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Alpha/Beta T-cell and B-cell Depleted Allogeneic Transplantation (IDE 13641) Followed by Blinatumomab Therapy for High-Risk B-Acute Lymphoblastic Leukemia: A Pilot Study

Short Title: Blinatumomab After TCR αβ/CD19 Depleted HCT

Version No. 1.3

Version Date: 7/12/2021

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PROTOCOL REVISION HISTORY

Version No.	Revision Date	Summary of Changes	Consent Revised Yes/No
1.1	10/22/2020	**FDA Requested changes as follows:** • Experimental Design revised to add Dexamethasone as pre-medication • Exhibit overt hematologic manifestation of relapse or persistent disease was added as bullet 4.3.8 in Exclusion criteria • Section 6.7 revised to add minimal organ functions and the pre-medication of Dexamethasone • Table 1-Cytokine release syndrome revised to state DEX to be given as first line and TOCI only if patient experiences refractory CRS for Grades 2 and 3 AE • Section 9.10 revised to add a statement referring to Appendix 3 which refers to the current USPI for volume calculations • Risk of Serious Adverse Reactions in Pediatric Patients due to Benzyl Alcohol Preservative was added to Section 9.10 • Blinatumomab AE List in Section 9.10 was revised as per the risks provided by Amgen • Appendix 3 revised to add "do not shake" to #3 discussing adding IV solution stabilizer • Appendix 3 revised to add a statement for Patients Weighing Under 22 kg • Appendix 3 revised to remove all volume calculation tables and refer to the current USPI for preparation Local Changes as follows: Removal of Lauren Pommert from list of Sub-Investigators	**Yes** • Added Dexamethasone as pre-medication • Added "Do not drive, operate heavy machinery, etc. under "Special Instructions" • Added CRS, Neurological problems, and Infections as separate bullets to "Health Risks or Problems" • Blina AE list was revised • Added drug monograph for Dexamethasone
1.2	2/8/2021	**Added the following to Section 5.4, 5.7.1, and 6.1:** Baseline blast samples will be sent for B-cell and T-cell clonality HTS assessment as part of the BBT, since B-lymphoblasts may have detectable T cell receptor (TCR) clones. If T-cell clonal sequences are detected on the baseline sample, T-cell clonality will continue to be sent along with B-cell clonality on subsequent samples.	Yes to add ClinicalTrials.gov ID Number

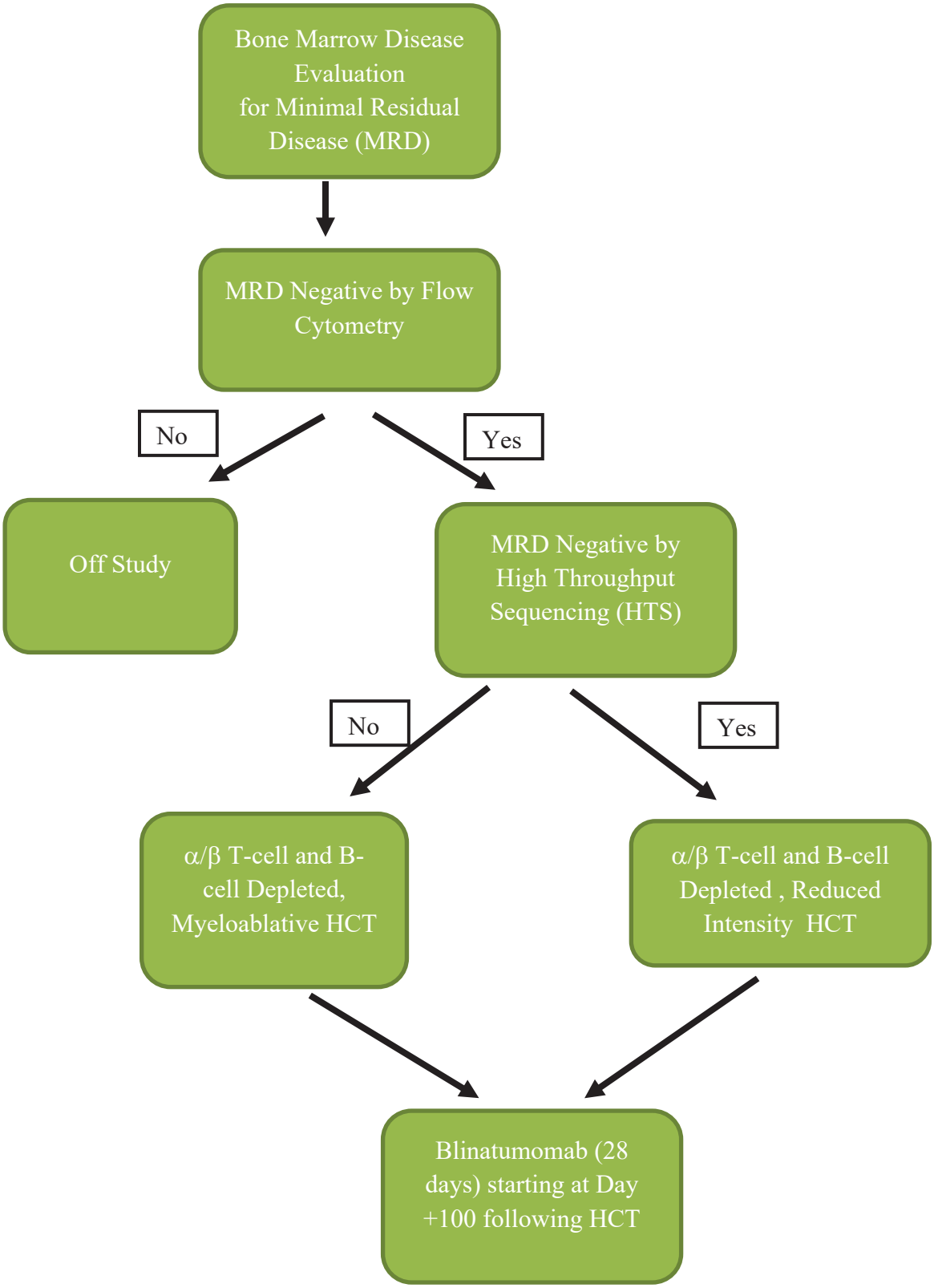
Version No.	Revision Date	Summary of Changes	Consent Revised Yes/No
		• **Rationale for Change:** The NGS as written will only test for B cell proteins to identify the leukemia clones present. Leukemia cells can also harbor T cell proteins and be used to identify leukemia clones. Thus, we want the NGS to test for BOTH B cell and T cell proteins to identify leukemia clones. This testing is done on the same sample that we are currently submitting. Subjects would need to be negative for both B and T cell clones to be eligible for the reduced intensity transplant (NGS negative)."	
1.3	7/12/2021	1. Added Kristin Page Chartrand as a Sub-I and removed Dr. Otto 2. Broadened the range of dates for dental evaluations to allow for greater flexibility for scheduling without a significant compromise in risk 3. Removed HLA typing from study calendar as this is done prior to enrolling 4. Changed EBV viral monitoring timepoint as EBV re-activation is extremely rare in the first month following HCT. 5. Changed IVIG dosing recommendation to "per institutional standard to allow for flexibility without impacting outcome 6. Tacrolimus was removed and immunosuppression requirements following stem cell infusions have been changed based on donor type and number of CD3+ TCRα/β+ cells/kg body weight. **Rationale:** The change in immunosuppression requirements is based on published and institutional experience. A multi-center study (Children's Wisconsin and Children's Hospital of Philadelphia) which was recently closed due to meeting accrual requirements demonstrated low rates of GVHD with higher numbers of allowed CD3+ TCRα/β+ cells than originally planned for in this protocol. Since the risk of GVHD is higher with haploidentical donor grafts, the immunosuppression requirements will be adjusted based on donor type (lower doses of CD3+ TCRα/β+ cells allowed without	Yes – Consent 2 – Treatment: Removed tacrolimus and updated the questionnaire section to provide more detail.

Version No.	Revision Date	Summary of Changes	Consent Revised Yes/No
		the use of immunosuppression). Also regarding the removal of tacrolimus; Based on the published and institutional experience noted above, tacrolimus will no longer be routinely used and MMF use alone will be implemented when CD3+ TCRα/β+ cell dose exceeded the noted limits. 7. Removal of ranitidine because it is not routinely used across institutions for anaphylaxis prophylaxis, this is no longer a requirement, but can be used if needed or desired. 8. CD34 dosing requirements updated. **Rationale:** CD34 dosing requirement changed to be the same across donor sources, but higher with haploidentical donors, and consistent across institutions, based on experience and published data. 9. Broadened timeframe window for evaluations occurring at the later timepoints post-HCT. **Rationale:** Patients will have less frequent follow-up visits at the transplant center as they get further from the acute post-transplant period. The expanded timeframe will allow for more flexibility in obtaining these required evaluations, without compromising clinical care. 10. Removal of uric acid from chem panel testing as it is not considered standard of care at all institutions. 11. MMF Dosing will be changed to 15 mg/kg every 8 hours based on published standard of care dosing. 12. Updated Section 8.3 to clarify what needs to be reported to DSMC and how to record them.	

ABSTRACT

Children, adolescents and young adults with relapsed or refractory B-acute lymphoblastic leukemia (B-ALL) often have dismal outcomes with chemotherapy alone. Despite allogeneic hematopoietic cell transplantation (HCT) providing a cure for some of these patients, relapse after HCT continues to be the most common reason for treatment failure. Patients undergoing HCT also risk suffering from graft-vs-host disease (GVHD) and, if they do not succumb to relapsed disease, may experience late effects related to myeloablative conditioning regimens, which have historically contained total body irradiation (TBI). Identifying novel ways to improve post-HCT outcomes in these high-risk patients has been challenging and currently no standard approach has been found. We are testing the ability of a biologically active therapy in blinatumomab, an anti-CD19/CD3 bispecific T-cell engager, to further reduce the risk of leukemia relapse following HCT to improve post-HCT outcomes. We will also utilize an alpha-beta T-cell and B-cell depleted graft to reduce the risk of GVHD along with a reduced intensity conditioning regimen without the use of TBI in patients who are minimal residual disease (MRD) negative using high throughput sequencing (HTS) prior to HCT. For those patients who remain HTS-MRD positive, we will utilize a myeloablative conditioning regimen, followed by blinatumomab. This multi-institutional pilot study will be limited to 25 (estimated 10-15 per stratum) evaluable children, adolescents and young adults with B-ALL, that have experienced a relapse or have high-risk disease.

