymphoid irradiation (TLI) 7Gy, Day -1 prior to transplant with a haploidentical donor Peripheral Blood Stem Cell graft depleted of TCR α/β+ and CD19+ cells. GVHD prophylaxis will consist of Mycophenolate mofetil (MMF) at a targeted dose of 20 mg/kg orally BID beginning Day -1 until Day +30. Tacrolimus will be administered starting Day +2 (through day +180) if TCRα/β+ cell content exceeds 1 × 10^5 cells/kg ideal BW of the patient. Rituximab will be administered on Day +2 if B cell content exceeds 1 x 10^5 cells/kg ideal BW of the patient. • Follow-up assessments will occur until 1 year post-transplantation. Per Amendment version 04/24/2020, follow-up assessments will occur until 2 years post-transplantation.

Statistical Considerations	• Fourteen transplant recipients (subjects) will be enrolled into the trial. With 14 subjects, the trial will have 0.86 power to reject the null hypothesis that the probability of 28-day engraftment $p \leq 0.7$ in favor of the alternative hypothesis that $p \geq 0.9$ according to a one-tailed test at a significance level of 0.2.

Treatment Schema:



MMF starting day -1 → through day +30

Tacrolimus starting day +2 → through day +180 (if TCRα/β+ cell content exceeds 1×10^5 cells/kg ideal BW)

Rituximab on day +2 (if B cell content exceeds 1×10^5 cells/kg ideal BW)

Table of Contents

List of Abbreviations and Definition of Terms

AABB	American Association of Blood Banks
ABG	Arterial Blood Gas
AER	Adverse Event Reporting
aGVHD	Acute Graft Versus Host Disease
ANC	Absolute Neutrophil Count
APC	Antigen Presenting Cell
ASCT	Autologous Stem Cell Transplantation
AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATG	Anti-Thymocyte Globulin
Bid	Twice daily
BMT	Bone Marrow Transplant
BMTCTN	Bone Marrow Transplant Clinical Trials Network
BM	Bone Marrow
BUN	Blood Urea Nitrogen
BW	Body Weight
CAEPR	Comprehensive Adverse Events and Potential Risks
CBC	Complete Blood Count
CD	Cluster of Differentiation
cGVHD	Chronic Graft Versus Host Disease
CLL	Chronic Lymphocytic Leukemia
CMV	Cytomegalovirus
CNS	Central nervous system
CRC	Clinical Research Committee
CT	Computed Tomography
CTCAE	Common Toxicity Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DLBCL	Diffuse Large B Cell Lymphoma
DL_{CO}	Carbon Monoxide Diffusion Capacity
DOT	Disease Oriented Team
DSMB/DSMC	Data Safety Monitoring Board/Committee
EBV	Epstein-Barr virus
ECG	Electrocardiography
ECHO	Echocardiography
FACT	Foundation for the Accreditation of Cell Therapy
FCBP	Female of Child Bearing Potential
FDA	Food and Drug Administration
FEV	Forced Expiratory Volume
G-CSF	Granulocyte Colony Stimulating Factor
GFR	Glomerular Filtration Rate
GVHD	Graft-versus-Host Disease

GVL	Graft Versus Lymphoma
HCT	Hematopoietic cell transplantation
HHCT	Haploidentical Hematopoietic Cell Transplantation
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HCT	Hematopoietic Cell Transplantation
IDE	Investigational Device Exemption
INR	International Normalized Ratio
IRB	Institutional Review Board
IV	Intravenous
Kg	Kilogram
KIR	Killer cell Immunoglobulin-like Receptor
KM	Kaplan-Meier
LDH	Lactate Dehydrogenase
LP	Lumbar Puncture
LVEF	Left Ventricular Ejection Fraction
MDRD	Modification of Diet in Renal Disease
MFI	Mean Fluorescence Intensity
MMF	Mycophenolate Mofetil
MRD	Matched Related Donor
NCI	National Cancer Institute
NK	Natural Killer (cell)
NHL	Non-Hodgkin lymphoma
OS	Overall Survival
PB	Peripheral Blood
PBSC	Peripheral Blood Stem Cell
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography
PFS	Progression Free Survival
PFT	Pulmonary Function Test
PI	Primary Investigator
PO	Per Os (by mouth)
PSR	Protocol Summary Report
PTLD	Post-Transplant Lymphoproliferative Disease
RBC	Red Blood Cell
RIC	Reduced Intensity Conditioning
RPR	Rapid Plasma Regain
SAE	Serious Adverse Event
SLL	Small Lymphocytic Lymphoma
SOP	Standard Operating Procedure
SPRT	Sequence Probability Ratio Tests
STR	Short Tandem Repeat
Sub-I	SubInvestigator

T cells	Thymus-derived white blood cells
TCR	T-Cell Receptor
TID	Three Times Daily
TLI	Total Lymphoid Irradiation
TNI	Total Nodal Irradiation
TPN	Total Parenteral Nutrition
Treg	T regulatory cell
TRM	Treatment Related Mortality
TSH	Thyroid Stimulating Hormone
UWBMT	University of Wisconsin Bone Marrow Transplant
UWCCC	University of Wisconsin Carbone Cancer Center
VOD	Veno-Occlusive Disease
WBC	White Blood Cell

1. Introduction

Patients with aggressive lymphoma (e.g., Hodgkin lymphoma, DLBCL) have a potentially curative option in frontline therapy. However, up to 50% of patients will relapse.[1-3] At relapse, if disease remains chemo-sensitive, there is still a curative option with high-dose chemotherapy consolidation and autologous stem cell transplant (ASCT).[4-9] However, many patients are ineligible for ASCT due to chemo-refractory disease, or will relapse after ASCT. There is no good standard alternative for such patients and often treatment options going forward are palliative, at best, in nature.

Patients with incurable lymphoma (e.g., follicular lymphoma, SLL, mantle cell lymphoma) can sometimes have an indolent course. A typical disease course, however, can include progressively briefer responses to treatment, leading ultimately to refractory and uncontrollable disease.

In both of these patient populations, there is a need for novel (and ideally curative) options. Allogeneic hematopoietic cell transplantation has been studied in lymphoma, both in aggressive histologies (typically after ASCT or for those refractory to chemotherapy and thus ineligible for ASCT) [10-18] and indolent histologies in relapse.[19-23] Studies support a potential for graft-versus-lymphoma (GVL) effect, though often at the cost of significant treatment related morbidity and mortality. Complications often arise as a result of graft-versus-host disease (GVHD). While rates of acute GVHD with reduced-intensity conditioning regimens may range relatively lower (10-20%), rates of extensive chronic GVHD range from 45-60% even with HLA matched donors.[22, 23] The optimal scenario would be one in which GVL could be optimized while minimizing GVHD.

To avoid serious GVHD and graft rejection in allogeneic hematopoietic cell transplantation, it has long been inevitable to find donors with the highest possible human leukocyte antigen (HLA) match to the recipient. The therapeutic use of hematopoietic cell transplantation (HCT) thus is limited by the availability of a suitable HLA-matched donor. A matched related donor can be found for only 30% of patients, thus 70% of patients have to rely on finding a matched unrelated donor.[24] Though donors can be identified for most of these patients, the search takes at least several weeks, if not months. If the aggressive course of the disease requires the fast identification of a suitable donor, this is too long for a significant number of patients. And in some instances, a donor ultimately cannot be found.

In order to find a solution, haploidentical hematopoietic cell transplantation (HHCT) using closely related, but only partially matched family donors has been exploited in several therapeutic settings, recently. In theory, virtually

every patient has a potentially suitable haploidentical related donor—parent, sibling, or child—and thus a successful strategy for HHCT may clearly be the solution for the 'lacking donor' problem. Yet, once again in only partially matched donors and recipients, the difficulties of resulting GVHD and graft failure arise, and initially trials of HHCT were complicated by a high incidence of GVHD, engraftment failure, and infectious complications resulting in an unacceptably high treatment related morbidity and mortality.[25] Recent efforts have therefore been directed on developing therapeutic strategies to minimize these complications. GVHD and graft rejection are primarily mediated by T cells of host and donor. Therefore, attempts to overcome the HLA-barrier have focused on strategies for effective host and graft T cell depletion.

1.1 CD34+ Enriched Stem Cell Grafts

An approach pioneered by the groups of Bachar-Lustig et al [26] and Aversa et al [27] was to overcome the rejection of T cell depleted bone marrow cells by using a 'megadose' of CD34+ (stem) cells (i.e. >10 × 10^6 CD34+ cells/kg) for transplantation. The harvesting of such megadoses was achieved by treating donors with hematopoietic growth factors prior to graft recovery. Using this setting, Aversa et al treated patients with advanced acute leukemia in different trials with haploidentical donors.[28] To avoid graft failure, intensive and highly straining patient conditioning regimens with total-body irradiation, thiotepa, fludarabine, and anti-thymocyte globulin (ATG) had to be used. Fast engraftment rates of neutrophils and platelets were observed together with a low rate of GVHD (<10%) and a promising disease-free survival.

Treatment-related mortality (TRM), however, was very high in these settings due to the intensive myeloablative conditioning regimens needed and a delayed immune reconstitution (due to T cell depletion) which resulted in a high number of serious infections or regimen related toxicities. Another major obstacle to this approach is achieving the 'megadose' of CD34+ stem cells necessary for overcoming major HLA-barriers. At doses below 10 × 10^6 CD34+ cells/kg BW of the recipient, rejection rates increased and engraftment and immune reconstitution were delayed (most pronounced in patients receiving less than 8 × 10^6 CD34+ cells/kg BW).[29]

1.2 CD3+ and CD19+ Depleted Stem Cell Grafts

The aim of another therapeutic approach therefore was to develop a strategy to improve engraftment independent from the infused stem cell doses, utilizing CD3 and CD19 depletion. A reduced intensity conditioning regimen (RIC) was developed to reduce TRM.[30, 31] The first promising

experiences were published for a pediatric population.[32-35] Here, the Miltenyi CliniMACS cell sorting system was used for CD3+/CD19+ depletion of the grafts using selective immunomagnetic beads. As discussed, profound T cell depletion is a fundamental prerequisite for successful HHCT to avoid severe GVHD. Meanwhile B cell depletion is obligatory to avoid EBV-related post-transplant lymphoproliferative disease (PTLD).

On the other hand, earlier investigations had demonstrated the important therapeutic potential of alloreactive natural killer (NK)-cells in the graft [36] (increased disease-free survival [37, 38], engraftment facilitating and graft-versus-tumor effects [39] and a smaller number of relapses and better survival [36]). NK-cells are also involved in the immune response against bacterial, viral and fungal infections. For example, in transplantation settings with KIR mismatched donors (thus more reactive NK cells), patients show lower incidences of CMV-reactivation.[40, 41]

It was therefore expected that grafts selectively depleted of T and B cells, which still contain not only CD34+ stem cells but also a significant number of graft facilitating cells such as NK-cells, monocytes and granulocytes, would lead to improved engraftment and better disease control as well as immune reconstitution.

Two phase I/II trials were initiated (adult patients with high risk myeloid and lymphoid malignancies and pediatric patients myeloid and lymphoid malignancies, solid tumors and non-malignant diseases).[27, 33] No G-CSF was administered post-transplantation and mycophenolate mofetil (MMF, 15 mg/kg bid) was used only if the T-cell content in the graft exceeded 5×10^4 CD3+ cells/kg. The conditioning regimen was well tolerated and engraftment was rapid (median time to >500 neutrophils/mcl was 13 days and to >20,000 platelets/mcl was 11 days). Furthermore, all but one patient engrafted with full donor chimerism by day 14–28 post-transplantation. In the trial with pediatric patients, graft rejection occurred in 13%. Engraftment kinetics thus were similar to those reported by Aversa et al after HHCT with CD34+- megadoses [27] although patients received a clearly lower dose of CD34+ cells/kg. After total nodal irradiation (TNI) based reconditioning and second haploidentical stem cell donation, final engraftment in pediatric patients was achieved in 100%.[33]

However, in adult patients, immune reconstitution was delayed due to the profoundly T-cell depleted grafts. NK-cell reconstitution was fast, probably due to the high NK-cell content of the CD3+/CD19+ depleted grafts. In this setting, 9 of 36 (25%) adult patients died due to transplant-related causes within the first 100 days and incidence of grade II–IV GVHD was 36%. Thus, incidence and degree of GVHD in adult patients after HHCT with

CD3+/CD19+ depleted grafts was higher than that reported for patients receiving HHCT with CD34+ selected grafts.[27]

Thus, this methodology allowed HHCT in an older or heavily pre-treated patient population even without megadoses of CD34+ stem cells. However, several factors still needed to be improved: the reconstitution of T cells, engraftment, and prevention of significant transplant-related morbidity/mortality.