EXPERIMENTAL DESIGN SCHEMA
Study population: Patients, ages ≤ 25 years, with B-ALL meeting study eligibility



Experimental Design Schema: HCT Conditioning Regimens

Reduced Intensity Conditioning Schema (HTS and Flow Cytometry MRD Negative)

Day	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	+1	…	+100
rATG* (1mg/kg)	X															
rATG* (3 mg/kg)		X	X	X												
Fludarabine					X	X	X	X								
Thiotepa									X							
Melphalan										X	X					
TCR-alpha/beta and CD19 depleted stem cell transplant													X			
Rituximab**														X		
Dexamethasone																X
Blinatumomab^																X

rATG: 1 mg/kg IV on Day -12 and 3 mg/kg on Days -11, -10, -9 (*No ATG for patients receiving unrelated donors)
Fludarabine: 40 mg/m^2 IV
Thiotepa: 5 mg/kg/dose IV at 8am and 8pm
Melphalan: 70 mg/m^2 IV
Rituximab 375 mg/m^2 IV day +1 **if patient EBV positive.
Dexamethasone: Adult patients 20 mg, Pediatric patients 5 mg/m^2 to a maximum dose of 20 mg
Blinatumomab: If ≥ 45 kg: 28 mcg/day on Days 1-28, If < 45 kg: 15 mcg/m^2/day on Days 1-28
^Only to be given if no evidence of > Grade 1 acute GVHD or any active infections within 1 week of blinatumomab infusion

Myeloablative Conditioning Schema (HTS Positive and Flow Cytometry Negative MRD)

Day	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	+1	…	+100
rATG*	X	X	X												
TBI				X	X	X									
Thiotepa							X	X							
Cyclophosphamide									X	X					
TCR-alpha/beta and CD19 depleted stem cell transplant												X			
Rituximab**													X		
Dexamethasone															X
Blinatumomab^															X

rATG: 3 mg/kg IV (*No ATG for patients receiving unrelated donors)
Total Body Irradiation (TBI): 1200 cGy/6 fractions; shielding of heart/lungs last two fractions: may be given before or after chemotherapy

Thiotepa: 5 mg/kg/dose IV
Cyclophosphamide: 60 mg/kg/dose IV
Rituximab 375 mg/m^2 IV on Day +1 if **patient EBV positive.
Dexamethasone: Adult patients 20 mg, Pediatric patients 5 mg/m^2 to a maximum dose of 20 mg
Blinatumomab: If ≥ 45 kg: 28 mcg/day on Days 1-28, If < 45 kg: 15 mcg/m^2/day on Days 1-28
^Only to be given if no evidence of > Grade 1 acute GVHD or any active infections within 1 week of blinatumomab infusion

PROTOCOL SUMMARY

Title	Alpha/Beta T-cell and B-cell Depleted Allogeneic Transplantation (IDE 13641) Followed by Blinatumomab Therapy for High-Risk B-Acute Lymphoblastic Leukemia: A Pilot Study
Protocol Short Name	Blinatumomab after TCR αβ/CD19 Depleted HCT
Principal Investigator/ Study Chair/ Coordinating Center/Sponsor-Investigator	Rachel Phelan, MD, MPH Pediatric Clinical Trials Office (CTO) 8701 Watertown Plank Road MFRC Suite 3018 Milwaukee, WI 53226
Study Sites	1) Children's Hospital of Wisconsin, Milwaukee, WI 2) American Family Children's Hospital, Madison WI
Clinical Trial Phase	Pilot Study
Study Disease	Relapsed/Refractory B-Acute Lymphoblastic Leukemia
Funding Source	Amgen
Main Eligibility Criteria	• Diagnosis of B-ALL with no evidence of minimal residual disease in the bone marrow by multi-parameter flow cytometry and/or next generation sequencing **and** that meets one of the following: a. In remission after first relapse or greater (≥ CR2) b. Patients with very-high risk biology ALL that is proceeding to HCT in first remission (e.g. Induction failure, Severe-hypodiploidy, Ph-like ALL) c. Patients in first remission who had persistent disease identified as end of consolidation (EOC) MRD > 0.01%. • Age ≤ 25 years at time of study enrollment • No evidence of active infection. • Patients who have experienced their relapse after HCT are eligible, provided they have no evidence of acute or chronic Graft-versus-Host Disease (GVHD) and are off all transplant immune suppression therapy (e.g. steroids, cyclosporine, tacrolimus). Steroid therapy for non-GVHD and/or non-leukemia therapy is acceptable. • Previously enrolled in Blinatumomab Bridging Therapy (BBT) trial

Study Rationale	Relapse of childhood B-Acute Lymphoblastic Leukemia (B-ALL) is a vexing clinical problem with high rates of subsequent relapse and death with current treatment approaches. Treatment with hematopoietic cell transplantation (HCT) typically involves the use of high doses of chemotherapy and/or radiation which can result in significant transplant-related morbidity/mortality as well as a substantial burden of late effects. Despite successfully undergoing HCT as treatment for relapsed/refractory ALL, patients still have a substantial rate of relapse following HCT and subsequent treatment options are limited. This trial will assess the feasibility of alpha/beta T-cell and B-cell depleted allogeneic transplantation followed by blinatumomab therapy for high-risk B-ALL as a means of reducing rates of subsequent relapse and improving survival, while also minimizing treatment-related morbidity/mortality and late effects.
Primary Objectives	To assess the feasibility of giving blinatumomab post-alpha/beta T-cell and B-cell depleted HCT
Secondary Objectives	1. Evaluate the tolerability of blinatumomab given post-HCT by evaluating the incidence of adverse effects attributed to blinatumomab therapy 2. To demonstrate that reduced intensity conditioning HCT results in acceptable outcomes compared to standard myeloablative HCT in patients who are flow cytometry and high throughput sequencing MRD negative pre-HCT. 3. Evaluate overall and disease-free survival at 1-year post-HCT 4. Evaluate incidence of graft failure, time to engraftment, transplant related mortality (TRM), relapse, acute GVHD, chronic GVHD 5. Evaluation of patient-reported outcomes 6. Measure the average length of stay following HCT 7. Evaluate persistence of MRD negativity assessed by flow cytometry and high throughput sequencing (HTS).
Exploratory Objectives	1. Analysis of immune cell phenotyping 2. Perform a functional assessment of lymphocyte subsets 3. Perform serum cytokine analysis 4. Evaluate the feasibility of HTSvs Flow Cytometry for MRD assessment following HCT to predict relapse
Study Design	Multi-center pilot study
Study Agent/ Intervention Description	Blinatumomab
Number of Subjects	25 (estimating 10 to 15 per stratum)
Duration of Follow up	1-year post-HCT
Estimated Time to Complete Enrollment:	30 - 36 months

LIST OF ABBREVIATIONS

AE	adverse event
ALL	acute lymphoblastic leukemia
ALT	Alanine Aminotransferase
AML	acute myeloid leukemia
ANC	absolute neutrophil count
APL	acute promyelocytic leukemia
AST	aspartate aminotransferase
AUC	area under the concentration time curve
BMT	bone marrow transplant
BP	blood pressure
BUN	blood urea nitrogen
CHW	Children's Hospital of Wisconsin
CI	confidence interval
CLL	chronic lymphocytic leukemia
CNS	central nervous system
CR	complete remission
CrCl	creatinine clearance calculator
CRF	case report forms
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTMS	Clinical Trials Management System
CTO	Clinical Trials Office
DFS	disease free survival
DLT	dose limiting toxicity
DSMC	data safety monitoring committee
DSMP	data safety monitoring plan
ECHO	echocardiogram
EFS	event free survival
EP	European Pharmacopeia
FISH	fluorescence in situ hybridisation
GFR	glomerular filtration rate
GVHD	graft versus host disease
HCT	hematopoietic cell transplantation
HgB	hemoglobin
HSCT	hematopoietic stem cell transplantation
HSV	herpes simplex virus
HTS	high throughput sequencing
IRB	Institutional Review Board
LDH	lactate dehydrogenase
MACC Fund Center	Midwest Athletes Against Childhood Cancer Fund Center
MCW	Medical College of Wisconsin
MCWCC DSMC	Medical College of Wisconsin Cancer Center Data Safety Monitoring Committee
MFC	multiparameter flow cytometry
MRD	minimal residual disease
MTD	maximum tolerated dose

MUGA	multi gated acquisition scan
NGS	next generation sequencing
NOS	not otherwise specified
NRM	non-relapse mortality
NS	normal saline
OnCore™	Online Enterprise Research Management Environment
ORR	overall response rate
OS	overall survival
PCP	pneumocystis carinii pneumonia
PCR	polymerase chain reaction
Pediatric CTO	Pediatric Cancer/Blood Disorder Clinical Trials Office
PI	principal investigator
PR	partial response
PVC	polyvinyl chloride
RFS	relapse free survival
SAE	serious adverse event
SIRS	systemic inflammatory response
SRC	Scientific Review Committee
SUSAR	suspected unexpected serious adverse reaction
UCBT	umbilical cord blood transplantation
ULN	upper limit of normal
UPIRSO	unanticipated problems involving risks to subjects or others
USP	United States Pharmacopeia
WBC	white blood count

1.0 BACKGROUND AND RATIONALE

1.1 HCT as Treatment for Relapsed/Refractory Pediatric B-Acute Lymphoblastic Leukemia

Children, adolescents and young adults with acute lymphoblastic leukemia (ALL) who relapse, have refractory disease or are identified as harboring high-risk features that predict greater rates of relapse, can be cured with allogeneic hematopoietic cell transplantation (allo-HCT)[1-5]. Post-HCT outcomes for pediatric patients in second remission (CR2) at 2-years based on registry data using the Center of International Bone Marrow Transplant Registry (CIBMTR) reports leukemia-free survival ranging 43-50% at 3-years depending on graft source and relapse as high as 65%[6]. The anti-leukemia effects of allo-HCT result from 1) chemotherapy and/or radiation used for the conditioning regimen and, 2) the graft-versus leukemia effect resulting from transplantation of the donor immune system[7,8]. Thousands of patients with hematological malignancies have been treated with human leukocyte antigen (HLA)-matched allo-HCT in the last twenty-five years[9,10]. Outcomes have also improved over time with better HLA typing, donor availability, and supportive cares. In the last 25 years, transplant related mortality (TRM) has reached an all-time low with rates previously >30% now dropping to 5%. Similarly, over the years, further improvement in survival rates for pediatric patients with acute lymphoblastic leukemia (ALL) treated with chemotherapy alone has been achieved through therapy intensification and risk-based stratification[10]. At the same time, new discoveries of high-risk (HR) ALL biology has been reported, for which conventional chemotherapy does not appear to cure. Examples of these new lesions with poor prognostic findings include deletions in IKZF1 (IKAROS), rearrangements of CRLF2, and ABL class fusions, all of which have been identified as a new class of HR ALL biology called Philadelphia-like B-ALL (Ph-like)[11-16]. However, despite improvements in HCT, relapse remains the primary reason for failure post-HCT in the range of 40%.[17]