1.3 TCRα/β+ and CD19+ Depleted Stem Cell Grafts

The significant impact of graft composition and conditioning regimen on engraftment has already been demonstrated by the fast engraftment kinetics observed in patients receiving CD3+/CD19+ depleted grafts compared to merely CD34+ enriched grafts. Cell species endangering transplantation outcome have meanwhile been identified in even more detail. Mainly the subsets of CD3+ cells with TCRα/β receptors mediate graft-versus-host activity while, on the contrary, CD3+ cells with TCRγ/δ receptors show the highly interesting graft-versus-tumor activity.[42-48] The beneficial effects of NK cells in the graft have already been discussed above. Furthermore, recent studies have revealed the existence of CD34-negative stem cells, which are probably precursors of CD34+ stem cells with a high repopulating capacity.[49] Additional graft facilitating cells have also been defined, such as CD8-positive T-cells, monocytes and antigen-presenting cells (APCs). [39, 50-54]

Thus, an efficient depletion of GVHD-mediating T cells presenting CD3 and TCRα/β as surface markers is imperative to prevent acute graft-versus-host disease, while leaving in the product a high number of CD34+ hematopoietic stem cells, as well as other important cells including NK cells, monocytes and TCRγ/δ cells that provide graft-versus tumor and anti-infectious activity. Further improvement of the cell product would be achieved by the depletion of CD19+ B cells because this reduces the risk for EBV-related PTLD. PTLD has previously been a major risk in transplantation settings.[55] In addition, there is growing evidence implicating B cells in the pathogenesis of acute and chronic GVHD [56], and rituximab used as prophylaxis against GVHD has been used successfully to minimize GVHD. [57, 58] Thus, CD19 depletion will serve both purposes, reducing the risk of PTLD as well as GVHD prophylaxis.

To facilitate this, a new cell sorting strategy for processing of the cell grafts has been developed using again the Miltenyi CliniMACS cell sorting system. The stem cell grafts are selectively depleted of TCRα/β+ and CD19+ cells by using paramagnetic microbeads in a single processing step

and the resulting cell grafts are rich in a variety of blood cells with diverse immunological properties.[59]

First clinical experiences in HHCT using TCRα/β- and CD19-depleted PBSC grafts have been obtained with pediatric patients in Tübingen.[60, 61] All were at extremely high risk and had poor prognosis. They were pretreated with a reduced-intensity conditioning (RIC) regimen and received no post-transplantation immunosuppression. In these pilot patients, engraftment and immune reconstitution were rapid. No acute side-effects were noted. By now, treatment of 23 pediatric patients with hematologic malignancies has been reported in Tübingen and Rome.[62]

No post-transplantation GVHD prophylactic immunosuppression was given. In all cases, engraftment was rapid, and immune recovery was markedly improved compared to patients after CD3+/CD19+ depletion. TCRγ/δ cells expanded faster than TCRα/β cells early after transplantation, though at day 100, TCRα/β cells were predominant. Only 7 of 23 patients experienced grade 1 and 2 acute GVHD and only 1 patient experienced transient grade 3 acute GVHD of the skin. There was no chronic GVHD.

In 23 children with nonmalignant disorders who received HHCT after TCRα/β/CD19 depletion, all but 4 patients engrafted and the latter were rescued by a second allograft.[63] Only 3 patients experienced skin-only grade 1 to 2 acute GVHD. No patient achieved visceral acute or chronic GVHD. With a median follow up of 18 months, 21 of 23 children are alive and disease free.

In summary, in all pilot patients treated so far, rapid and sustained engraftment, rapid immune reconstitution, and a low incidence of GVHD were observed. Cell sorting using the CliniMACS device proved to be efficient since a high TCRα/β log depletion was achieved and the TCRα/β/CD19 depletion was demonstrated to be effective and feasible while good recovery rates for stem cells and innate effector cells were observed with a high viability of the resulting cells in the transplant.

1.4 Relevance of haploidentical stem cell transplantation in lymphoma

Allogeneic HCT and especially the transplantation of a haploidentical cell graft, carries substantial, well known risks that have to be weighed against the risk of the malignancy as well as the consideration of other treatment options. For patients entering this study, an allogeneic HCT has been deemed necessary by the treating physician.

For patients with follicular lymphoma, small lymphocytic lymphoma/chronic lymphocytic leukemia, and mantle cell lymphoma), outcomes after matched

related donor (MRD) HCT are reasonable with progression-free survival (PFS) and overall survival (OS) after transplant ranging from 40-80% at 3-5 years.[22, 23, 64] Thus, for these patients, the unavailability of a matched related donor will be a requirement prior to consideration on this trial for haploidentical donor transplantation, which would be meant to provide a transplantation alternative that would otherwise not be available. It is important to consider that the reasonable outcomes with MRD HCT in these disease histologies do not incorporate the significant amount of GVHD seen in these patients that can be a life altering problem even in the setting of their initial hematologic disease being cured. Ultimately, if this trial and subsequent study reveals that TCRα/β/CD19 depletion does indeed result in lower rates of GVHD (as hypothesized), it will be important to compare the efficacy of MRD HCT with HHCT in patients with these lymphomas and/or consider testing this method of cell processing (TCRα/β/CD19 depletion) with MRD HCT.

For the patients with aggressive histologies, outcomes with MRD HCT are not as favorable, and with rarer NHLs, not as well studied. Typically, PFS and OS at 3-5 years after transplantation for aggressive lymphomas ranges 20-40%. [65-67] It remains to be seen whether HHCT may actually be a more favorable alternative compared to MRD HCT. It can be hypothesized that there may be a stronger immune effect (GVL) against the disease in the setting of a partial mismatch. Interestingly, in a multi-center retrospective analysis evaluating alternative donors in transplantation of patients with relapsed/refractory Hodgkin lymphoma, haploidentical transplantation was associated with improved outcomes when compared to MRD HCT, including improved progression-free survival, lower relapse rates, and lower rates of GVHD.[68] In this study, however, T cell depletion was employed through post-transplant cyclophosphamide. Nonetheless, it still suggested a benefit of HHCT over MRD HCT in aggressive lymphoma.

Thus, we would not prioritize a haploidentical donor over a matched related donor for follicular lymphoma, CLL/SLL, and mantle cell lymphoma, where disease control is reasonable with MRD HCT. However, for all of the other histologies, we feel using a haploidentical donor may improve disease control and is reasonable to consider as the first option even if a MRD is available. This is reflected in the eligibility criteria for this protocol.

Patients eligible for this trial have severe disease and have a poor long-term outcome if transplantation is not performed. The transplantation of TCRα/β and CD19 depleted haploidentical stem cell grafts offers considerable potential benefits. Due to the efficient TCRα/β cell depletion, similar GVHD rates are expected as observed with CD34+-selected or CD3/19-depleted grafts. However, GVHD as the most severe potential risk is addressed in this trial through the institution of statistical stopping

guidelines and continuous monitoring of the patient with the possibility to intervene if engraftment failure is too frequent or GVHD ensues (see Section 11.5 Sequential monitoring of engraftment failure and GVHD).

Benefits of this cell processing plan are expected to be mediated by beneficial effector cells which are retained in the graft during the depletion procedure. These cells are expected to facilitate engraftment, exert GVL effects, reduce the risk of infections, and improve immune reconstitution. Especially the latter is currently one of the major challenges after stem cell transplantation. Additionally, these beneficial cells offer the advantage of using dose- and toxicity-reduced conditioning regimens and the efficient and selective depletion of TCRα/β+ cells allows the reduction of immunosuppression post-transplantation for GVHD prophylaxis. In summary, this is expected to translate into a reduced treatment-related toxicity.

Preliminary data from transplantation of single patients with TCRα/β- and CD19-depleted haploidentical stem cell grafts show a low rate of acute and chronic GVHD as well as high engraftment rates and a fast recovery of the immune system compared to data published for patients transplanted with CD34-selected or CD3/CD19-depleted hematopoietic stem cell grafts. Therefore, overall, the available information suggests that the present study has a favorable risk-benefit ratio.

1.5 Purpose of this protocol

There are few efficacious treatment options for patients with Hodgkin lymphoma and aggressive non-Hodgkin lymphoma who have previously failed ASCT or who are ineligible for ASCT due to progressive disease. There are also limited options for patients with indolent lymphomas who have suffered multiple relapses. We propose to conduct a pilot study that will provide a potentially curative option for patients who do not otherwise have a viable transplant option with high-risk lymphoid malignancies, based on the hypotheses mentioned above. We have chosen a conditioning regimen that should be myelosuppressive enough to allow engraftment of a haploidentical stem cell graft, without being fully myeloablative. This regimen also includes chemotherapy drugs and radiation techniques that are effective against lymphoma. The primary purpose of this study is to establish that this conditioning regimen along with this novel stem cell processing method is sufficient to lead to engraftment of donor cells in a reasonable period of time. The secondary purpose of this study is to confirm that rates of GVHD are comparable to prior published data of allogeneic stem cell transplant in this patient population. The hope is that engraftment will be achieved in all patients, and that rates of GVHD will, if anything, be lower than prior observed rates due to the selective depletion

of TCRα/β+ cells, and that other secondary endpoints of this trial will be hypothesis-generating to evolve into a larger study of similar patients in which efficacy of this treatment can be better understood.

2. Study Aims / Study Objectives

Patients with relapsed/refractory aggressive lymphoma have a high relapse rate and mortality following standard transplant protocols. Patients with indolent lymphoma have better outcomes following standard transplant, however, a matched related donor is often not available and rates of GVHD leave room for improvement. We hope to improve on the outcome of these patients by TCRα/β+ cell depletion using peripheral blood stem cells from a HLA-haploidentical to provide a suitable donor, optimize a GVL effect, and minimize GVHD.

2.1 Primary Hypothesis

- The rate of engraftment (defined as ANC >500/mcl and platelets > 20K/mcl without transfusion in the prior 7 days) at Day +28 after transplantation is no less than 90%

2.2 Secondary Hypotheses

- Acute GVHD grade III/IV by Day +100 is below 30%
- Chronic GVHD (severe) by Day+ 180 is below 40%

To study these hypotheses, we propose to conduct a clinical trial with the following objectives:

2.3 Primary Objective:

To determine engraftment at 28 days after transplantation following TCRα/β and CD19 cell depletion using peripheral blood stem cells from a HLA-haploidentical donor.

2.4 Secondary Objectives:

Acute GVHD – The cumulative incidence of grade III – IV acute GVHD by Day +100 will be determined. The GVHD grade will be determined by the IBMTR Severity Index criteria (Appendix A).

Chronic GVHD – The cumulative incidence of severe chronic GVHD by Day +180 will be recorded and defined according to the NIH consensus criteria (Appendix B).

Graft failure rate – defined as < 5% donor chimerism in the CD3 and/or CD33 selected cell populations at any time in the study follow up period once initial engraftment has been achieved.

Treatment-related mortality rate – defined as death from any cause other than disease progression

Progression-free survival – defined as time before any progression by either PET/CT or bone marrow (Appendix C).

Overall survival – after enrollment on study

3. Selection of Study Patients

Study entry is open to adult patients regardless of gender, race or ethnic background.

3.1 Recipient Inclusion Criteria

3.1.1 Age 18 to 75 years

3.1.2 Patients must meet one of the following disease criteria within 24 months of registration. Salvage therapy is allowed between the patient meeting one of the below criterion and registration. Patients will be considered eligible regardless of their current disease status (i.e. complete remission, partial remission, stable disease, progressive disease) unless otherwise noted below as long as one of the below criterion has been met within the previous 24 months:

3.1.2.1 Relapsed/refractory Hodgkin lymphoma after ASCT

3.1.2.2 Relapsed/refractory Hodgkin lymphoma, deemed ineligible for ASCT due to refractory disease

3.1.2.3 Relapsed/refractory diffuse large B cell lymphoma after ASCT (history of transformed lymphoma is acceptable). Disease must be in at least complete remission or partial remission with the use of salvage therapy before study treatment commences.

3.1.2.4 Relapsed/refractory diffuse large B cell lymphoma, deemed ineligible for ASCT due to refractory disease (history of transformed lymphoma is acceptable). Disease must be in at least complete remission or partial remission with the use of salvage therapy before study treatment commences.

3.1.2.5 Relapsed/refractory T cell lymphoma relapsed after at least 1 prior line of therapy

3.1.2.6 Relapsed/refractory follicular lymphoma relapsed after at least 1 prior line of therapy

3.1.2.7 Relapsed/refractory mantle cell lymphoma relapsed after at least 1 prior line of therapy

3.1.2.8 Relapsed/refractory small lymphocytic lymphoma/chronic lymphocytic leukemia relapsed after at least 1 prior line of therapy

3.1.2.9 Relapsed/refractory non-Hodgkin Lymphoma, if not specified above, relapsed after at least 1 prior line of therapy

3.1.3 Karnofsky score of 60% or better (“Requires occasional assistance, but is able to care for most of his/her needs”).

3.1.4 Pulmonary: DLCO (corrected for hemoglobin) > 40%; and FEV1 > 50%

3.1.5 Cardiac: EF ≥ 50%. No uncontrolled angina or active cardiac symptoms consistent with congestive heart failure (class III or IV), by the New York Heart Association criteria. No symptomatic ventricular arrhythmias or ECG evidence of active ischemia. No evidence by echocardiography of severe valvular stenosis or regurgitation.

3.1.6 Renal: estimated GFR by MDRD formula > 40 mL/min/1.73m^2

3.1.7 Women of child bearing potential must have a negative serum or urine pregnancy test within 14 days prior to study registration and agree to use adequate birth control during study treatment. A female of childbearing potential (FCBP) is a sexually mature female who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

3.1.8 Voluntary written consent

3.2 Recipient Exclusion Criteria

3.2.1 Active CNS lymphoma within two weeks of registration. Patients with a history of CNS involvement must have adequate treatment as defined by at least two negative spinal fluid assessments separated by at least one week. (Otherwise LP is not required if no clinical suspicion or evidence of CNS involvement.) Patients who have received cranial radiation therapy must still be eligible to receive total lymphoid irradiation to 7 Gy.

3.2.2 New or active infection as determined by fever, unexplained pulmonary infiltrate or sinusitis on radiographic assessment. Infections diagnosed within 4 weeks of registration must be determined to be controlled or resolving prior to treatment.

3.2.3 Presence of HIV (Ab positivity), or active hepatitis A, B or C infection (active infection will be defined as hepatitis A IgM Ab positivity, hepatitis B surface Ag positivity, or hepatitis C PCR positivity, respectively)

3.2.4 Allergy or hypersensitivity to agents used within the treatment protocol.

3.2.5 For an indolent lymphoma histology (follicular lymphoma, SLL/CLL) or mantle cell lymphoma, the patient should not have an HLA-matched sibling, who would be an eligible donor, available.

3.2.6 History of prior mediastinal radiation

3.2.7 Reported illicit drug use

3.2.8 Vulnerable population in groups, i.e., prisoners, those lacking consent capacity, non-English speaking, illiterate, pregnant females.

3.3 Donor Inclusion Criteria

3.3.1 Donors must match at least one allele of HLA-A, B, C, DR ***and*** DP (or permissive mismatch in the case of DP) by high resolution typing. An HLA-matched family member is ineligible to serve as a donor. Eligible donors include biological parents, siblings, half-siblings or children.

3.3.2 Age ≥ 18 and ≤ 70 years.

3.3.3 Donors must meet the selection criteria prior to the start of the recipient's pre-transplant conditioning regimen as defined by the Foundation for the Accreditation of Cell Therapy (FACT) and will be screened according to the American Association of Blood Banks (AABB) guidelines and UW BMT program SOP.

3.4 Donor Exclusion Criteria

3.4.1 Recipient derived anti-donor HLA antibodies identified as "unacceptable" by Luminex assay. Exceptions may be made by the Director of the Histocompatibility Laboratory in conjunction with the recipient's physician and a plan for desensitization.

3.4.2 Not suitable for donation according to UW BMT program donor selection SOP.

3.4.3 Vulnerable population groups, i.e., prisoners, those lacking consent capacity, non-English speaking, illiterate, pregnant females.

4. Research Design and Methods

Allogeneic HCT is a potentially curative option for patients with relapsed aggressive lymphoma and relapsed indolent lymphoma. This study is a pilot study intended to study clinical outcome and toxicity of a novel transplant approach for patients with high-risk lymphomas. Research subjects will be identified among patients referred to the UWCCC for treatment of advanced lymphomas. The patients will be treated and followed within the UW BMT program according to our approved standard-operating-procedures for clinical management of patients undergoing HSCT. All clinical care will be provided by the UW BMT program and follow-up thereafter according to the clinical needs of the patients and what is considered standard of care. There will be no scheduled visits or evaluation on dates that do not correspond to those indicated by best practice.

4.1 Study Data Collection and Monitoring

The study will report clinical data using the OnCore database using electronic case report forms. These case report forms will be designed to collect specific information, including lab values, analysis of GVHD, chimerism data, staging evaluation, and survival data, in order to collect appropriate data to achieve the specific objectives of the study. Key study personnel are trained on the use of OnCore and will comply with protocol specific instructions and all measures to maintain confidentiality. In addition, specific transplant related information will be collected and recorded in the UW BMT program database according to the program standard operating procedures, which will assure confidentiality according to HIPAA regulations. Essential study documents will be retained in accordance with the University of Wisconsin record retention policy and all regulatory agencies that govern and oversee clinical trials.

4.2 Privacy and confidentiality

The collection of sensitive patient (study subject) information will be limited to the amount necessary to achieve the aims of the study. Records identifying subjects will be kept confidential and to the extent permitted by applicable laws and/or regulations will not be made public. If the results of the trial are published, subject identity will remain confidential.

5. Registration Procedures

5.1 Patients cannot begin protocol treatment prior to registration

Registration will occur in OnCore after the patient has signed the subject consent and eligibility is confirmed, but before any protocol treatment has been administered. Any patient signing consent, but not enrolled in the study, will be recorded as a screen failure. To be eligible for registration to this study, the patient must meet each criteria listed on the eligibility checklist based on the eligibility assessment documented in the patient's medical record.

5.2 Removal from study

Eligibility criteria will be verified and ineligible patients will proceed off study (i.e. will not be registered). No further follow up will be obtained in that situation. Patients may withdraw fully from the study at any point, however, given the nature of this trial, once the initial registration and intervention with conditioning followed by transplantation occurs, it is not advisable for a patient to withdraw from close follow up and management as is dictated in the protocol for their own well-being. However, if a patient still wishes to withdraw, they will be allowed to do so and advised to continue at least off protocol care per the BMT program standard operating procedures. If they choose this, no further follow up will be obtained for purposes of the study. Similarly, the investigator may remove a patient from study, however, in the best interest of the patient, this should only be done if the patient is not able to comply with follow up as dictated in the protocol and in that situation, it would be advised to follow at least off protocol care per the BMT program standard operating procedures. If this happens, no further follow up will be obtained for purposes of the study.

6. Treatment / Intervention Plan

Day -5 to -2	Fludarabine 40 mg/m^2 IV daily
Day -5 to -4	Cyclophosphamide 60 mg/kg IV daily, Mesna 60 mg/kg IV over 24 hours starting prior to cyclophosphamide
Day -1	Total Lymphoid Irradiation 7 Gy (in one fraction)
Day -1	Begin MMF (through Day +30)
Day 0	Peripheral Blood Stem Cell Transplant
Day +1	Second day of PBSC infusion (if needed, see Section 6.3)
Day +2	Begin Tacrolimus (through Day +180) ONLY if graft TCRα/β+ cell content is over 1 × 10^5 cells/kg ideal BW of the patient

Day +2	Rituximab 375 mg/m^2 IV once ONLY if graft B cell content exceeds 1 x 10^5 cells/kg ideal BW of the patient.

6.1 Dose Calculations

Dosing of all medications will be according to actual body weight.

6.2 Preparative Regimen

Fludarabine 40 mg/m^2/day will be administered by intravenous infusion over approximately 30 minutes on Days -5 through -2 for a total dose of 160 mg/m^2. **For patients who have an estimated or measured GFR < 70 mL/min/1.73 m^2, the fludarabine dose should be reduced by 20%.** Fludarabine dosing is based on the last calculated GFR prior to the start of conditioning. The fludarabine dose should be the same on Days -5 to -2, even if the patient's creatinine changes.

Cyclophosphamide 60 mg/kg/day will be administered by intravenous infusion over approximately 2 hours on Days -5 and -4 for a total dose of 120 mg/kg. Mesna 60 mg/kg will be administered IV over 24 hours on Days -5 and -4, starting prior to cyclophosphamide. **Cyclophosphamide should be given following fludarabine.**

Total Lymphoid Irradiation (TLI) is to be administered on Day -1. A single dose of 7 Gy is to be administered using 3D conformal radiation therapy or intensity-modulated radiation therapy as per standard UW techniques. TLI will encompass the cervical, supraclavicular, infraclavicular, axillary, hilar, mediastinal, para-aortic, iliac, and inguinal lymph node regions. In addition, the spleen will be included if present.

6.3 Preparation of the Hematopoietic Stem Cell Graft

Mobilization and Collection of Donor PBSC
Donors will self-administer G-CSF subcutaneously per UW SOP. Appropriate teaching will be provided. The morning of the stem cell blood product collection after subcutaneous injection of G-CSF, the donor will report to UW Hospital Infusion Center for laboratory work to include: WBC, Differential, Platelets, Hematocrit, and CD 34+ analysis. The donor will then undergo a 2-3 blood volume apheresis at the University of Wisconsin Hospital and Clinics Infusion Center.

Cell Processing

The manufacturing process of TCRα/β- and CD19-depleted cell grafts and quality control will be performed according to validated procedures and documented according to institutional guidelines. The graft will be depleted of α/β+T cells and CD19+ B cells using the CliniMACS® magnetic cell separation system, anti-CD19 microbeads and the anti-TCR α/β microbead kit containing biotinylated monoclonal anti-TCR α/β antibody and anti-biotin microbeads (Miltenyi Biotec) as described in the IDE, and the CliniMACS user manual (version 2.40, manual publication date: July 2011). Labeling and graft processing will be performed in accordance with the manufacturer's guidelines and SOPs developed by the Stem Cell Processing facility at University of Wisconsin. A median log >4 depletion of α/β T cells and log > 3 depletion of B cells is expected using this system, per published performance data provided by the manufacturer. The cellular composition of the graft will be evaluated by flow cytometry before and after the processing steps using extensive, lineage specific antibody panels. For further details on the mechanism of function of the CliniMACS device see the CMC section of the IDE.

The specification for the final formulation of the cell product has been set according to findings from previous studies [41, 42, 48, 50]. For transplantation, the following graft composition is targeted:

- Number of viable CD34+ cells ≥4 × 10^6 cells/kg ideal BW of the patient
- Number of CD3+ TCRα/β+ cells ≤1.5 × 10^5 cells/kg ideal BW of the patient
- Number of B cells ≤1 × 10^5 cells/kg ideal BW of the patient

The graft must contain ≥4 × 10^6 CD34+ cells/kg ideal BW of the patient. This will be prioritized in graft composition with the actions listed below if this or other targets cannot be jointly achieved.