A retrospective case control study performed in the Nordic countries found that ALL patients with very high-risk (VHR) features who received a standard myeloablative HCT in CR1 had a DFS advantage at 10-years over patients who only received chemotherapy alone (73% vs. 50%; P = 0.02). The authors concluded that myeloablative HCT should be considered in CR1 for children with VHR ALL due to risk for unsuccessful salvage post-relapse and poor survival rates for HCT in CR2[18]. Over the years the paradigm appears to have shifted from HCT in CR1 to more intensive multiagent chemotherapy for previously considered HR patient groups.[19-22] But at the same time, the definition of HR leukemia is changing given recent discoveries with new genomic lesions and continued MRD testing which has identified patients at the highest risk of treatment failure. Myeloablative HCT in CR1 should still be considered when a patient's predicted EFS falls below 50% with conventional chemotherapy. In the past, survival benefits of HCT were offset by high rates of TRM, but as these rates continue to drop to well below 10% in children, HCT should remain an option to improve survival rates for these HR patients.

Myeloablative HCT is an excellent option that accelerates the initiation of potentially curative therapy. However, improvements in graft processing methodology and post-transplantation therapy are required to 1) further enhance anti-tumor efficacy of transplantation to prevent cancer recurrence, 2) provide maximal innate immune protection against infections following transplantation during the interim before adequate adaptive immune function is restored and 3) increase the rate of engraftment.

There is mounting evidence to support that for patients in hematological remission, the presence of minimal residual disease (MRD), identified immediately prior to HCT, is associated with higher rates of relapse following HCT. In fact, identifying MRD prior to HCT has become one of the most prognostic findings in ALL predicting post-HCT outcomes.[23-31] Currently both deep sequencing approaches, such as high throughput sequencing (HTS), and multiparameter flow cytometry are considered standard assays for MRD detection in patients with ALL, with flow cytometry used more often in North America and deep sequencing in Europe. The Children's Oncology Group (COG) study ASCT0431 is an example of how predictive pre-HCT MRD can be.[17] There were 144 pediatric patients enrolled with relapse ALL, where those who achieved MRD negativity pre-HCT identified by flow cytometry (FC) reported significantly less relapse at 1-year (20% versus 60%, p=0.4). Even more impressive was the predictability of relapse using high throughput sequencing (HTS) for MRD detection which was superior to FC.[32] Patients on the ASCT0431 study who achieved HTS-MRD negativity pre-HCT had no relapses compared to those who were HTS-MRD positive (0% versus 16%, p=0.02) and significantly greater overall survival (96% versus 77%, p=0.003). Additionally, post-HCT HTS-MRD detection was better at predicting relapse than FC-MRD (p<0.0001), especially early after HCT (day 30 FC-MRD positive relapse rate, 35%; HTS-MRD positive relapse rate, 67%; p=0.004).

The impressive results reported by Pulsipher and colleagues in the ASCT0431 study for patients who achieved HTS-MRD negativity pre-HCT, where no relapses were reported, suggest that standard myeloablative conditioning HCT may not be necessary for cure and raises the question of reduced intensity conditioning for this subset of patients. Therefore, introducing more novel HCT approaches such as using reduced intensity conditioning with alpha/beta T cell/B cell graft depletion for patients who are MRD negative prior to HCT by HTS to decrease the known late effects and increased morbidity that can be associated with myeloablative HCT, and yet retain the excellent survival seen with current HCT outcomes, is a promising approach and the primary focus of this study.

1.2 Emerging Role of γδ T cells

Preclinical and early clinical results using adoptive immunotherapies for a number of cancers with various cellular components such as NK cells, γδ T cells and dendritic cells emphasize the importance of a functioning, tumor-reactive immune system for cancer[33-39]. γδ T cells comprise about 3-6% of the human peripheral lymphocytes but are important for the primary response to infectious agents. γδ T cells also have potent anti-tumor effects and play a crucial role in cancer immunosurveillance[40-46]. γδ T cells are not alloreactive and facilitate engraftment after transplantation in rodent models without causing GVHD[47]. Additionally, γδ T cells exert antileukemic activity and after transplant, patients with high γδ T cell counts had significantly better 5-year survival than those with normal or low counts (70.8 vs. 19.6%, p=0001), while there was no difference in GVHD[41]. γδ T cells from G-CSF mobilized donors are highly cytotoxic and produce anti-tumor immunostimulatory cytokines such as IFNγ and TNFα and interleukin-6[43].

1.3 Rationale for Use of TCR αβ/CD19 Depleted Stem Cell Product for HCT

Given the potential advantages of using T cell depletion strategies surrounding HCT, primarily to mitigate GVHD, approximately 2 decades ago, Miltenyi Biotec introduced a commercially available platform for T-cell depletion using an automated antibody/magnetic beads column

system. Humanitarian Exemption IDE approval has been obtained for CD34+ cell selection as a method of T-cell depletion by this company. Given the very slow immune recovery and higher rates of infection and rejection with traditional T cell depletion methodologies, newer antibody combinations using this platform have been introduced. Most recently, investigators throughout the world have shown promising outcomes using a TCRαβ+/CD19+ depletion approach. This approach removes the cells most closely linked with GVHD (TCRαβ cells) while preserving γδ T cells and other key white blood cells in the product. In addition, the approach removes CD19+ B-cells to minimize the risk of EBV-lymphoproliferative disease.

Preclinical cell selection experiments on the CliniMACS® device using a biotinylated anti-TCR αβ antibody and anti-biotin microbeads, show that it is possible to deplete a mean of 99.99% of αβ T cells from peripheral blood mononuclear cell (PBMC) products. The mean CD34+ recovery was 66% (comparable to conventional CD34+ positive selection). High numbers of γδ T cells (mean: $3.4x10^8$ γδ T cells) and NK cells (mean: $8x10^8$ NK cells) remained in the graft, with recoveries averaging 92% and 80%, respectively. Using the clinical grade reagents now available in Europe and using the two-step combined TCR αβ/CD19 depletion, similar data were reported by Handgretinger[48].

Chaleff et al. demonstrated rapid HCT engraftment in a murine NOD/SCID $IL2^{rnull}$ model.[49] Higher overall stem cell engraftment and a significantly larger number of circulating CD45+ cells (leukocytes) in general and in particular, γδ T cells, was seen in bone marrow and thymus at 8 weeks after transplant when compared to mice transplanted with CD133+ stem cells.[49]

The first clinical trial using this TCR αβ T cell/CD19+ depletion process for patients with hematologic malignancies and non-malignant disorders was performed in Europe, namely in Germany (National Coordinating Investigator: R. Handgretinger, Eudra 2011-005562-38) and in Italy (PI: F. Locatelli, NCT01810120). Initial results from 17 patients from the German site and preliminary findings from the Italian site were presented at the December 2013 Annual Meeting of the American Society of Hematology (ASH). Results from twenty-three children with non-malignant disorders from this clinical trial were reported and showed that this graft processing strategy was safe and effective for haploidentical donor HCT.[50] A high dose of CD34+ stem cells (median 16.8 x 10^6/kg recipient body weight, minimum 10.2 x 10^6/kg) was infused and all but four patients engrafted, who were rescued successfully by a second allograft. A median dose of 40,000 αβ T cells/kg was infused with three patients experiencing skin-only Grade I-II acute GVHD and importantly no chronic GVHD. After a median follow-up of 18 months (range 5-40), 21/23 patients are alive and disease-free. The experience by the Italian group led to a subsequent trial in children with acute leukemia using the same alpha/beta T cell/B cell depletion.[51] Eighty children with ALL (n=56) or AML (n=24) with a median age of 6.6 (range, 0.4 to 16.8) years who received their first HCT between 2011 and 2014 enrolled to this prospective trial. All patients received an alpha/beta T cell and B cell depleted myeloablative haploidentical HCT. Twenty-four patients (30%) developed Grade I/II GVHD (skin only) with the remaining 70% not reporting any Grade GVHD. Clinically limited, skin-only chronic GVHD was reported in only 4 patients (5%). The 5-year cumulative incidence of TRM for the cohort was 5% and with a median follow-up of 46 (range, 26 to 60) months, the incidence of relapse was 24%. The 5-year OS was 72% for the whole cohort (71% for ALL and 68% for AML).[51]

A more recent publication by Bertaina et al reports on a multicenter retrospective analysis of children with leukemia who received HCT with a matched unrelated donor, mismatched unrelated donor or haploidentical donor with TCR αβ/CD19 depleted grafts. In this report, 127 patients underwent matched unrelated donor transplant, 118 had mismatched unrelated donor transplant and 98 had alpha/beta TCR depleted haploidentical grafts. It was notable that non-relapse mortality and leukemia-free survival was similar between the matched unrelated donor group and the haploidentical group using αβ/CD19 depleted grafts (p=0.435 and p=0.470, respectively). Importantly, Grade 3-4 acute GVHD rates were significantly less than the MUD group (p<0.001).[52]

Given the success of alpha/beta T cell and B cell depletion in hematologic malignancies, we have been performing this graft engineering model here at Children's Hospital of Wisconsin as part of a clinical trial with the Children's Hospital of Philadelphia for children, adolescents and young adults with acute leukemia followed by a myeloablative HCT (NCT02323867). This has since become one of our standard conditioning approaches for HCT in pediatric patients with acute leukemia combining αβ T cell/CD19 B cell depletion followed by myeloablative HCT. Our institution has 13 patients who have enrolled, 9 who are at least 6-months post-HCT. One patient has relapsed post-HCT and 3 patients have developed low-grade acute GVHD (Grade 1/2). No patients at our site have had DLT or TRM. The most recent study progress report demonstrated 57 total patients transplanted on this protocol. Sixteen of the 57 patients had died at the time of this report, with 5 of those patients having death attributed to transplant-related mortality and the remaining from either relapsed disease or other late effects of transplant (personal communication). This data as well as other prior studies demonstrate that post-HCT relapse can still occur and thus incorporating novel post-HCT therapies that may effectively target and further limit relapse, are of great interest.