- If after one day of stem cell collection and TCRα/β- and CD19-depletion, a minimum cell dose of 4 × 10^6 CD34+ cells/kg ideal BW of the patient is not reached, a second day of collection is allowed (on Day 0) and stem cells will be infused on Day +1, following initial infusion on Day 0.
- If after one day of stem cell collection and TCRα/β- and CD19-depletion, the number of CD3+ TCRα/β+ cells exceeds 1.5 × 10^5 cells/kg ideal BW of the patient, product will be reduced to bring the CD3+ TCRα/β+ cell count to ≤1.5 × 10^5 cells/kg ideal BW of the

patient. If in doing this, the CD34+ cell content falls below the above goal, a second day of collection is allowed (on Day 0) and stem cells will be infused on Day +1, following initial infusion on Day 0.

- For any graft with TCRα/β+ cell content greater than 1×10^5 cells/kg ideal BW of the patient, the patient will be placed on tacrolimus starting Day +2, in addition to MMF for immunosuppression. Specifications for tacrolimus dosing included in Section 6.5.

- If B cell content exceeds 1×10^5 cells/kg ideal BW of the patient, rituximab (375 mg/m2) will be administered post-transplant Day +2.

  If a second apheresis of the same donor needs to be performed, this would be known and performed on Day 0 and the second infusion administered on Day+1. A CD34 selection will be performed if a second day of collection is needed, rather than a TCRα/β- and CD19 cell depletion. The rationale and algorithm for cell processing based on graft content is further detailed in the CMC section of the IDE.

Packaging and Labeling
Labeling of final HSC graft product will be performed in accordance with the SOPs developed by the Stem Cell Processing facility at the University of Wisconsin. The graft is intended for direct administration after completion of the preparation process. However, if administration must be delayed for medical reasons, the product has a shelf-life of 72 hours, calculated from the end of apheresis with storage at 5 ± 3°C. The graft product will be delivered to the UW BMT Unit in sterile bags.

6.4 Stem Cell Transplant

On Day 0, patients will receive the HSC graft product. A sample of the product to be infused will be sent for flow cytometry to determine the content of CD34+, CD19 +, CD56+, TCRα/β, and TCRγ/δ cells.

Administration of stem cell product
For transplantation, patients will receive the TCRα/β- and CD19-depleted HSC graft intravenously on Day 0 after the appropriate premedication. Resuscitation equipment and emergency medications will be on hand in case of infusion reaction. If the graft contains less

than 4 x 10^6 CD34+ cells/kg patient BW, a second graft may be administered to boost the number of hematopoietic stem cells that the patient receives on Day +1.

Nursing staff will remain at the patient's bedside during the entire stem cell graft infusion. Patients will be monitored for adverse effects of the infusion such as rash, acute allergic reaction, bronchospasm, respiratory distress, and hemodynamic instability. If severe acute reactions occur (defined as CTCAE grade 4 - life-threatening consequences; urgent intervention indicated), the infusion will be stopped until the patient is stabilized. Monitoring and supportive care will be provided during the cell infusion according to institutional guidelines.

6.5 Graft-vs-Host Disease Prophylaxis

Alpha-beta T cell depletion will be performed on the stem cell product prior to infusion – as detailed above in Section 6.3. This in and of itself serves as a prevention of GVHD.

Mycophenolate Mofetil (MMF) will be given at a targeted dose of 20 mg/kg PO BID (based upon actual body weight and rounded up to the nearest pill size) with the maximum total daily dose not to exceed 3 grams (1.5g PO BID). MMF prophylaxis will be started on Day -1 and discontinued after the last dose on Day +30.

Tacrolimus (if applicable) will be given at a dose of 0.12 mg/kg/day oral in two divided doses (or 0.04 mg/kg/day IV if unable to tolerate oral) rounded to the nearest 0.5 mg with dose adjusted to maintain a trough level of 5-15 ng/mL beginning Day +2 until Day +180. Taper can be started at any point after day +90, as seen fit by the treating physician and in accordance with the BMT SOP, with a goal of the medication being completely discontinued by day 180, in the absence of GVHD. **Tacrolimus will ONLY be given if graft TCRα/β+ cell content is over 1×10^5 cells/kg ideal BW of the patient**

Rituximab (if applicable) will be administered at a dose of 375 mg/m^2 by intravenous infusion per standard protocol for rituximab infusion on Day +2 **ONLY if graft B cell content exceeds 1 x 10^5 cells/kg ideal BW of the patient. Per UW institutional practice, rituximab may be rounded to the nearest 50mg dose as long as within 10% of original calculated dose.**

6.6 Indwelling Central Venous Catheter

Patients will have a central venous catheter for the administration of IV medications and transfusion of blood products.

6.7 Supportive Care

Patients will receive the standard supportive care provided to HCT patients according to the UW BMT SOPs.

7. Adverse Event Reporting Requirements

7.1 Purpose

An adverse event (AE) is any untoward medical occurrence in a clinical investigation subject that is enrolled in a clinical trial. An AE may be any unfavorable or unintended sign, symptom, or disease temporally associated with participation in a clinical trial. While some events may not initially appear to be associated with the use of the study treatment, a relationship may not emerge until sufficient numbers of reports accumulate over the course of the study. Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner for timelier monitoring of patient safety and care. The following sections provide information about expedited reporting.

7.2 General AE reporting requirements

The study period during which all AEs and SAEs must be reported begins upon initiation of study treatment and ends at the 12 month post-transplant follow-up visit; Please note that with this Amendment version 04/24/2020, study follow-up has been extended to 24 months as specified in section 7.4.4. Both AEs and SAEs will be reported using Common Terminology Criteria for Adverse Events (CTCAE Version 4.0). In general, HSCT is performed by first administrating myelotoxic and immunosuppressive medications followed by infusion of allogeneic stem cells. There is a predictable and expected period of severe hematologic toxicity followed by recovery as the allogeneic stem cells engraft (grow) and produce blood cells. This early post-transplant toxicity is predictable, expected and typically resolved by one month after transplantation. Consequently, reporting requirements for expected AEs and SAEs until Day +30 will follow exceptions to SAE reporting guidelines outlined in

Table 2. Beyond Day +30 (Day 31 to 365), the AE and SAE reporting will be determined according to Table 1 below. In addition, section 7.4.4 has additional follow-up monitoring as delineated by protocol Amendment version 04/24/2020.

7.3 Serious Adverse Events (SAE) Review and Oversight Requirements

7.3.1 Serious Adverse Event – Reported Within 24 Hours

Serious Adverse Events requiring reporting within 24 hours (as described in the protocol) must also be reported to the Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu within one business day. The OnCore SAE Details Report must be submitted along with other report materials as appropriate (NCI CTEP-AERs form or FDA Medwatch Form #3500 and/or any other documentation available at that time of initial reporting). The DSMC Chair will review the information and determine if immediate action is required. Within 10 working days, all available subsequent SAE documentation must be submitted electronically along with a 24 hour follow-up SAE Details Report and a completed UWCCC SAE Routing Form to saenotify@uwcarbone.wisc.edu. All information is entered and tracked in the UWCCC database.

The Principal Investigator notifies all investigators involved with the study at the UWCCC, the IRB, the sponsor, and the funding agency (all as applicable) and provides documentation of these notifications to the DSMC.

If the SAE occurs on a clinical trial in which the UW PI serves as the sponsor-investigator, the PI reviews the event to determine whether the SAE requires reporting to the FDA and other participating investigators.

See Section 7.4 for detailed instructions on SAE reporting.

7.3.2 Serious Adverse Event – Reported within 10 Days

Serious Adverse Events requiring reporting within 10 days (as described in the protocol) must also be reported to the Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu. The OnCore SAE Details Report must be submitted along with other report materials as appropriate (NCI CTEP-AERs form or FDA Medwatch Form #3500 and/or any other documentation available at that time of initial reporting). The DSMC Chair will review the information and determine if further action is required. All information is entered and tracked in the UWCCC database.

The Principal Investigator notifies all investigators involved with the study at the UWCCC, the IRB, the sponsor, and the funding agency and provides documentation of these notifications to the DSMC.

If the SAE occurs on a clinical trial in which the UW PI serves as the sponsor-investigator, the PI reviews the event to determine whether the SAE requires reporting to the FDA and other participating investigators.

For a multiple-institutional clinical trial the PI is responsible for ensuring SAEs are reported to the FDA as well as to all participating investigators.

See Section 7.4 for detailed instructions on SAE reporting.

7.3.3 Sponsor-Investigator Responsibilities for SAE Review

The UWCCC Principal Investigator is acting as a Principle Investigator and Sponsor-Investigator. The IDE Holder assumes responsibilities of the study sponsor in accordance with FDA 21 CFR 312.32. In this capacity, the UWCCC PI reviews all reports of serious adverse events occurring on the study at the UWCCC and participating external sites and makes a determination of 1) **suspectedness** (i.e., whether there is a reasonable possibility that the device caused the AE); and 2) **unexpectedness** (the event is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed) in the context of this study. SAE with suspected causality to study device and deemed unexpected are reported as IDE Safety Reports by the UWCCC PI to the FDA, all participating investigators on the study, and the external global sponsor (if applicable) within 15 calendar days. All fatal or life-threatening SAE that are unexpected and have suspected causality to the study device will be reported by the UWCCC PI to the FDA, all participating investigators on the study, and the external global sponsor (if applicable) within 7 calendar days.

Refer to Section **7.4.3** for UWCCC PI instructions for reporting to the FDA.

7.3.4 Study Progress Review

Protocol Summary Reports (PSR) are required to be submitted to the DSMC in the timeframe determined by the risk level of the study (quarterly; semi-annually; or annually). The PSR provides a cumulative report of SAEs, as well as instances of non-compliance, protocol deviations, and unanticipated problems, toxicities and responses that have occurred on the protocol in the timeframe specified. PSRs for those protocols scheduled for review are reviewed at each DSMC meeting.

Protocol Summary Reports enable DSMC committee members to assess whether significant benefits or risks are occurring that would warrant study suspension or closure. This information is evaluated by the DSMC in conjunction with other reports of quality assurance activities (e.g., reports from Internal Audits, Quality Assurance Reviews, etc.) occurring since the prior review of the protocol by the DSMC. Additionally, the DSMC requires the study team to submit external DSMB or DSMC reports, external monitoring findings for industry-sponsored studies, and any other pertinent study-related information.

In the event that there is significant risk warranting study suspension or closure, the DSMC will notify the PI of the DSMC findings and ensure the appropriate action is taken for the protocol (e.g., suspension or closure). The DSMC ensures that the PI reports any temporary or permanent suspension of a clinical trial to the sponsor (e.g., NCI Program Director, Industry Sponsor Medical Monitor, Cooperative Group Study Chair, etc.) and other appropriate agencies. DSMC findings and requirements for follow-up action are submitted to the CRC.

7.4 Expedited Reporting of Serious Adverse Events

Depending on the nature, severity, and attribution of the serious adverse event an SAE report will be phoned in, submitted in writing, or both according to Table 1 below. All serious adverse events must also be reported to the UWCCC Data and Safety Monitoring Committee Chair. All serious adverse events must also be reported to the UW IRB (if applicable), and any sponsor/funding agency not already included in the list.

Determine the reporting time line for the SAE in question by using the following tables. Then refer to following sections A, B and C.

Table 1

FDA Reporting Requirements for Serious Adverse Events (21 CRF Part 312)
NOTE: Investigators MUST immediately report to *Peiman Hematti, IDE holder,* and any other parties outlined in the protocol ANY Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64).
An adverse event is considered serious if it results in ANY of the following outcomes:

1) Death.
2) A life-threatening adverse event.
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria* MUST be immediately reported to the UWCCC within the timeframes detailed in the table below:

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in hospitalization ≥ 24 hrs	10 Calendar Days			24-Hour; 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required		10 Calendar Days	

** Exceptions to SAE Reporting: Table 1 applies to events that occur 31 to 365 days after HSCT. Refer to Table 2 for SAE expedited reporting requirements within 30 days after HSCT. Specific protocol exceptions to expedited reporting (SPEER) are listed in the CAEPR Table below.* Per Amendment version 04/24/2020, see section 7.4.4 below. Reporting timelines do not apply because these events were not collected in real time. They will be collected and reported as they are reviewed by the study team with approval of this Amendment.

Expedited AE reporting timelines are defined as:

- 24-Hour; 5 Calendar Days – The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- 10 Calendar Days – A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE

Table 2: SAE reporting requirements for events that occur within 30 days after HSCT

	Grade 1 – 3	Grade 4	Grade 4	Grade 5	Grade 5
		Unexpected	**Expected**	**Unexpected**	**Expected**
Unrelated Unlikely	Not required	Not required	Not required	10 Calendar days	10 Calendar days

Possible Probable Definite	Not required	10 Calendar days	Not required	24-Hrs; 5 Calendar Days	10 Calendar days

7.4.1 SAE Requiring 24 Hour Reporting Occurs at UWCCC:

1. **Report to the UWCCC:**

   Reference the **SAE SOP** (Standard Operating Procedure) and the **SAE Reporting Workflow** on the UWCCC website (https://kb.wisc.edu/uwccc/internal) for specific instructions on how and what to report to the UWCCC for 24 hour initial and follow-up reports. **A follow-up report is required to be submitted within 10 days of the initial 24 hour report.**

   For this protocol, the following UWCCC entities are required to be notified:

   a) saenotify@uwcarbone.wisc.edu
   b) UWCCC PI, Vaishalee P Kenkre, MD: vpkenkre@medicine.wisc.edu
   c) Hematology DOT Program Manager
   d) Any other appropriate parties listed on the SAE Routing Form (for follow-up reports only)

2. **Report to the IRB:**
   Consult the UW-IRB website for reporting guidelines (https://kb.wisc.edu/hsirbs)

7.4.2 SAE Requiring 10 Day Reporting Occurs at UWCCC:

1. **Report to the UWCCC:**
   Reference the **SAE SOP** and the **SAE Reporting Workflow** on the UWCCC website (https://kb.wisc.edu/uwccc/internal) for specific instructions on how and what to report to the UWCCC for 10 day reports.

   For this protocol, the following UWCCC entities are required to be notified:

   a) saenotify@uwcarbone.wisc.edu
   b) Any appropriate parties listed on the SAE Routing Form

2. **Report to the IRB:**
   Consult the UW-IRB website for reporting guidelines (https://kb.wisc.edu/hsirbs)

7.4.3 Other Reporting Requirements

Reporting to the FDA:
The FDA does not need to be automatically notified of every SAE that occurs during the clinical trial. For every SAE, the PI (or Sub-I in absence of the PI) must complete the UWCCC Sponsor-Investigator Determination of FDA Reporting for Institutional Trials. The PI or Sub-I will evaluate the SAE to determine if reporting to the FDA is necessary.

SAE with suspected causality to study device and deemed unexpected are reported as IDE Safety Reports by the UWCCC PI to the FDA, all participating investigators on the study, and the external global sponsor (if applicable) within 15 calendar days. All fatal or life-threatening SAE that are unexpected and have suspected causality to the study device will be reported by the UWCCC PI to the FDA, all participating investigators on the study, and the external global sponsor (if applicable) within 7 calendar days.

Reportable Serious Adverse Events occurring on studies on which a UW PI is acting as sponsor-investigator must be reported to the FDA within the appropriate time frame. Mandatory and voluntary reporting guidelines and instructions are outlined on the FDA website:
http://www.fda.gov/Safety/MedWatch/HowToReport/default.htm

7.4.4 Extended Safety Monitoring

Per Amendment version 04/24/2020, study follow-up is being retroactively extended to 2 years post-transplant. Reporting timelines listed above for UWCCC, IRB, and FDA cannot be applied, because these data points are being collected retrospectively. They will be collected and reported as they are reviewed by the study team following the approval of the amendment. In the additional year of follow up that is being added, data on the following will be specifically collected and entered at 18 months and 24 months post-transplant:

Survival (alive/dead)

Disease status (progression or stable)

Start of any further disease directed therapy

Grade 2-3 infections in the prior 6 month period

Hospitalizations in the prior 6 month period

Non protocol specified transplant

Secondary graft failure

Reportable adverse events (what was previously defined in Table 1 as SAEs reportable to DSMC)

Unanticipated problems reportable to IRB

Secondary cancers

7.5 Risks and Toxicities

Fludarabine – myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, autoimmune hemolytic anemia, autoimmune thrombocytopenia, paresthesias, peripheral neuropathy, renal and pulmonary toxicity (interstitial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered almost exclusively after very high doses of fludarabine monophosphate. Tumor lysis syndrome, complicating fludarabine monophosphate therapy has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed in both pre-treated patients receiving fludarabine and in individuals receiving fludarabine combined with other agents.

Cyclophosphamide – cardiomyopathy, skin rash, mucositis, sterility, fluid weight gain/edema, alopecia and hemolytic/anemia. Hematologic including leukopenia, thrombocytopenia, anemia, pancytopenia; gingivitis, glossitis, pharyngitis, stomatitis, enteritis; nausea/vomiting, anorexia, diarrhea; hematemesis, melena; photosensitivity; nephropathy: hemorrhagic cystitis, dysuria, azotemia, hematuria, renal failure.

Total Lymphoid Irradiation – acute - fatigue, nausea & vomiting, diarrhea, mild skin redness, dry mouth; late – secondary malignancy, early cardiac disease, infertility, azoospermia, menopause induction

Mycophenolate Mofetil – pancytopenia, nausea, vomiting, diarrhea, hypertension, headache, dizziness, insomnia, hyperglycemia, electrolyte imbalances, rash, and leg cramps/bone pain, reversible renal insufficiency, hypertension, hyperglycemia, hypomagnesemia, hypokalemia, and neurologic toxicity.

Tacrolimus – reversible renal insufficiency, hypertension, hyperglycemia, hypomagnesemia, hyperkalemia, and neurological toxicity (tremor and headache)

Rituximab – Immediate: Fever, chills, rigors (especially with first dose), nausea, asthenia, lethargy, malaise, vomiting, headache, throat irritation, abdominal pain, back pain, hypotension, hypertension, arrhythmias, diarrhea, rash during infusion, cough, bronchospasm, dyspnea, rhinitis, dizziness, night sweats, myalgia, arthralgia, pruritis, urticarial, anxiety, flushing, syncope, anaphylactic reactions, fatal infusion reaction complex (including hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fib or cardiogenic shock), fatal cardiovascular events (in rheumatoid arthritis patients), tumor lysis syndrome (especially with > 25,000/mm circulating malignant cells or high tumor burden) and associated renal failure, serum sickness, hypocalcemia, seizure; Prompt: Lymphopenia, infectious events (bacterial, viral, fungal), Leukopenia, neutropenia, angioedema, peripheral edema, hyperglycemia, elevated LDH, sinusitis, Thrombocytopenia, anemia, transient red cell aplasia (1 case), hemolytic anemia (2 cases), mucocutaneous reactions including paraneoplastic pemphigus, Stevens-Johnson syndrome, lichenoid dermatitis, vesiculobullous dermatitis, toxic epidermal necrolysis, Delayed to late: Bowel obstruction and/or perforation, late onset neutropenia, fatal cardiac failure, bronchiolitis obliterans, interstitial pneumonitis, hepatitis B virus reactivation with fulminant hepatitis, hepatic failure, and death, (new, reactivated, or exacerbated) viral infections (including JC virus, CMV, herpes simplex, parvovirus B19, VZV, West Nile virus [WNV], hepatitis C), Waldenstrom's (hyperviscosity), progressive multifocal leukoencephalopathy (PML) caused by activation of the JC virus.

Comprehensive Adverse Events and Potential Risks (CAEPR) List for BMT.

Adverse Events with at least possible relationship to allogeneic stem cell transplantation			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (> 20%)	**Less Likely (<20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Pancytopenia			Anemia, thrombocytopenia, neutropenia (gr.3)
Febrile neutropenia			Febrile neutropenia (gr. 3,4 before day 30)
	Immune Hemolytic anemia		
		Microangiopathic hemolytic anemia	
CARDIAC DISORDERS			
	Atrial fibrillation		
GASTROINTESTINAL DISORDERS			
	Abdominal pain		Diarrhea (gr. 3)
	Constipation		Nausea/vomiting (gr.3)
Diarrhea			
Oral mucositis			
Nausea and vomiting			
Anorexia			Anorexia (gr. 4 before day 30)
Colitis			
GENERAL DISORDERS			
Edema			
Fever			
		Multi-organ failure	
Pain			
	Non-cardiac chest pain		
HEPATOBILIARY DISORDERS			
		Hepatic failure	
		Veno-occlusive disease	
IMMUNE SYSTEM DISORDERS			
Graft-vs-host disease			
		Cytokine release syndrome	
INFECTIONS AND INFESTTIONS			
	Bladder infection		
	Catheter related infection		
	Bacteremia		

Adverse Events with at least possible relationship to allogeneic stem cell transplantation			Specific Protocol Exceptions to Expedited Reporting (SPEER)
		Sepsis	
	Lung infection		
	Pustular rash		
	Sinusitis		
INVESTIGATIONS			
	AST, ALT, bili, Alkaline phosphatase increased		
	Creatinine increased		
	Lymphocytes decreased		Lymphocytosis (gr. 3)
	Weight gain/loss		
	WBC, HGB, PLTs decreased		Anemia, thrombocytopenia, neutropenia (gr.3)
METABOLISM AND NUTRITIONAL DISORDERS			
	Multiple electrolyte abnormalities		Dehydration (gr.3), Hyponatremia (gr.3), hypokalemia (gr.3)
	Iron overload		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
		Avascular necrosis	
		Fibrosis deep connective tissue	
	Muscle weakness		
	Osteoporosis		
RENAL AND URINARY DISORDERS			
		Acute kidney injury	
	Proteinuria		
REPRODUCTIVE SYSTEM			
Azoospermia			
Dysmenorrhea			
Infertility			
RESPIRATORY DISORDERS			
		ARDS	
		Bronchiolitis obliterans (bronchial obstruction)	
		Pneumonitis	
		Pulmonary edema	
		Pulmonary fibrosis	
		Respiratory failure	
SKIN AND SUBCUTANEOUS DISORDERS			
	Erythroderma		
	Skin induration		
	Rash maculo-papular		

Adverse Events with at least possible relationship to allogeneic stem cell transplantation			Specific Protocol Exceptions to Expedited Reporting (SPEER)
VASCULAR DISORDERS			
	Capillary leak syndrome		
	Hypertension		
	Thromboembolic event		

8. Study Endpoints

8.1 Primary Endpoint

The primary endpoint is engraftment at 28 days from the time of transplantation. Neutrophil engraftment is defined as ANC ≥ 500/mcl for three consecutive measurements on different days. The first of the three days will be designated the day of recovery. The only competing event for neutrophil recovery is death without neutrophil recovery. Platelet engraftment is defined as the first day of a platelet count > 20,000/mm^3 with no platelet transfusions in the preceding 7 days

8.2 Secondary Endpoints

Acute GVHD – The cumulative incidence of grade III – IV acute GVHD by Day +100 will be determined. The GVHD grade will be determined by the IBMTR Severity Index criteria (Appendix A).

Chronic GVHD – The cumulative incidence of severe chronic GVHD by Day +180 will be recorded and defined according to the NIH consensus criteria (Appendix B).

Graft failure – defined as < 5% donor chimerism in the CD3 and/or CD33 selected cell populations at any time during the study follow up period once initial engraftment has been achieved.

Treatment-related mortality – defined as death from any cause other than disease progression

Progression-free survival – defined as time before any progression by either PET/CT or bone marrow (Appendix C).

Overall survival – after enrollment on study

9. Schedule of Evaluations

All evaluations are considered part of the standard of care evaluation of a patient for allogeneic stem cell transplantation, except the TCRα/β and TCRγ/δ portion of the lymphocyte subset analysis, which is considered for research purposes. Informed consent must be obtained within 42 days of registration, all other pre-transplant evaluations must be completed within 28 days of registration unless otherwise stated below. Scheduled evaluations post-transplant may be performed within 3 days from the targeted date prior to Day +28 and within 7 days thereafter.