1.4 Rationale for Studying Blinatumomab Post-HCT in Relapsed/Refractory B-ALL

Relapse of B-ALL following HCT is a vexing clinical problem with high rates of subsequent death with current treatment approaches. At the point of relapse, patients have often been exposed to numerous treatment modalities which have resulted in comorbidities which may preclude them from more intensive treatments to again regain remission. They may also have limited treatment options left to pursue. With this in mind, a focus on relapse prevention following HCT has become an increasing area of interest.

Several drugs have been evaluated as a form of "maintenance therapy" following HCT for hematologic diseases as an attempt to prevent relapse. Targeted agents, such as tyrosine kinase inhibitors, histone deacetylase inhibitors, and hypomethylating agents have all been trialed to either prevent disease recurrence or treat early signs of disease following HCT[53-57]. However, these trials have been relatively small and thus larger prospective trials are needed in this arena.

Blinatumomab is a promising novel agent for the treatment of B-lineage lymphoid malignancies.[58-64] Blinatumomab is a bispecific single-chain antibody that targets the CD19 antigen and redirects CD3+ T-cells for selective lysis of tumor cells. In a Phase 2 trial of adult B-ALL, patients who were in a complete hematologic remission (< 5% blasts in the bone marrow) but with MRD persistence or had relapsed after Induction and Consolidation therapy who had re-achieved a complete remission but remained with MRD, received blinatumomab as a 4-week continuous

intravenous infusion at a dose of 15 μg/m^2/24 hours (max dose of 28 μg/24 hours).[61] Of 21 treated patients, 16 became MRD negative as assessed by quantitative polymerase chain reaction (QT-PCR) for either rearrangements of immunoglobulin or T-cell receptor genes, or specific genetic aberrations. Among the 16 responders, 12 had been molecularly refractory to previous chemotherapy. Probability for relapse-free survival was 78% at a median follow-up of 405 days. Blinatumomab was similarly effective and well tolerated in a small series of pediatric cases.[59]

Additionally, blinatumomab has been evaluated in children with relapsed/refractory B-ALL in an Amgen-sponsored Phase 1/2 study conducted by the COG (MT103-205/AALL1121) and the I-BFM European childhood leukemia cooperative group with extremely promising results. Forty-nine patients were treated in the Phase 1 portion and 44 patients in the Phase 2.[65] Four patients had dose-limiting toxicities in cycle 1 of the Phase 1 component. Three experienced Grade 4 cytokine-release syndrome (one attributed to Grade 5 cardiac failure); one had fatal respiratory failure. The maximum-tolerated dosage was 15 μg/m^2/d as a continuous infusion for 28 days. Blinatumomab pharmacokinetics was linear across dosage levels and consistent among age groups. Based on the Phase 1 data, the recommended blinatumomab dosage for children with relapsed/refractory ALL who have >5% leukemia in their bone marrow, was 5 μg/m^2/d (max dose 9 μg/d) for the first 7 days to help mitigate cytokine release syndrome (CRS), followed by 15 μg/m^2/d thereafter. Among the 70 patients who received the recommended dosage, 27 (39%; 95% CI, 27% to 51%) achieved complete remission within the first two cycles, 14 (52%) of whom achieved complete MRD response. The most frequent Grade ≥ 3 adverse events were anemia (36%), thrombocytopenia (21%), and hypokalemia (17%). Three patients (4%) and 1 patient (1%) had cytokine-release syndrome of Grade 3 and 4, respectively. Two patients (3%) interrupted treatment after Grade 2 seizures. This trial was the first such trial in pediatrics and demonstrated anti-leukemic activity of single-agent blinatumomab with complete MRD response in children with relapsed/refractory B-ALL. Blinatumomab has since moved to a Phase 3 trial, in combination with chemotherapy, conducted at >100 COG institutions for children, adolescents and young adults with 1st relapse B-ALL (AALL1331; NCT02101853). Blinatumomab was subsequently FDA approved in 2017 for pediatric and adult patients with relapsed or refractory B-ALL and later in 2018 the approval was expanded by the FDA to both children and adults who are in a complete remission (<5% blasts in the marrow) but still have MRD.

Blinatumomab has been successfully used in the post-HCT setting in both children and adults with B-ALL with the ability to induce remission in several case reports.[66-68] Of the publications referenced, there were no severe adverse events (SAE) reported. Any toxicity attributed to blinatumomab was also minimal. A single adult patient appeared to develop mild GVHD following their blinatumomab infusion, which was treated successfully with a course of steroids. It was hypothesized that the GVHD was the result of suppression of B-regulatory cells, possibly translating to a decrease in T regulatory cells and leading to graft-vs-leukemia which manifested as GVHD.[13,68] In the report by Handgretinger et al., 3 pediatric patients with B-ALL, who all suffered a post-HCT relapse, were successfully re-induced with a single 28-day cycle of blinatumomab achieving a complete morphologic remission and no evidence of MRD by PCR analysis (MRD <10^{-4}). All 3 patients tolerated the blinatumomab therapy incredibly well with no Grade 3/4 toxicity or report of GVHD. The authors concluded that their findings warrant clinical trials in pediatric patients with relapsed/refractory B-ALL using blinatumomab before and after HCT.[59]

Despite the promising outcomes reported with blinatumomab in the relapse or refractory disease setting prior to HCT, as well as a few case reports using it post-HCT with good remission responses, blinatumomab has not been evaluated prospectively in the post-HCT setting as a form of maintenance therapy in the context of a clinical trial. This Pilot study will evaluate the efficacy of blinatumomab as a form of maintenance therapy following HCT to prevent disease relapse. This trial will determine if blinatumomab used as maintenance therapy following HCT can reduce rates of subsequent relapse and improve survival. If successful, this strategy could become standard of care for patients going to HCT for B-ALL.

1.5 Rationale for Reduced Intensity Conditioning HCT in Pediatric ALL

Over the past 10 years considerable experience has been gained using reduced-intensity conditioning (RIC) regimens for HCT. Although several different regimens have been tested, one unifying feature is that they have the potential for acceptable rates of donor engraftment and lower TRM relative to conventional or dose-intensive myeloablative conditioning. Because most children tolerate conventional dose-intensive conditioning and TRM increases with age, RIC has mainly been reserved for older patients or those with poor performance status.

One fundamental difference between RIC and myeloablative conditioning is the mechanism of disease control. With myeloablative conditioning, relapse protection is provided by the dose-intensive chemotherapy and/or total body irradiation (TBI) and the allogeneic, graft-versus-leukemia (GVL) effect. In contrast, the lower dose of chemotherapy and/or irradiation associated with RIC may provide little up-front disease control, and thus, the efficacy of RIC has been ascribed to the post-HCT GVL effect. Although GVL reactions are difficult to document in real time, both the kinetics of disease regrowth and responsiveness to GVL have had bearing on patient selection for RIC. Thus, RIC has been more commonly used in patients with chronic leukemia and indolent lymphoma.[69] Enthusiasm for using RIC for ALL has been appropriately guarded, because of the poor responsiveness to post-HCT immune-based approaches in ALL, including rapid tapering of immune suppression and donor lymphocyte infusion (DLI) following relapse.[70,71]

Under certain circumstances, RIC may be indicated for patients with ALL who require HCT but are ineligible for a dose-intensive conditioning. Such indications include: poor performance status, active infections, significant organ dysfunction, or advanced age. Transplant outcome data after various RIC regimens for ALL are few and, with two exceptions, are limited to reports in adults. To date, 7 reports have focused on the outcomes of HCT with RIC for ALL[72-78], whereas other reports have included patients with ALL and other leukemias.[79-81] The report by Verneris et al. reported pediatric outcomes using the Center of International Bone Marrow Transplant Registry (CIBMTR) which included 38 children, adolescents and young adults with ALL who received a RIC HCT.[78] The median age was 12 years and 47% had performance scores <90%. While 13% of patients were in first remission (CR1) at time of HCT, 60% were in CR2 or greater and the remaining 22% of patients had active disease. Disease-free survival (DFS) at 3-years was 30%, relapse 37% and TRM was 40%. Despite this study reporting relatively good survival outcomes (DFS of 30%) using a RIC approach in heavily pre-treated and poorly conditioned pediatric patients, relatively high rates of relapse remain. A recent abstract presented at ASH also reported on a Phase I/II clinical trial looking at using TCR alpha/beta and CD19 depletion for adult and pediatric patients with hematologic malignancies also supports the use of a reduced intensity

regimen, with outcomes similar to what has been seen with myeloablative regimens (OS of 62% and DFS of 53% and a relapse rate of 34%).[82]

Given the challenge that reduced intensity conditioning regimens have in eradicating residual leukemia present at time of HCT, introducing a post-HCT therapy such as blinatumomab, that has shown to be effective in selectively targeting CD19+ cells, universally present in B-ALL, is one approach to lower relapse post-HCT when RIC is used. Additionally, the report by Pulsipher and colleagues identified pediatric patients with ALL that were HTS-MRD negative pre-HCT had 0% relapse and excellent 2-year survival (>95%) and could be prime candidates for RIC to avoid the late effects and morbidity associated with a myeloablative HCT.[32] Thus, this study: Alpha/Beta T-cell and B-cell depleted allogeneic transplantation followed by blinatumomab therapy for HR B-ALL: A pilot study, will partner the two above concepts of using HTS-MRD assessment to identify patients who may benefit from a RIC approach and introducing post-HCT maintenance therapy with a B-cell targeted agent with proven efficacy in B-ALL, blinatumomab.

1.6 Use of Patient-Reported Outcomes

Gaining the perspective of the patient though the use of patient-reported outcomes (PROs) measures is being viewed as increasingly important in the clinical research arena. As research develops in this area, it has become increasingly clear that PROs can give clinicians and researchers critical information that may not be attainable through routine history taking or other research methods.[83-87]

Although it is acknowledged that gaining the patient and parent perspective on well-being through PROs is important in this field, there has been debate regarding what the best tool is for this purpose. A recent publication by Shaw et. al highlights the different tools that have been in use and the need to agree upon a commonly used tool in order to compare outcomes across studies and PROMIS tools are mentioned as the option that was free, easy to access and is not burdensome.[88] A task force to harmonize PRO assessments in the field of HCT is underway and the use of PROMIS tools is being incorporated into more clinical trials.[89,90]

A recent study at our institution looked at PROs compared to clinician documentation during pediatric cancer treatment. Using the NIH-funded Patient Reported Outcomes Measurement Information System (PROMIS) to administer surveys looking at multiple dimensions of functioning, patients demonstrated impaired function related to any symptom measured at 24% of clinic visits. Of those clinic visits where PROMIS identified impaired function, that symptom was not documented by the clinician 80% of the time. These included physicians missing important issues such as fatigue, pain, anxiety, and depression. We will be using the PROMIS surveys in this study as a standardized way of assessing patient/caregiver viewed function in this patient population.[91]

2.0 HYPOTHESIS AND OBJECTIVES

2.1 Hypothesis

Blinatumomab will be feasible to administer post-HCT for children, adolescents and young adults with relapsed or refractory B-ALL undergoing alpha-beta T-cell and B-cell depleted HCT.