	Pre-BMT Evaluation	**Weekly – Day +1 to engraftment[12]**	**Day 28 Restaging**	**Weekly Day +28 to Day +84**	**3 month restaging (Day +91 – Day +105)**	**Months 6, 9, 12[16]**
Informed Consent	X					
Medical History[1]	X					
Toxicity Assessment	X	X	X	X	X	X
Physical Exam	X	X	X	X	X	X
Performance Status	X		X	At month 2	X	X
GVHD evaluation[2]		Twice weekly	X	X	X	X
CMV PCR[3]		X	X	X	X	X
EBV PCR			X	Every 2 weeks	X	X
CBC	X	Twice weekly[13]	X	X	X	X
Magnesium	X	Twice weekly		X	X	X
Total bilirubin	X	X	X	X	X	X
AST	X	X	X	X	X	X
ALT	X	X	X	X	X	X
Alkaline phosphatase	X					
Electrolytes, Creatinine, BUN	X	Twice weekly		X	X	X
Quantitative Immunoglobulins	X				X	
Uric acid, INR, Ferritin[4], Calcium, Phosphate	X					
Lymphocyte subset analysis[5]			X		X	X[5]
Tacrolimus level (if on this medication)		X	X	X	X	X[14]
Pre-transplant viral panel[6]	X					

	Pre-BMT Evaluation	Weekly – Day +1 to engraftment[12]	Day 28 Restaging	Weekly Day +28 to Day +84	3 month restaging (Day +91 – Day +105)	Months 6, 9, 12[16]
Urinalysis with microscopy and culture if indicated	X					
Pregnancy test[7]	X					
Luminex screen[8]	X					
CD3, CD33 STR (PB)	X		X	At month 2	X	X
Panorex[9]	X					
CT sinuses	X					
Disease Restaging by PET or CT scan[10]	X				X	X[15]
BM aspirate and biopsy	X					
ECG	X					
Cardiac ECHO	X					
PFTs with ABG	X					
LP[11]	X					

1. Patients should be specifically asked if they engage in illicit drug use and their answer should be documented in the medical chart.
2. Refer to Appendix A for acute GVHD grading scales
3. Weekly CMV PCR starting Day +14 for those at risk for CMV infection (CMV positive donor or recipient) until Day +100 and then at 6, 9 and 12 months
4. Ferritin is only required if recipient has a prior history of RBC transfusions.
5. Lymphocyte subset analysis includes by flow cytometry the cell counts/percentages of: CD3+, TCRα/β, TCRγ/δ, CD4, CD8, CD19 +, CD16/CD56. Testing to be completed by 1 month, 3 months, 6 months, and 12 months post HCT.
6. Viral panel to include: HIV 1 and 2 antibody; HTLV I/II antibody screen; Hepatitis A total antibody, if positive IgM Ab; Hepatitis B surface antigen, surface antibody and core antibody; Hepatitis C antibody, if positive Hepatitis C PCR; Syphilis testing, if positive repeat to confirm; Toxoplasma antibody IgM. For patients negative for CMV or not previously tested, order CMV IgG/IgM. For patients who have been previously tested and positive, order CMV PCR. For Hodgkin's patients only, order EBV IgG and IgM antibodies.
7. Urine or serum pregnancy test within 14 days of registration ONLY for females of childbearing potential. A female of childbearing potential (FCBP) is a sexually mature female who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).
8. Consider ordering luminex upon identification of donors to help facilitate donor selection. May then need to be repeated within 28 days of protocol registration.
9. Separate Panorex scan not required if CT sinus includes an evaluation of teeth.
10. Pre-transplant disease restaging by PET, rather than CT, is suggested for all disease types other than SLL/CLL, but is not required. A CT is acceptable if PET cannot be attained. Post-transplant disease restaging for all disease types may be performed by CT, but (for all disease types except SLL/CLL) is preferable by PET until complete remission has been attained. At all time points the type of imaging used for disease restaging is left to physician discretion.

11. LP to be performed within 14 days of registration ONLY for those patients who meet criteria described in Exclusion Criteria 3.3.1 (i.e., if clinical suspicion of CNS involvement, then LP needed to rule out. If patient previously had involvement, but 2 negative LPs as stated, and no further suspicion of recurrence, no need to repeat LP at this time.)
12. Twice weekly assessments should be performed until patient meets criteria for both neutrophil and platelet engraftment.
13. During the transplant admission, it is permissible to obtain a wbc, hemoglobin and platelet count twice weekly in lieu of a full CBC until the patient shows signs of cell recovery (to be determined by clinical staff), at which point a full CBC will be ordered to monitor for neutrophil engraftment. WBC count not required until Day +7.
14. Tacrolimus level only required (if applicable) at month 6.
15. Disease restaging by PET or CT only required at months 6 and 12.
16. Data to be collected at months 18 and 24 as described in section 7.4.4

10. Correlative Assays

Reconstitution of T, B and NK cell subsets (lymphocyte subset analysis) will be assessed by immune cell phenotyping. Cell counts/percentages of CD3+, TCRα/β, TCRγ/δ, CD4, CD8, CD19+, CD16//CD56 will be evaluated using samples collected at 1 month, 3 months, 6 months, and 12 months post-transplant (see Section 11.6 Correlative Analysis).

11. Statistical Considerations

11.1 Objectives

As a pilot trial, the objective is to generate preliminary data on engraftment after TCRα/β cell depletion from a haploidentical cell product, and also observe clinical outcome such as acute GVHD, chronic GVHD, graft failure, treatment-related mortality, progression-free survival, overall survival and immune markers listed in Section 10.0.

11.2 Endpoints

The primary endpoint of the study is engraftment at 28 days after transplant. Any patient who is enrolled and receives HHCT will be accounted for in the final assessment. Secondary endpoints include aGVHD grade III-IV by Day +100, cGVHD (severe) at Day +180, graft failure at any point during study follow up and after initial engraftment, treatment-related mortality, progression-free survival, and overall survival. Immune markers as measured by cell counts of CD3+, TCRα/β, TCRγ/δ, CD4, CD8, CD19+, CD16, and CD56 will be obtained 1 month, 3 months, 6 months, and 12 months post-transplant.

11.3 Analysis plan

Times to event such acute and chronic GVHD, graft failure, treatment-related mortality, PFS, and OS will be analyzed using Kaplan-Meier (KM) method, and the incidence rates of Day +28 engraftment, Day +100 aGVHD grade III-IV, Day +180 cGVHD (severe), and Day +100 OS will be obtained from the KM estimates along with 95% confidence intervals. Binary outcome variables such as grade 3 or worse non-hematologic toxicity, will be summarized with a proportion and a 95% confidence interval.

11.4 Sample size justification

The sample size is limited by the feasibility over 2 years. Fourteen transplant subjects and 14 donor subjects will be enrolled into the trial. With 14 transplant subjects, the trial will have 0.86 power to reject the null hypothesis that the probability of 28-day engraftment p is ≤ 0.7 in favor of the alternative hypothesis that p is ≥ 0.9 according to a one-tailed test at a significance level of 0.2. Considering this being a limited pilot study, it was felt important to have a reasonably high power at the expense of a significance level much higher than typical.

Given the sample size of 14, the study has 0.93 power to reject the null hypothesis that the probability of 100-day aGVHD grade III-IV p is ≥ 0.6 in favor of the alternative hypothesis p is ≤ 0.3 according to a one-tailed test at a significance level of 0.2. Similarly, the study has 0.93 power to reject the null hypothesis that the probability of 180-day cGVHD p is ≥ 0.7 in favor of the alternative hypothesis p is ≤ 0.4 according to a one-tailed test at a significance level of 0.2.

11.5 Sequential monitoring of engraftment failure and GVHD

In order to ensure that patients are not subject to treatment with higher engraftment failure and higher acute or chronic GVHD, engraftment failure and GVHD will be monitored continuously based on sequential probability ratio tests (SPRTs). If the engraftment failure rate or acute or chronic GvHD rate appears high by crossing the SPRT boundary for the null hypothesis for any of the three outcome measures, the study will be suspended and the UWCCC Data and Safety Monitoring Committee (DSMC) will be notified. After investigation of the outcome as of the SPRT boundary crossing, the PI in consultation with other investigators, study statistician, and the UWCCC DSMC will make the

decision whether to continue the trial or to terminate for patient safety concern.

The following three tables give the SPRT boundaries for suspension of the study for 28 day engraftment failure, 100 day aGVHD grade III-IV and 180 day severe cGVHD. No adjustment for multiplicity for three outcome measures has been made as being anti-conservative is desirable to ensure adequate treatment and safety of patients.

Number of	SPRT boundaries* for 28-day engraftment failure													
Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Events		2	2	3	3	3	3	3	3	4	4	4	4	4

Number of	SPRT boundaries for 100-day aGVHD grade III-IV													
Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Events				4	5	5	6	6	6	7	7	8	8	9

Number of	SPRT boundaries for 180-day severe cGVHD													
Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Events					5	6	6	7	7	8	9	9	10	10

* If the number patients with event is equal to boundary, suspend the study.
Blank entries imply no decision can be made due to insufficient data.

11.6 Correlative analysis

Longitudinal analysis will be performed on the immune markers using repeated measures generalized mixed linear models. Also analysis of association between immune markers and clinical outcomes be performed to evaluate whether the trajectory of the immune markers predict clinical outcome. These correlative analyses will be exploratory in nature.

12. Record Retention

The UW BMT Program maintains a HIPAA compliant clinical database that is used to comply with FACT and Center for International Blood and Marrow Transplant Research (CIBMTR) reporting requirements. Patient, disease, treatment and outcome data are collected and reported as required by regulatory and quality reporting requirements. Patients will also be registered in the OnCore database within the UWCCC.

13. Patient Consent and Peer Judgment

Current FDA, NCI, state, federal and institutional regulations concerning informed consent will be followed.

14. Data and Safety Monitoring

Oversight and Monitoring Plan

The UWCCC Data and Safety Monitoring Committee (DSMC) is responsible for the regular review and monitoring of all ongoing clinical research in the UWCCC. A summary of DSMC activities are as follows:

- Reviews all clinical trials conducted at the UWCCC for subject safety, protocol compliance, and data integrity.
- Reviews all Serious Adverse Events (SAE) requiring expedited reporting, as defined in the protocol, for all clinical trials conducted at the UWCCC, and studies conducted at external sites for which UWCCC acts as an oversight body.
- Reviews all reports generated through the UWCCC DSMS elements (Internal Audits, Quality Assurance Reviews, Response Reviews, Compliance Reviews, and Protocol Summary Reports)
- Notifies the protocol Principal Investigator of DSMC decisions and, if applicable, any requirements for corrective action related to data or safety issues.
- Notifies the CRC of DSMC decisions and any correspondence from the DSMC to the protocol Principal Investigator.
- Works in conjunction with the UW Health Sciences IRB in the review of relevant safety information as well as protocol deviations, non-compliance, and unanticipated problems reported by the UWCCC research staff.
- Ensures that notification of SAEs requiring expedited reporting is provided to external sites participating in multi-institutional clinical trials coordinated by the UWCCC.

Monitoring And Reporting Guidelines

Data related to these trials undergo review of subject safety at regularly scheduled DOT meetings where the results of each subject's treatment are discussed and the discussion is documented in the DOT meeting minutes. The discussion includes the number of subjects enrolled, significant toxicities, dose adjustments, and responses observed. Protocol Summary Reports are submitted by the study team for review by the DSMC. Any unanticipated problems, complications, and adverse events will be reported to the investigator upon occurrence, and reported to the IRB in accordance with posted guidance.

UWCCC quality assurance and monitoring activities are determined by study sponsorship and risk level of the protocol as determined by the PRMC. All protocols (including Intervention Trials, Non-Intervention Trials, Behavioral and Nutritional Studies, and trials conducted under a Training Grant) are evaluated by the PRMC at the time of committee review. UWCC monitoring requirements for trials without an acceptable external DSMB are as follows:

a) Intensive Monitoring

Protocols subject to intensive monitoring generally include UW Institutional Phase I and Institutional Trials of any phase involving recombinant DNA/gene transfer. These protocols undergo continuous review of data and subject safety at weekly Phase I/Disease Oriented Team (DOT) meetings where the results of each subject's treatment are discussed and the discussion is documented in the DOT meeting minutes. The discussion includes the number of subjects enrolled, significant toxicities, dose adjustments, and responses observed. Protocol Summary Reports are submitted on a quarterly basis by the study team for review by the DSMC.

15. References

1. Feugier, P., et al., *Long-term results of the R-CHOP study in the treatment of elderly patients with diffuse large B-cell lymphoma: a study by the Groupe d'Etude des Lymphomes de l'Adulte.* J Clin Oncol, 2005. **23**(18): p. 4117-26.
2. Pfreundschuh, M., et al., *CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group.* Lancet Oncol, 2006. **7**(5): p. 379-91.
3. Engert, A., et al., *Escalated-dose BEACOPP in the treatment of patients with advanced-stage Hodgkin's lymphoma: 10 years of follow-up of the GHSG HD9 study.* J Clin Oncol, 2009. **27**(27): p. 4548-54.
4. Philip, T., et al., *High-dose therapy and autologous bone marrow transplantation after failure of conventional chemotherapy in adults with intermediate-grade or high-grade non-Hodgkin's lymphoma.* N Engl J Med, 1987. **316**(24): p. 1493-8.
5. Philip, T., et al., *Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma.* N Engl J Med, 1995. **333**(23): p. 1540-5.
6. Atkins, C.D., *Autologous bone marrow transplantation in relapsed non-Hodgkin's lymphoma.* N Engl J Med, 1996. **334**(15): p. 990; author reply 991-2.
7. Seshadri, T., et al., *Salvage therapy for relapsed/refractory diffuse large B cell lymphoma.* Biol Blood Marrow Transplant, 2008. **14**(3): p. 259-67.
8. Rancea, M., et al., *High-dose chemotherapy followed by autologous stem cell transplantation for patients with relapsed/refractory Hodgkin lymphoma.* Cochrane Database Syst Rev, 2013. **6**: p. CD009411.
9. Josting, A., et al., *Treatment of primary progressive Hodgkin's and aggressive non-Hodgkin's lymphoma: is there a chance for cure?* J Clin Oncol, 2000. **18**(2): p. 332-9.
10. Chopra, R., et al., *Autologous versus allogeneic bone marrow transplantation for non-*

Hodgkin's lymphoma: a case-controlled analysis of the European Bone Marrow Transplant Group Registry data. J Clin Oncol, 1992. **10**(11): p. 1690-5.
11. Doocey, R.T., et al., *Allogeneic haematopoietic stem-cell transplantation for relapsed and refractory aggressive histology non-Hodgkin lymphoma.* Br J Haematol, 2005. **131**(2): p. 223-30.
12. Dann, E.J., C.K. Daugherty, and R.A. Larson, *Allogeneic bone marrow transplantation for relapsed and refractory Hodgkin's disease and non-Hodgkin's lymphoma.* Bone Marrow Transplant, 1997. **20**(5): p. 369-74.
13. van Besien, K., et al., *Allogeneic transplantation for recurrent or refractory non-Hodgkin's lymphoma with poor prognostic features after conditioning with thiotepa, busulfan, and cyclophosphamide: experience in 44 consecutive patients.* Biol Blood Marrow Transplant, 1997. **3**(3): p. 150-6.
14. Ratanatharathorn, V., et al., *Prospective comparative trial of autologous versus allogeneic bone marrow transplantation in patients with non-Hodgkin's lymphoma.* Blood, 1994. **84**(4): p. 1050-5.
15. Sarina, B., et al., *Allogeneic transplantation improves the overall and progression-free survival of Hodgkin lymphoma patients relapsing after autologous transplantation: a retrospective study based on the time of HLA typing and donor availability.* Blood, 2010. **115**(18): p. 3671-7.
16. Sureda, A., et al., *Reduced-intensity conditioning compared with conventional allogeneic stem-cell transplantation in relapsed or refractory Hodgkin's lymphoma: an analysis from the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation.* J Clin Oncol, 2008. **26**(3): p. 455-62.
17. Peggs, K.S., et al., *Clinical evidence of a graft-versus-Hodgkin's-lymphoma effect after reduced-intensity allogeneic transplantation.* Lancet, 2005. **365**(9475): p. 1934-41.
18. Alvarez, I., et al., *Nonmyeloablative stem cell transplantation is an effective therapy for refractory or relapsed hodgkin lymphoma: results of a spanish prospective cooperative protocol.* Biol Blood Marrow Transplant, 2006. **12**(2): p. 172-83.
19. Khouri, I.F., et al., *Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality.* Blood, 2001. **98**(13): p. 3595-9.
20. Khouri, I.F., et al., *Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies.* J Clin Oncol, 1998. **16**(8): p. 2817-24.
21. Rodriguez, R., et al., *Comparison of reduced-intensity and conventional myeloablative regimens for allogeneic transplantation in non-Hodgkin's lymphoma.* Biol Blood Marrow Transplant, 2006. **12**(12): p. 1326-34.
22. Rezvani, A.R., et al., *Nonmyeloablative allogeneic hematopoietic cell transplantation in relapsed, refractory, and transformed indolent non-Hodgkin's lymphoma.* J Clin Oncol, 2008. **26**(2): p. 211-7.
23. Khouri, I.F., et al., *Eight-year experience with allogeneic stem cell transplantation for relapsed follicular lymphoma after nonmyeloablative conditioning with fludarabine, cyclophosphamide, and rituximab.* Blood, 2008. **111**(12): p. 5530-6.
24. PJ, M., *Overview of Marrow Transplantation Immunology*, in *Thomas' Hematopoietic cell transplantation*, F.S. Blume KG, Appelbaum FR, Editor. 2004, Blackwell Science: Malden, MA. p. 16-30.
25. Anasetti, C., et al., *Effect of HLA incompatibility on graft-versus-host disease, relapse, and survival after marrow transplantation for patients with leukemia or lymphoma.* Hum Immunol, 1990. **29**(2): p. 79-91.
26. Bachar-Lustig, E., et al., *Megadose of T cell-depleted bone marrow overcomes MHC barriers in sublethally irradiated mice.* Nat Med, 1995. **1**(12): p. 1268-73.
27. Aversa, F., et al., *Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse.* J Clin Oncol, 2005. **23**(15): p. 3447-54.
28. Aversa, F., et al., *Treatment of high-risk acute leukemia with T-cell-depleted stem cells*

from related donors with one fully mismatched HLA haplotype. N Engl J Med, 1998. **339**(17): p. 1186-93.
29. Lamb, L.S., Jr. and R.D. Lopez, *gammadelta T cells: a new frontier for immunotherapy?* Biol Blood Marrow Transplant, 2005. **11**(3): p. 161-8.
30. Bethge, W.A., et al., *Haploidentical allogeneic hematopoietic cell transplantation in adults with reduced-intensity conditioning and CD3/CD19 depletion: fast engraftment and low toxicity.* Exp Hematol, 2006. **34**(12): p. 1746-52.
31. Bethge, W.A., et al., *Haploidentical allogeneic hematopoietic cell transplantation in adults using CD3/CD19 depletion and reduced intensity conditioning: an update.* Blood Cells Mol Dis, 2008. **40**(1): p. 13-9.
32. Barfield, R.C., et al., *A one-step large-scale method for T- and B-cell depletion of mobilized PBSC for allogeneic transplantation.* Cytotherapy, 2004. **6**(1): p. 1-6.
33. Benaim, E., et al., *Rapid engraftment after reduced intensity conditioning (RIC) and transplantation of T-cell depleted large numbers of CD34+ stem and CD56+ natural killer (NK) cells obtained from mobilized haploidentical donors*, in *ASH*2003.
34. Chen, X., et al., *Rapid immune reconstitution after a reduced-intensity conditioning regimen and a CD3-depleted haploidentical stem cell graft for paediatric refractory haematological malignancies.* Br J Haematol, 2006. **135**(4): p. 524-32.
35. G, H., et al., *CD3 depleted hematopoietic peripheral blood stem cell grafts in children with refractory hematologic malignancies undergoing transplantation from mismatched related donors*, in *ASH*2005.
36. Ruggeri, L., et al., *Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants.* Science, 2002. **295**(5562): p. 2097-100.
37. Ruggeri, L., et al., *Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value.* Blood, 2007. **110**(1): p. 433-40.
38. Lang, P., et al., *Transplantation of a combination of CD133+ and CD34+ selected progenitor cells from alternative donors.* Br J Haematol, 2004. **124**(1): p. 72-9.
39. Ruggeri, L., et al., *Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation.* Blood, 1999. **94**(1): p. 333-9.
40. Morrison, B.E., et al., *Chemokine-mediated recruitment of NK cells is a critical host defense mechanism in invasive aspergillosis.* J Clin Invest, 2003. **112**(12): p. 1862-70.
41. Cook, M., et al., *Donor KIR genotype has a major influence on the rate of cytomegalovirus reactivation following T-cell replete stem cell transplantation.* Blood, 2006. **107**(3): p. 1230-2.
42. Beetz, S., et al., *Human gamma delta T cells: candidates for the development of immunotherapeutic strategies.* Immunol Res, 2007. **37**(2): p. 97-111.
43. Blazar, B.R., et al., *Murine gamma/delta-expressing T cells affect alloengraftment via the recognition of nonclassical major histocompatibility complex class Ib antigens.* Blood, 1996. **87**(10): p. 4463-72.
44. Bonneville, M. and E. Scotet, *Human Vgamma9Vdelta2 T cells: promising new leads for immunotherapy of infections and tumors.* Curr Opin Immunol, 2006. **18**(5): p. 539-46.
45. Drobyski, W.R., D. Majewski, and G. Hanson, *Graft-facilitating doses of ex vivo activated gammadelta T cells do not cause lethal murine graft-vs.-host disease.* Biol Blood Marrow Transplant, 1999. **5**(4): p. 222-30.
46. Glucksberg, H., et al., *Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors.* Transplantation, 1974. **18**(4): p. 295-304.
47. Klingebiel, T., et al., *Results and factors influencing outcome after fully haploidentical hematopoietic stem cell transplantation in children with very high-risk acute lymphoblastic leukemia: impact of center size: an analysis on behalf of the Acute Leukemia and Pediatric Disease Working Parties of the European Blood and Marrow Transplant group.* Blood, 2010. **115**(17): p. 3437-46.
48. Viey, E., et al., *Phosphostim-activated gamma delta T cells kill autologous metastatic renal cell carcinoma.* J Immunol, 2005. **174**(3): p. 1338-47.