2.2 Primary Objectives

To assess the feasibility of giving blinatumomab post-alpha/beta T-cell and B-cell depleted HCT

2.3 Secondary Objectives

- Evaluate the tolerability of blinatumomab given post-HCT by evaluating the incidence of adverse effects attributed to blinatumomab therapy
- To demonstrate that reduced intensity conditioning HCT results in acceptable outcomes compared to standard myeloablative HCT in patients who are flow cytometry and high throughput sequencing MRD negative pre-HCT.
- Evaluate the overall and disease-free survival at 1-year post-HCT
- Evaluate persistence of MRD negativity assessed by flow cytometry and high throughput sequencing
- Evaluate incidence of primary and secondary graft failure, time to engraftment, TRM, relapse, acute GVHD, chronic GVHD
- Evaluation of patient-reported outcomes following HCT
- Measure the average length of stay following HCT

2.4 Exploratory Objectives

- Analysis of immune cell phenotyping post-HCT
- Perform a functional assessment of lymphocyte subsets post-HCT
- Perform serum cytokine analysis post-HCT
- Evaluate the feasibility of High Throughput Sequencing (HTS) vs Flow Cytometry for MRD assessment following HCT to predict relapse

3.0 STUDY DESIGN

3.1 General Description

This is a non-randomized, multi-center pilot study evaluating the feasibility of blinatumomab following an alpha/beta T-cell and B-cell depleted allogeneic hematopoietic cell transplant. All eligible subjects will receive an alpha/beta T-cell and B-cell depleted transplant followed by a single cycle of maintenance blinatumomab therapy starting 100 days following HCT. Subjects will be assigned to the myeloablative conditioning stratum if prior to HCT their bone marrow evaluation is minimal residual disease (MRD) negative by flow cytometry (FC-MRD negative) (performed at the University of Washington, Brent Wood, MD), but positive by high throughput sequencing (HTS) (performed at Adaptive Technologies, Seattle, WA). If subjects are MRD negative by both FC and HTS prior to HCT, they will be assigned to the reduced intensity conditioning (RIC) stratum of the study.

3.2 Number of Subjects

For this pilot study, the planned number of total subjects is 25 with an estimation of 10-15 per stratum: 10-15 subjects in the FC-MRD negative only/myeloablative conditioning stratum and an

additional 10-15 subjects in the FC-MRD negative **AND** HTS-MRD negative stratum.

3.3 Primary Completion

The study will reach primary completion 30 to 36-months from the time the study opens to accrual.

3.4 Study Completion

The study will reach study completion 42-48-months from the time the study opens to accrual.

4.0 PATIENT SELECTION

Study entry is open to patients regardless of gender or ethnic background. While there will be every effort to seek out and include females and minority patients, the patient population is expected to be no different than that of other acute leukemia studies at the Medical College of Wisconsin.

4.1 Eligibility Criteria

Patients must have baseline evaluations performed prior to the start of conditioning for HCT and must meet all inclusion and exclusion criteria. In addition, the patient must be thoroughly informed about all study aspects, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient and/or caregiver if the patient is less than 18 years of age prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

4.2 Inclusion Criteria

4.2.1 Diagnosis of B-ALL with no evidence of minimal residual disease in the bone marrow by multi-parameter flow cytometry (FC-MRD negative, <0.01%) and meet at least one of the following:

a. In remission after first relapse or greater (≥ CR2)

b. Very-high risk biology ALL that is proceeding to HCT in first remission (e.g. Induction failure, Severe-hypodiploidy, Ph-like ALL)

c. First remission with persistent disease identified as end of consolidation (EOC) MRD > 0.01%.

4.2.2 Patients must have an available unrelated or haploidentical donor

4.2.3 Age ≤ 25 years at time of study enrollment

4.2.4 Karnofsky Performance Status ≥ 60% for patients 16 years and older and Lansky Play Score ≥ 60 for patients under 16 years of age (see Appendix 1)

4.2.5 Have acceptable organ function as defined within 14 days of study registration:

Renal: creatinine clearance or radioisotope GFR ≥ 60 mL/min/1.73m^2

Hepatic: ALT < 5 x upper limit of normal (ULN) and total bilirubin ≤ 3 mg/dL

Cardiac: left ventricular ejection fraction ≥ 40% by ECHO/MUGA

Pulmonary: No evidence of dyspnea at rest. No supplemental oxygen requirement. If measured, carbon monoxide diffusion capacity (DLCO) > 50%.

Central Nervous System: Based on clinical exam, no concern for/evidence of active CNS infection. Patients with fully treated prior CNS infections are eligible. Patients with seizure disorders may be enrolled if seizures are well-controlled on anticonvulsant therapy.

4.2.6 Patients who have experienced their relapse after HCT are eligible, provided they have no evidence of acute or chronic Graft-versus-Host Disease (GVHD) and are off all transplant immune suppression therapy for at least 7-days (e.g. steroids, cyclosporine, tacrolimus). Steroid therapy for non-GVHD and/or non-leukemia therapy is acceptable.

4.2.7 Immunotherapy: At least 42 days after the completion of any type of immunotherapy aside from blinatumomab (e.g. tumor vaccines or CAR T-cell therapy).

4.2.8 XRT: Cranial or craniospinal XRT is prohibited during protocol therapy. ≥ 90 days must have elapsed if prior TBI, cranial or craniospinal XRT

4.2.9 Sexually active females of child-bearing potential must agree to use adequate contraception (diaphragm, birth control pills, injections, intrauterine device [IUD], surgical sterilization, subcutaneous implants, or abstinence, etc.) for the duration of treatment and for 2 months after the completion of blinatumomab therapy. Sexually active men must agree to use barrier contraceptive for the duration of treatment and for 2 months after the completion of blinatumomab therapy.

4.2.10 Voluntary written consent before performance of any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the subject at any time without prejudice to future medical care.

4.2.11 All patients enrolled in this study must have been enrolled in the Blinatumomab Bridging Therapy (BBT) Trial (Burke IIT)

4.3 Exclusion Criteria

4.3.1 Active extramedullary disease or presence of chloromatous disease.

4.3.2 Receiving concomitant chemotherapy, radiation therapy; immunotherapy or other anti-cancer therapy for treatment of disease other than is specified in the protocol.

4.3.3 Systemic fungal, bacterial, viral, or other infection not controlled (defined as exhibiting ongoing signs/symptoms related to the infection and without improvement, despite appropriate antibiotics or other treatment). Patients with possible fungal infections must have had at least 2 weeks of appropriate anti-fungal antibiotics and be asymptomatic.

4.3.4 Pregnant or lactating. The agents used in this study are known to be teratogenic to a fetus and there is no information on the excretion of agents into breast milk. All females of childbearing potential must have a blood test or urine study within 7 days prior to registration to rule out pregnancy.

4.3.5 Known allergy to any chemotherapies or targeted agents included in this protocol.

4.3.6 Participating in a concomitant Phase 1 or 2 study involving treatment of disease.

4.3.7 Active malignancy other than B-ALL.

4.3.8 Exhibit overt hematologic manifestation of relapse or persistent disease

4.4 Donor Eligibility

4.4.1 Allowed Donor Sources

1. Fully matched sibling donors are not allowed.
2. Unrelated donors. HLA typing of at least 10 alleles is required. Donor must be matched at 9/10 or 10/10 alleles (HLA A, B, C, DRB1, DQB1).
3. Haploidentical matched family members. Minimum match level full haploidentical (at least 5/10; HLA A, B, C, DRB1, DQB1 alleles), but use of haploidentical donors with extra matches (e.g. 6, 7, or 8/10) encouraged.
4. High resolution typing at all loci to be performed.

4.4.2 Inclusion Criteria for Donors

Donor eligibility will be determined in compliance with Code of Federal Regulations 21 CFR 1271, subpart C. For a donor to be eligible, the donor must meet donor criteria for human cells, tissues and cellular and tissue-based products. Specifically, a donor is eligible under these provisions only if:

- Donor screening in accordance with 1271.75 indicates that the donor:
  - Is free from risk factors for, and clinical evidence of, infection due to relevant communicable disease agents and diseases; **and**
  - Is free from communicable disease risks associated with xenotransplantation; **and**
- The results of donor testing for relevant communicable disease agents in accordance with 1271.80 and 1271.85 are negative or nonreactive, except as provided in 1271.80(d)(1).
- If a donor does not meet these criteria, he/she is not eligible.
- Donor must be willing to undergo G-CSF mobilization and stem cell apheresis.

5.0 STUDY ENTRY AND WITHDRAWAL; STUDY PROCEDURES

5.1 Required Preregistration Screening Tests and Procedures

Screening assessments must be performed within 21 days prior to enrollment. Any results falling outside of the reference ranges may be repeated at the investigator's discretion. All on-study visit procedures are allowed a window of ± 2 days unless otherwise noted. Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation.

A written, signed informed consent form (ICF) and a Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained before any study-specific assessments are initiated. A copy of the signed ICF and HIPAA form will be given to the subject and a copy will be filed in the medical record. The original will be kept on file with the study records.

All patients who are consented will be registered in the Online Enterprise Research Management Environment (OnCore™), the MCW Cancer Center and Pediatric Clinical Trials Office (CTO) Clinical Trial Management System. The system is password protected and meets HIPAA requirements.

5.2 Registration Process

Upon completion of the screening evaluation, eligibility confirmation and obtaining written consent, the patient will be registered in the study file by the Department of Pediatrics Division of Hematology/Oncology/BMT MACC Fund Center Clinical Trials Office (CTO).

5.3 Pretreatment Period

5.3.1 Screening/Baseline Assessments

The screening procedures and baseline assessments must be completed within 21-days of the beginning of conditioning chemotherapy:

- Physical examination
- Vital signs
- Complete medical history
- Baseline conditions assessment
- Performance status (Karnofsky/Lansky Performance Score)
- Disease Evaluation
  - Bone marrow (BM) aspirate and/or biopsy (morphology, cytogenetics, FISH, and minimal residual disease via HTS and flow cytometry). Baseline blast samples will be sent for B-cell and T-cell clonality HTS assessment as part of the BBT, since B-lymphoblasts may have detectable T cell receptor (TCR) clones. If T-cell clonal sequences are detected on the baseline sample, T-cell clonality will continue to be sent along with B-cell clonality on subsequent samples.
  - Lumbar puncture (CSF cytology and cell count)
- History of prior treatments and any residual toxicity relating to prior treatment
- Baseline medications

- Complete blood count (CBC) with differential and platelet count
- Blood chemistry assessment, including:
  - Alkaline phosphatase, aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, calcium, phosphorus, magnesium, blood urea nitrogen (BUN), creatinine, total protein, albumin, glucose, potassium, sodium, chloride, bicarbonate, lactate dehydrogenase (LDH)
  - Thyroid function tests: thyroid-stimulating hormone (TSH), free thyroxine (FT4)
- Coagulation assessment, including prothrombin time, partial thromboplastin time, international normalized ratio (PT/PTT/INR)
- IgG level
- Urinalysis
- Serum or urine pregnancy test within 7 days prior to the start of conditioning
- Imaging (CT or MRI) of chest/abdomen/pelvis/sinuses (C/A/P/S) to evaluate for occult infection
- Electrocardiogram (ECG)
- Cardiac assessment (ECHO, MUGA, etc.)
- Pulmonary function testing (including DLCO), if able
- Renal GFR
- Dental Evaluation per institutional standards (May be performed up to 60 days prior to the start of conditioning)
- Infectious disease markers per institutional standards

Study Calendar

Assessments	Baseline	Conditioning	HCT	Post-HCT																Follow-Up		
	Day -34 to -13	Day -12 to -1	Day 0	Day +7	Day +14	Day +19	Day +21	Day +28	Day +35	Day +42	Day +49	Day +56	Day +63	Day +70	Day +77	Day +84	Day +91	Day +100	Day +135	Day +180	Day +270	Day +365
Inclusion/Exclusion Criteria	X																					
Demographics	X																					
Medical History[A]	X																					
Physical exam, vitals, weight	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Performance Status	X							X										X		X		X
AE evaluation		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Echocardiogram	X																					X
CT or MRI of C/A/P/S	X																					
Pulmonary Function[B]	X																			X		X
12-lead ECG	X																					
Disease evaluation (BM and CSF)	X							X										X		X		X

Assessments	Baseline	Conditioning	HCT	Post-HCT																Follow-Up		
	Day -34 to -13	Day -12 to -1	Day 0	Day +7	Day +14	Day +19	Day +21	Day +28	Day +35	Day +42	Day +49	Day +56	Day +63	Day +70	Day +77	Day +84	Day +91	Day +100	Day +135	Day +180	Day +270	Day +365
FC MRD	X							X										X		X		X
HTS MRD	X							X										X		X		X
Dental evaluation[C]	X																					
CBC w/ differential	X			X	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
IgG level	X							X				X				X				X		X
Chemistry Panel[D]	X			X	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Thyroid function tests	X																					
Coagulation assessment	X																	X				
Urine analysis	X																	X				
Pregnancy Test[E]	X																					
ABO Rh blood typing	X																					
Viral Studies				X	X		X	X	X	X	X	X	X	X	X	X	X	X				
Infectious Disease Markers	X																					
Infection Assessment				X	X		X	X	X	X	X	X	X	X	X	X	X	X				
GFR	X																					
GVHD clinical assessment				X	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Chimerism[F]								X										X		X		X
Immune cell phenotyping						X											X		X	X		
Functional assessment of lymphocyte subsets						X											X		X	X		
Plasma multiplex cytokine analysis						X											X		X	X		
PROMIS measures	X																	X		X		X

Note: Timing of protocol therapy administration, evaluations and response assessment (disease evaluation) studies are based on schedules derived from the experimental design or on established standards of care. Disease evaluations may require adjustments by ± 2 weeks and other assessments may require minor adjustment by ± 14 days for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues.

A. Includes concomitant medications related to that prior treatment/medical history
B. Pulmonary evaluation to include LV, DLCO, and spirometry for patients ≥ 7 years; pulse oximetry for patients < 7 years.
C. May be performed up to 60 days prior to the start of conditioning
D. Chemistry panel includes: electrolytes, magnesium, calcium, phosphate, BUN, creatinine, glucose, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, LDH
E. Only females of child-bearing age are required to have urine pregnancy testing.
F. Blood (whole and sorted) and Bone Marrow (unsorted) should be sent at each timepoint.

5.4 Study Procedures During the Post-HCT Period

- Weekly up to Day +100:
  - Physical Exam
  - Vital signs
  - Body weight
  - GVHD clinical assessment
  - Infection assessment
  - CBC with differential and platelet count
  - PCR analysis of viral reactivation/infection (including EBV [starting at Day +28], CMV and Adenovirus)
  - Chemistry panel
  - Adverse event (AE) evaluation
- Days +28, +100, +180, +365 (+/- 14 days):
  - Performance score (Lansky/Karnofsky)
- Days +19, +91, +135, +180 (+/- 14 days):
  - Immune cell phenotyping
  - Functional assessment of lymphocyte subsets
  - Plasma multiplex cytokine analysis
- Days +28, +100, +180, +1 year
  - High throughput sequencing (HTS) and flow cytometry for minimal residual disease from bone marrow. Baseline blast samples will be sent for B-cell and T-cell clonality HTS assessment as part of the BBT, since B-lymphoblasts may have detectable T cell receptor (TCR) clones. If T-cell clonal sequences are detected on the baseline sample, T-cell clonality will continue to be sent along with B-cell clonality on subsequent samples.
  - Bone marrow biopsy/aspirate with morphology, cytogenetics, FISH as indicated
  - Lumbar puncture (CSF cytology and cell count)
- Days Baseline (pre-HCT work-up), +100, +180, +1year (+/- 14 days):
  - PROMIS measures

5.5 Study Procedures, Prior to Post-HCT Blinatumomab Day 1

- Physical examination
- Vital signs
- Performance status
- Evaluation of adverse events
- Concomitant medications
- CBC with differential and platelet count
- Blood chemistry assessment, including:
  - Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, glucose, potassium, sodium, chloride, bicarbonate, LDH
- Coagulation assessment, including PT/PTT/INR
- Urinalysis

5.6 Study Procedures, During Treatment

Inpatient visits begin on Day -12 when the conditioning regimen begins, and patients remain hospitalized during conditioning, transplantation and during the post-transplant recovery period. Hospital discharge time point depends on the unique medical condition of each patient and is therefore, variable. Follow-up assessments after discharge will be performed/obtained in the outpatient setting, unless the subject has been readmitted to the hospital.

5.7 End of Treatment

5.7.1 End of Treatment Procedures

To be completed within 60 days (± 14 days) following the completion of blinatumomab (approximately Day +180 following allo-HCT).

Bone marrow aspirate and/or biopsy (morphology, cytogenetics, FISH and minimal residual disease via HTS and flow cytometry). Baseline blast samples will be sent for B-cell and T-cell clonality HTS assessment as part of the BBT, since B-lymphoblasts may have detectable T cell receptor (TCR) clones. If T-cell clonal sequences are detected on the baseline sample, T-cell clonality will continue to be sent along with B-cell clonality on subsequent samples.

- Lumbar puncture (CSF cytology and cell count)

5.8 Post-Treatment

5.8.1 Follow-Up Visits

All subjects, including those who discontinue protocol therapy early, will be followed for 12 months from HCT infusion (Day 0).

These visits will include (+/-14 days) a minimum of:

- Outpatient Visit at 6 months after transplant
- Outpatient Visit at 9 months after transplant
- Outpatient Visit at 1 year after transplant (final study visit)

Follow-up assessments may occur with the subject's local health care provider after the Day +100 post-HSCT assessment with PI approval.

Routine outcomes (treatment related mortality, disease free survival) will be abstracted from the Blood and Marrow Transplantation (BMT) medical record and institutional database through the 3rd year post-transplant follow-up.

5.9 Long-Term/Survival Follow-Up Procedures

Long-Term follow-up care will be provided according to the treating institution's SOP.

5.10 Study Withdrawal Procedures

5.10.1 Duration of Therapy

Treatment will consist of conditioning chemotherapy, hematopoietic cell transfusion, post-HCT care, and blinatumomab at Day +100. Subjects will receive protocol therapy unless:

- Subject withdraws consent or is non-compliant
- Disease progression
- Inter-current illness that prevents further administration of treatment
- Unacceptable toxicity

5.10.2 Subject-Initiated Withdrawal

A subject may decide to withdraw from the study at any time prior to the start of conditioning for HCT.

5.10.3 Investigator-Initiated Withdrawal

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

5.10.4 Sponsor-Initiated Withdrawal

Sponsor's decision to discontinue the study.

5.10.5 Withdrawal Documentation Procedure

The reason for study withdrawal and the date the subject was removed from the study must be documented in the case report form (CRF).

5.10.6 Replacement of Subjects who Withdraw

Subjects who withdraw from the study for any reason prior to receiving at least one day of blinatumomab will be replaced until the target sample size of 10-15 patients in each stratum is reached. If a patient does not withdraw his/her consent, they will be followed up for one year after HCT.

6.0 TREATMENT PLAN

6.1 Overview of Treatment Plan

Treatment begins with administration of a reduced-intensity conditioning (RIC) regimen of ATG, fludarabine, thiotepa, and melphalan if the patient was found to be MRD negative by BOTH flow cytometry (FC) and high throughput sequencing (HTS). Baseline blast samples will be sent for B-cell and T-cell clonality HTS assessment as part of the BBT, since B-lymphoblasts may have detectable T cell receptor (TCR) clones. If T-cell clonal sequences are detected on the baseline sample, T-cell clonality will continue to be sent along with B-cell clonality on subsequent samples and both are required to be negative to be eligible for the RIC regimen. If the patient was MRD negative by FC ONLY (HTS positive), they will receive a myeloablative conditioning regimen consisting of total body irradiation, cyclophosphamide, and thiotepa. Patients will be transplanted on Day 0 with matched unrelated OR haploidentical donor PBSC grafts depleted of TCRα/β+ and CD19+ cells. Graft composition is targeted to contain ≥ 4 x 10^6 cells/kg patient BW of viable CD34+ cells while minimizing the number of CD3+TCRα/β+ cells in the graft. As prophylaxis for GVHD, all patients receiving grafts containing >100,000 CD3+ TCRα/β+ cells/kg BW for matched unrelated donors and >500,000 CD3+ TCRαβ+ cells/kg BW will be administered mycophenolate mofetil for 28 days followed by a rapid taper based on the patient's clinical status. If patients are known EBV positive, a single dose of rituximab will be administered for PTLD prophylaxis. All patients will receive a single cycle of blinatumomab 100 days following HCT.