49. Zanjani, E.D., et al., *Reversible expression of CD34 by adult human bone marrow long-term engrafting hematopoietic stem cells.* Exp Hematol, 2003. **31**(5): p. 406-12.
50. Bornhauser, M., et al., *Stable engraftment after megadose blood stem cell transplantation across the HLA barrier: the case for natural killer cells as graft-facilitating cells.* Transplantation, 1999. **68**(1): p. 87-8.
51. Grimes, H.L., et al., *Graft facilitating cells are derived from hematopoietic stem cells and functionally require CD3, but are distinct from T lymphocytes.* Exp Hematol, 2004. **32**(10): p. 946-54.
52. Fugier-Vivier, I.J., et al., *Plasmacytoid precursor dendritic cells facilitate allogeneic hematopoietic stem cell engraftment.* J Exp Med, 2005. **201**(3): p. 373-83.
53. Jacquet, E.G., et al., *Facilitating cells as a venue to establish mixed chimerism and tolerance.* Pediatr Transplant, 2003. **7**(5): p. 348-57.
54. Tanaka, J., et al., *The role of accessory cells in allogeneic peripheral blood stem cell transplantation.* Int J Hematol, 1999. **69**(2): p. 70-4.
55. Gottschalk, S., C.M. Rooney, and H.E. Heslop, *Post-transplant lymphoproliferative disorders.* Annu Rev Med, 2005. **56**: p. 29-44.
56. Cutler, C. and J.H. Antin, *Chronic graft-versus-host disease.* Curr Opin Oncol, 2006. **18**(2): p. 126-31.
57. Cutler, C., et al., *Rituximab prophylaxis prevents corticosteroid-requiring chronic GVHD after allogeneic peripheral blood stem cell transplantation: results of a phase 2 trial.* Blood, 2013. **122**(8): p. 1510-7.
58. Michallet, M., et al., *Rituximab, fludarabine, and total body irradiation as conditioning regimen before allogeneic hematopoietic stem cell transplantation for advanced chronic lymphocytic leukemia: long-term prospective multicenter study.* Exp Hematol, 2013. **41**(2): p. 127-33.
59. Chaleff, S., et al., *A large-scale method for the selective depletion of alphabeta T lymphocytes from PBSC for allogeneic transplantation.* Cytotherapy, 2007. **9**(8): p. 746-54.
60. Lang P, S.M., Teltschik H-M, Feuchtinger T, Pfeiffer M, Schilbach K, Handgretinger R, *First clinical results with a-b+ T-cell depleted haplo-identical stem cells in children.*, in *Tandem Meetings*. p. S135-136.
61. Schumm M, L.P., Bethge W, Vogel W, Faul C, Feuchtinger T, Pfeiffer M, Handgretinger R, *Depletion of TcRab+ and CD19-positive cells from leukapheresis products with the CliniMACS device.*, in *BMT Tandem Meetings*2011. p. S339-340.
62. Handgretinger R, L.P., Feuchtinger TF et al., *Transplantation of TCRαβ/CD19 depleted stem cells from haploidentical donors*, in *ASH meeting*2011.
63. Bertaina, A., et al., *HLA-haploidentical stem cell transplantation after removal of alphabeta+ T and B cells in children with nonmalignant disorders.* Blood, 2014. **124**(5): p. 822-6.
64. Fenske, T.S., et al., *Autologous or reduced-intensity conditioning allogeneic hematopoietic cell transplantation for chemotherapy-sensitive mantle-cell lymphoma: analysis of transplantation timing and modality.* J Clin Oncol, 2014. **32**(4): p. 273-81.
65. van Kampen, R.J., et al., *Allogeneic stem-cell transplantation as salvage therapy for patients with diffuse large B-cell non-Hodgkin's lymphoma relapsing after an autologous stem-cell transplantation: an analysis of the European Group for Blood and Marrow Transplantation Registry.* J Clin Oncol, 2011. **29**(10): p. 1342-8.
66. Thomson, K.J., et al., *Favorable long-term survival after reduced-intensity allogeneic transplantation for multiple-relapse aggressive non-Hodgkin's lymphoma.* J Clin Oncol, 2009. **27**(3): p. 426-32.
67. Rezvani, A.R., et al., *Non-myeloablative allogeneic haematopoietic cell transplantation for relapsed diffuse large B-cell lymphoma: a multicentre experience.* Br J Haematol, 2008. **143**(3): p. 395-403.
68. Burroughs, L.M., et al., *Comparison of outcomes of HLA-matched related, unrelated, or HLA-haploidentical related hematopoietic cell transplantation following nonmyeloablative conditioning for relapsed or refractory Hodgkin lymphoma.* Biol Blood Marrow Transplant,

2008. **14**(11): p. 1279-87.
69. Rowlings, P.A., et al., *IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade.* Br J Haematol, 1997. **97**(4): p. 855-64.
70. Jagasia, M.H., et al., *National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report.* Biol Blood Marrow Transplant, 2015. **21**(3): p. 389-401 e1.
71. Cheson, B.D., et al., *Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification.* J Clin Oncol, 2014. **32**(27): p. 3059-68.
72. Hallek, M., et al., *Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines.* Blood, 2008. **111**(12): p. 5446-56.

Appendices

Appendix A

ACUTE GRAFT VERSUS HOST DISEASE

Investigators should document on a weekly basis (beginning with the day of transplant) the raw data for the GVHD target organs either in the medical record directly or on a trial-specific worksheet. This should include the extent of skin rash, if any; the bilirubin; the daily stool output; or number of stools per day for an outpatient. The weekly record should reflect the worst representative days of the preceding week for each target organ involvement. Biopsy confirmation of target organs is recommended in most circumstances to confirm the diagnosis acute GVHD. Grading will be determined by the International Bone Marrow Transplant Registry (IBMTR) Severity Index grading system (A, B, C, D corresponding to I, II, III, and IV, respectively).[69]

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	Generalized erythroderma	> 1500 mL/d	Bilirubin 6-15 mg/dl
4	Generalized erythroderma with bullae	> 2000 mL/d and severe abdominal pain ± ileus	Bilirubin > 15 mg/dl

Grade	Skin	GI	Liver
A	Stage 1	none	none
B	Stage 2	Stage 1-2	Stage 1-2
C	Stage 3	Stage 3	Stage 3

D	Stage 4	Stage 4	Stage 4

Appendix B

CHRONIC GRAFT VERSUS HOST DISEASE

The diagnosis of chronic GVHD is based on both clinical and histopathologic findings for each organ system. Examples of pathognomonic and possible manifestations of chronic GVHD are outlined below; possible manifestations should be further evaluated to rule out other potential non-chronic GVHD etiologies. For example, pancreatic enzyme insufficiency may cause malabsorption and weight loss independent of chronic GVHD.

Time since transplant will not be used to distinguish acute from chronic GVHD (e.g., chronic GVHD may occur before Day 100, and acute GVHD may occur after Day 100). If acute GVHD is suspected after Day 60 or chronic GVHD is suspected before Day 100, biopsies are strongly encouraged to confirm the diagnosis. Additionally, biopsies are encouraged to confirm the diagnosis of chronic GVHD in patients with "possible" manifestations.

Definite and Possible Manifestations of Chronic GVHD

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

Clinical scoring and assessment of severity will be determined by the NIH consensus criteria (severity briefly detailed below) and the treatment algorithm will be according to the UW BMT program SOP regarding chronic GVHD.[70]

Mild cGVHD	1 or 2 organs involved with no more than score 1, plus lung score 0
Moderate cGVHD	3 or more organs involved with no more than score 1 OR at least 1 organ (not lung) with score of 2 OR lung score 1
Severe cGVHD	At least 1 organ with score of 3 OR lung score of 2 or 3

Appendix C

DISEASE RESPONSE CRITERIA AND EVALUATION

Criteria for response assessment and evaluation for progression for Hodgkin and non-Hodgkin lymphoma (not CLL) will be according to the Lugano classification briefly outlined below and further outlined in the referenced publication.[71]

Response and site	PET-CT–Based Response	CT-Based Response
Complete Response	*Complete metabolic response*	*Complete radiologic response (all of the following):*
Lymph nodes and extralymphatic sites	Score of 1, 2, or 3 with or without residual mass on 5 point scale. It is recognized that in Waldeyer's ring or extranodal sites with high physiologic uptake or with activation within spleen or marrow (eg, with chemotherapy or myeloid colony-stimulating factors), uptake may be greater than normal mediastinum and/or liver. In this circumstance, complete metabolic response may be inferred if uptake at sites of initial involvement is no greater than surrounding normal tissue even if the tissue has high physiologic uptake	Target nodes/nodal masses must regress to ≤ 1.5 cm in longest transverse diameter of a lesion. No extralymphatic sites of disease
Nonmeasured lesion	Not applicable	Absent
Organ enlargement	Not applicable	Regress to normal
New lesions	None	None
Bone marrow	No evidence of FDG-avid disease in marrow	Normal by morphology; if indeterminant, IHC negative
Partial Response	*Partial metabolic response*	*Partial remission (all of the following):*
Lymph nodes and extralymphatic sites	Score 4 or 5 with reduced uptake compared with baseline and residual mass(es) of any size.	≥50% decrease in sum of the product of the perpendicular diameters (SPD) for multiple lesions of up to 6 target measurable nodes and extranodal sites. When a lesion is too small to measure on CT, assign 5mmx5mm as the default value. When no longer visible, 0x0mm. For a node > 5mmx5mm, but smaller than normal, use actual measurement for calculation
Nonmeasured lesion	Not applicable	Absent/normal, regressed, but no increase
Organ enlargement	Not applicable	Spleen must have regressed by >50% in length beyond normal
New lesions	None	None
Bone marrow	Residual uptake higher than uptake in normal marrow but reduced	Not applicable

	compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). If there are persistent focal changes in the marrow in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy or an interval scan	
No response or stable disease	*No metabolic response*	*Stable disease*
Target nodes/nodal masses, extranodal lesions	Score of 4 or 5 with no significant change in FDG uptake from baseline	<50% decrease from baseline in SPD of up to 6 dominant, measurable nodes and extranodal sites; no criteria for progressive disease are met
Nonmeasured lesion	Not applicable	No increase consistent with progression
Organ enlargement	Not applicable	No increase consistent with progression
New lesions	None	None
Bone marrow	No change from baseline	Not applicable
Progressive disease	*Progressive metabolic disease*	*Progressive disease requires at least one of the following:*
Individual target nodes/nodal masses	Score of 4 or 5 with an increase in intensity of uptake from baseline and/or	Progression of cross product of largest transverse diameter and perpendicular diameter (PPD):
Extranodal lesions	New FDG-avid foci consistent with lymphoma	An individual node/lesion must be abnormal with: Longest transverse diameter > 1.5 cm and Increase by ≥ 50% from PPD nadir and An increase in largest transverse diameter (or shortest axis perpendicular to the largest transverse diameter) from nadir 0.5 cm for lesions ≤ 2cm 1.0 cm for lesions > 2cm In the setting of splenomegaly, the splenic length must increase by > 50% of the extent of its prior increase beyond baseline (eg, a 15-cm spleen must increase to > 16 cm). If no prior splenomegaly, must increase by at least 2 cm from baseline New or recurrent splenomegaly
Nonmeasured lesions	None	New or clear progression of pre-existing nonmeasured lesions
New lesions	New FDG-avid foci consistent with lymphoma. If uncertain, biopsy or interval scan may be considered	Regrowth of previously resolved lesions A new node > 1.5 cm in any axis A new extranodal site > 1.0 cm in any axis; if < 1.0 cm in any axis, its presence must be unequivocal and must be attributable to lymphoma

		Assessable disease of any size unequivocally attributable to lymphoma
Bone marrow	New or recurrent FDG-avid foci	New or recurrent involvement

Criteria for response assessment and evaluation for progression for CLL will be according to the International Workshop on Chronic Lymphocytic Leukemia briefly outlined below and further outlined in the referenced publication.[72]

Parameter	CR*	PR*	PD*
Group A			
Lymphadenopathy+	None > 1.5cm	Decrease ≥ 50%	Increase ≥ 50%
Hepatomegaly	None	Decrease ≥ 50%	Increase ≥ 50%
Splenomegaly	None	Decrease ≥ 50%	Increase ≥ 50%
Blood lymphocytes	< 4000/mcl	Decrease ≥ 50% from baseline	Increase ≥ 50% over baseline
Marrow	Normocellular, < 30% lymphocytes, no B-lymphoid nodules.	50% reduction in marrow infiltrate, or B-lymphoid nodules	
Group B			
Platelet count	>100, 000/mcl	>100, 000/mcl or increase ≥ 50% over baseline	Decrease of ≥ 50% from baseline secondary to CLL
Hemoglobin	>11g/dl	>11g/dl or increase ≥ 50% over baseline	Decrease of ≥ 2g/dl from baseline secondary to CLL
Neutrophils	>1500/mcl	>1500/mcl or ≥ 50% improvement over baseline	

*CR (complete remission): all of the criteria have to be met, and patients have to lack disease-related constitutional symptoms; PR (partial remission): at least two of the above criteria of group A plus one of the criteria of group B have to be met; SD is absence of progressive disease (PD) and failure to achieve at least a PR; PD: at least one of the above criteria of group A or group B has to be met.

+Sum of the products of multiple lymph nodes (as evaluated by CT scans at visits scheduled to have imaging or by physical exam that can be confirmed by CT if needed).