6.2 Reduced Intensity Arm (MRD Negative Disease Status by Both Flow Cytometry and Next Generation Sequencing)

Treatment begins with administration of a reduced-intensity conditioning regimen of ATG, fludarabine, thiotepa, and melphalan. Patients will be transplanted on Day 0 with donor PBSC grafts depleted of TCRα/β+ and CD19+ cells. In the unlikely event that an insufficient number of CD34+ cells are in the HSC graft; a stem cell boost will be provided as soon as possible. This will be obtained by a second apheresis of the donor. Graft composition, as specified in the IDE, is targeted to contain ≥ 4 x 10^6 cells/kg patient BW of viable CD34+ cells while minimizing the number of CD3+TCRα/β+ cells in the graft. If the patient is EBV positive, a single dose of rituximab will be administered for PTLD prophylaxis.

6.3 Overview of Reduced-Intensity Conditioning Regimen

Prior to transplantation, patients will receive a reduced-intensity conditioning regimen of ATG (haploidentical donor source only), fludarabine, thiotepa, and melphalan (see Reduced-Intensity Conditioning Regimen table below). Coordination of mobilization and apheresis of the donor with the conditioning of the patient will proceed so that HSC graft infusion occurs on Day 0 (see Schema).

Reduced-Intensity Conditioning Regimen				
Day	**Medication**	**Dosage**	**Route**	**Dose**
-12	Rabbit ATG*	1 mg/kg	IV over at least 6 hours	1 of 4
-11	Rabbit ATG	3 mg/kg	IV over at least 4 hours	2 of 4
-10	Rabbit ATG	3 mg/kg	IV over at least 4 hours	3 of 4

Reduced-Intensity Conditioning Regimen				
Day	**Medication**	**Dosage**	**Route**	**Dose**
-9	Rabbit ATG	3 mg/kg	IV over at least 4 hours	4 of 4
-8	Fludarabine	40 mg/m^2	IV over 30 minutes	1 of 4
-7	Fludarabine	40 mg/m^2	IV over 30 minutes	2 of 4
-6	Fludarabine	40 mg/m^2	IV over 30 minutes	3 of 4
-5	Fludarabine	40 mg/m^2	IV over 30 minutes	4 of 4
-4	Thiotepa	5 mg/kg bid q 12 hours	Dose 1: IV over 4 hours Dose 2: IV over 4 hours	1 of 2 2 of 2
-3	Melphalan	70 mg/m^2	IV bolus	1 of 2
-2	Melphalan	70 mg/m^2	IV bolus	2 of 2

**if Dose 1 of ATG (administered over at least 6 hours) is well tolerated, may decrease infusion duration to a period of at least 4 hours for subsequent doses 3 mg/kg IV over at least four hours on Day -11, -10, -9*

6.4 Reduced Intensity Conditioning Agents and Guidelines for Administration

Note that the protocol treatment schedule may require minor adjustment by ± 24 hours for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia- scheduling issues.

6.4.1 Rabbit ATG (Haploidentical Donor Source Only) Administration Guidelines

Intravenous infusion through a high-flow vein Days: -12, -11, -10, -9

Dose: 1 mg/kg IV over at least six hours on Day -12

6.4.1.2 Rabbit ATG Special Nursing Instructions, Precautions and Monitoring

- Administer through an in-line 0.22 µm filter.
- Pre-medicate with methylprednisolone, diphenhydramine, and acetaminophen 30-60 minutes prior to the infusion to reduce the incidence and intensity of side effects during the infusion.
- Have emergency medications readily available.
- Always keep appropriate resuscitation equipment at the patient's bedside while rabbit ATG is being administered.
- Observe the patient continuously for possible allergic reactions throughout the infusions.

6.4.2 Fludarabine Administration Guidelines

Intravenous infusion over 30 minutes Days: -8, -7, -6, and -5.

Dose: 40 mg/m^2/day

6.4.2.1 Fludarabine Special Nursing Instructions, Precautions and Monitoring

- Use of fludarabine in combination with pentostatin is not recommended due to the risk of severe pulmonary toxicity.

6.4.3 Thiotepa Administration Guidelines

Intravenous infusion over 4 hours Day: -4

Dose: 5 mg/kg/dose, bid q 12 hours

6.4.3.1 Thiotepa Special Nursing Instructions, Precautions and Monitoring

- Protect from light at all times
- Filter solutions through a 0.22µm filter prior to administration; if solution remains opaque after filtration, it should not be used.
- Bathe patient and change linen according to institutional guidelines after administration to avoid contact dermatitis and discoloration of the skin.

6.4.4 Melphalan Administration Guidelines

Intravenous infusion through a peripheral or central line Days: -3 and -2

Dose: 70 mg/m^2 day

6.4.4.1 Melphalan Special Nursing Instructions, Precautions and Monitoring

- Provide fluid management per institutional standards
- Infusion must be completed within 60 minutes of product reconstitution.

6.5 Myeloablative Conditioning (MRD Negative Disease Status by Flow Cytometry, but MRD Positive by NGS)

Prior to transplantation, patients will receive a myeloablative conditioning regimen of ATG (haploidentical donor source only), consisting of Total Body Irradiation, cyclophosphamide, and thiotepa (see Myeloablative Conditioning Regimen table below). Patients will be transplanted on Day 0 with donor PBSC grafts depleted of TCRα/β+ and CD19+ cells. In the unlikely event that an insufficient number of CD34+ cells are in the HSC graft, a stem cell boost will be provided as soon as possible. This will be obtained by a second apheresis of the donor. Graft composition is targeted to contain ≥ 5 x 10^6 cells/kg patient BW of viable CD34+ cells while minimizing the number of CD3+TCRα/β+ cells in the graft. If the patient is EBV positive, a single dose of rituximab will be administered for PTLD prophylaxis.

Myeloablative Conditioning Regimen				
Day	**Medication**	**Dosage**	**Route**	**Dose**
-11	Rabbit ATG	3 mg/kg	IV over at least 6 hours	1 of 3
-10	Rabbit ATG	3 mg/kg	IV over at least 4 hours	2 of 3
-9	Rabbit ATG	3 mg/kg	IV over at least 4 hours	3 of 3
-8	TBI	200 cGy bid		
-7	TBI	200 cGy bid		
-6	TBI	200 cGy bid		

Myeloablative Conditioning Regimen				
Day	**Medication**	**Dosage**	**Route**	**Dose**
-5	Thiotepa	5 mg/kg	IV	1 of 2
-4	Thiotepa	5 mg/kg	IV	2 of 2
-3	Cyclophosphamide	60 mg/kg	IV	1 of 2
-2	Cyclophosphamide	60 mg/kg	IV	2 of 2

6.6 Myeloablative Conditioning Agents and Guidelines for Administration

Note that the protocol treatment schedule may require minor adjustment by ± 24 hours for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia- scheduling issues.

6.6.1 Rabbit ATG (Haploidentical Donor Source Only) Administration Guidelines

Intravenous infusion through a high-flow vein Days: -11, -10, -9

Dose: 3 mg/kg IV over at least six hours on Day -11

if Dose 1 of ATG (administered over at least 6 hours) is well tolerated, may decrease infusion duration to a period of at least 4 hours for subsequent doses 3 mg/kg IV over at least four hours on Day -10, -9

6.6.1.1 Rabbit ATG Special Nursing Instructions, Precautions and Monitoring

- Administer through an in-line 0.22 µm filter.
- Pre-medicate with methylprednisolone, diphenhydramine, and acetaminophen 30-60 minutes prior to the infusion to reduce the incidence and intensity of side effects during the infusion.
- Have emergency medications readily available.
- Always keep appropriate resuscitation equipment at the patient's bedside while rabbit ATG is being administered.
- Observe the patient continuously for possible allergic reactions throughout the infusions.

6.6.2 Thiotepa Administration Guidelines

Intravenous infusion over 4 hours

Day: -5, -4

Dose: 5 mg/kg/dose

6.6.2.1 Thiotepa Special Nursing Instructions, Precautions and Monitoring

- Protect from light at all times.
- Filter solutions through a 0.22µm filter prior to administration; if solution remains opaque after filtration, it should not be used.

- Bathe patient and change linen according to institutional guidelines after administration to avoid contact dermatitis and discoloration of the skin.

6.6.3 Cyclophosphamide Administration Guidelines

Intravenous infusion through a peripheral or central line Days: -3 and -2

Dose: 60 mg/kg/dose

6.6.3.1 Cyclophosphamide Special Nursing Instructions, Precautions and Monitoring

- Provide fluid management and mesna per institutional standards

6.7 Blinatumomab (Both Strata)*

***Only to be given if no evidence of > Grade 1 acute GVHD or any active infections within 1 week of blinatumomab infusion**

Minimal Organ Functions to be Met Before the Start of the Blinatumomab Infusion

Patients must meet the following criteria (prior to blinatumomab infusion)
- No oxygen requirement with oxygen saturations > 90%.
- AST, ALT < 5x upper limit of normal for age; bilirubin < 2 mg/dL.
- Hemoglobin > 8 mg/dL prior to infusion. (May be transfusion dependent).
- Renal function: serum creatinine < 2 x normal for age.
- Exhibit overt hematologic manifestations of relapse or persistent disease. Evidence of recurrent/persistent disease based primarily on flow cytometry, cytogenetics, chimerism analysis, or other molecular studies does not by itself represent grounds for exclusion.

Administration Guidelines: Intravenous infusion through a central line starting Day +100 +/- 7 days **Blinatumomab will be given as a 28-day continuous infusion**		
	Patient Weight Greater Than or Equal to 45 kg (*Fixed-dose*)	**Patient Weight Less Than 45 kg (*BSA-based dose*)**
Days 1-28	28 mcg/day	15 mcg/m^2/day (not to exceed 28 mcg/day)
Blinatumomab Special Nursing Instructions, Precautions and Monitoring • Must be administered through a central line at rate of 5 mL/hr IV over 24 hours through an acceptable IV line. Only **PVC non-DEHP lines with a 0.2 µm inline filter are acceptable**. • Do not flush the IV line as it will create an IV bolus to be administered into the patient. For outpatient administration, use FDA approved pumps. • Only the exact volume should be administered; any remaining overfill should be discarded appropriately. • Hospitalization is recommended for the first 3 days of Cycle		

Administration Guidelines: Intravenous infusion through a central line starting Day +100 +/- 7 days **Blinatumomab will be given as a 28-day continuous infusion**		
	Patient Weight Greater Than or Equal to 45 kg (*Fixed-dose*)	**Patient Weight Less Than 45 kg (*BSA-based dose*)**
• Pre-medicate with dexamethasone o Adults, premedicate with 20 mg of dexamethasone 1 hour prior to the first dose of Blinatumomab in the first cycle and when restarting an infusion after an interruption of 4 or more hours o Pediatric patients, premedicate with 5 mg/m^2 of dexamethasone, to a maximum dose of 20 mg, prior to the first dose of Blinatumomab in the first cycle and when restarting an infusion after an interruption of 4 or more hours		

6.8 Transplantation with TCRα/β- and CD19-Depleted HSC Graft

6.8.1 Mobilization and Collection of Donor PBSC

- Donor (or guardian) must sign the consent for donation of stem cells following explanation of risks and benefits.
- Donor should receive a recommended G-CSF regimen of 15 mcg/kg/d starting Day-5, with leukapheresis on Day -1, and 0 (if necessary). Collection center SOP's apply. Target CD34+ dose/kg patient weight to be collected is 10-20 x 10^6. Based upon prior experience, we anticipate collecting sufficient donor cells in one or two collections.
- Unrelated donors: Peripheral stem cells collected as per standards of the National Marrow Donor Program.

6.8.2 Manufacturing Process

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 IND, 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 the Medical College of Wisconsin and the University of Wisconsin. A median log 4.8 depletion of α/β T cells and log 3.5 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.

6.8.3 Specification for Final Formulation of Stem Cell Graft

Targeted Graft Composition		
Cell Type	**Target Number/kg Patient BW in Graft**	**Action**
CD3+TCRαß	≤ 25,000	•If greater than target of 1 x 10^5 per kg for haploidentical donor or greater than target of 5 x 10^5 per kg for URD→administer mycophenolate mofetil (20 mg/kg BID Day +1 until Day +30, then taper) •The maximum limit of TCRα/β+ cells for release is 5.0 x 10^5 cells/kg.
CD19+	≤ 1 x 10^5	N/A
CD34+	Minimum of ≥ 4 x 10^6 Goal of ≥ 5 x 10^6 for unrelated donors Goal of ≥ 10 x 10^6 for haploidentical donors	•If less than 4 x 10^6 cells/kg→stem cell boost will be administered after the initial graft. Stem cell boost will be obtained by second apheresis of donor.

6.8.3.1 **Release Criteria** for the graft serves to maximize CD34+ cells while minimizing CD3+ TCRα/β+ cells in the graft. The maximum limit of TCRα/β+ cells for release is < 5.0 x 10^5 cells/kg.

6.8.3.2 TCRα/β- and CD19-depleted HSC graft will be released from the Stem Cell Processing facility to the BMT Unit for patient infusion after flow cytometry and standard quality control testing has been completed.

6.8.3.3 A final product containing < 70% viable CD34+ cells will not be administered and in such cases, an application for compassionate use of the investigational device under this IDE via an IDE supplement will be submitted.

6.8.3.4 In the event that the final product tests positive for Gram stain, the PI (or PI designee) and treating physician will be notified prior to release so that appropriate clinical action can be taken, including initiation of appropriate, empiric broad- spectrum antibiotic treatment. Moreover, testing will be initiated to identify the contaminating organism and its antibiotic susceptibility. Investigation into the source of contamination and corrective action will be implemented.

6.9 Packaging and Labeling

Labeling of final HSC graft product will be performed in accordance with the SOPs at each participating institution. 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 Pediatric BMT Unit in sterile bags that are packed for transport in outer sterile overwrap packaging.

6.10 Administration of CD3+TCRα/β+/CD19+ Depleted HSC Graft

For transplantation, patients will receive the TCRα/β- and CD19-depleted HSC graft intravenously on Day 0 after the appropriate premedication per institutional guidelines. Resuscitation equipment and emergency medications will be on hand in case of infusion reaction.

Patients receiving grafts containing >1x 10^5 for haploidentical donors and >5 x 10^5 for URD CD3+ TCRα/β+ cells/kg BW will be administered mycophenolate mofetil for 28 days followed by a rapid taper based on the patient's clinical status.

If the patient is EBV positive, prophylactic rituximab (375 mg/m^2 IV on Day 1) will be administered on Day +1.

If insufficient stem cell content (≤ 4 x 10^6 CD34+ cells/kg patient BW of the initial HSC graft is due to poor mobilization of the donor, the second apheresis product will be processed using TCRα/β- and CD19-depletion.

If insufficient stem cell content of the initial HSC graft is due to inefficient TCRα/β cell depletion, the second apheresis product will be processed using CD34+ selection.

6.11 Monitoring During Transfusion of Graft

Nursing staff will remain at the patient's bedside during the entire CD3+TCRα/β+/CD19+ depleted hematopoietic stem cell graft infusion. Patients will be monitored for adverse effects of the infusion such as rash, acute allergic reaction, bronchospasm, respiratory distress, and acute vascular leak syndrome. 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 institutional guidelines.

6.12 Graft Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the PI is required to maintain accurate accountability records throughout the study. The administration of the HSC grafts will be documented in the CRF and in the patient's medical file. All partially used HSC grafts will be disposed of according to facility SOPs for disposal of cellular product and/or biohazard waste.

6.13 Prophylaxis and Supportive Care

6.13.1 General

All supportive measures consistent with optimal patient care will be given throughout the study. Institutional standards for general supportive care during the conditioning regimen will be maintained including antiemetic prophylaxis and treatment. In addition, institutional guidelines will be followed for general supportive care after transplantation and will include antimicrobial agents, nutritional support and blood product support as necessary.

6.13.2 Venous Access

Patients will have an appropriate long-term central venous access placed according to institutional standard practice, prior to initiation of the conditioning regimen.

6.13.3 Isolation

Recipients will be maintained in single occupancy rooms with protective isolation per institutional guidelines.

6.13.4 Nutrition

Parenteral nutrition will be initiated depending on the patient's needs.

6.13.5 Blood Products

All blood products, except the infused CD3+TCR α/β- and CD19-depleted stem cells, will be irradiated in accordance with institutional standards. Recipients who are CMV negative will receive leukocyte depleted blood products from study entry.

6.13.6 Anti-Allergic Prophylaxis

Note that during the administration of Rabbit ATG, HSC graft and rituximab (if administered), resuscitation equipment and emergency medication shall be readily available in case of an infusion reaction and the following pre-medications will be administered per institutional guidelines.

6.13.7 Post-Transplant Lymphoproliferative Disease

A history of EBV may predispose patients to PTLD, which could be life threatening. If the patient is EBV positive, a single dose of rituximab will be administered on Day +1 (375 mg/m^2). PCR analysis for EBV infection/reactivation will be performed per institutional standards. Treatment of PTLD will be performed per institutional standards at the discretion of the treating physician.

6.13.8 Prophylaxis of Viral, Bacterial, and Fungal Infections

Patients will be treated per institutional guidelines with appropriate prophylaxis strategies for this patient population.

- IgG will be measured per institutional standards; if IgG < 400 mg/dL, then IVIG will be administered at a dose per institutional standards.

6.13.9 Management of Suspected or Confirmed Viral Reactivation

6.13.9.1 If EBV reactivation is suspected based on a positive PCR assessment:

- If EBV viral load ≥ 1,000 copies/mL, then treat with appropriate anti- viral therapy per institutional standards
- If EBV viral load < 1,000 copies/mL, then repeat assessment; if repeat testing

demonstrates:
- same or lower viral load, then monitor twice weekly until undetectable
- a higher viral load, then treat with appropriate anti-viral therapy per institutional standards

6.13.9.2 If CMV-, ADV- reactivation is suspected because of positive PCR or antibody assays, specific preemptive antiviral therapy will be instituted in accordance with institutional standards.

6.13.10 Management of Bacterial Infections

Antibiotic treatment will be administered per institutional guidelines.

6.13.11 Management of ABO Incompatibility

All patients with ABO incompatibility to their donor should be evaluated and treated according to institutional standards.

6.13.12 Management of Thrombocytopenia/Anemia

Irradiated leukocyte-poor platelets or irradiated packed red blood cells will be administered according to institutional standards.

6.13.13 Management of Febrile Neutropenia

Broad-spectrum antibiotics will be administered intravenously according to institutional guidelines.

6.13.14 Prophylaxis and Management of Acute GVHD

As prophylaxis for GVHD, all patients receiving grafts containing >1x 10^5 for haploidentical donors and >5 x 10^5 for URD CD3+ TCRα/β+ cells/kg BW will be administered mycophenolate mofetil at a dose of 15 mg/kg given over 2 hours (total maximum dose of 2 g/day), every 8 hours for 28 days followed by a rapid taper based on the patient's clinical status.

Recipients who develop acute GVHD will be treated according to institutional guidelines at the discretion of the PI (or PI designee).

6.13.15 Management of Graft Failure

Primary (absolute ANC < 500/µL at Day +28) or secondary graft failure (initial neutrophil engraftment followed by a persistent decline in ANC < 500/µl that is unresponsive to growth factor therapy) will be considered a treatment failure. Graft failure is considered a treatment related medical emergency necessitating a second transplant. An individualized plan utilizing stem cells from the best available donor will be developed by the PI (or PI designee) and BMT team based on the clinical status of the patient.

6.13.16 Management of Post-Transplantation Relapse

Patients who relapse or progress following protocol treatment will be treated according to individual treatment decision.

6.14 Follow-Up Period

Subjects will be followed for 1-year post-HCT or post-blinatumomab (last cycle given), whichever occurs later or after removal from the study treatment or until death, whichever occurs first.

Subjects removed from the study treatment for unacceptable SAEs will be followed until resolution or stabilization of the adverse event. SAEs will be followed until completion.

7.0 DOSING DELAYS/DOSE MODIFICATIONS

7.1 Dose Limiting Toxicity: Blinatumomab

The most frequent serious adverse events noted in patients treated with blinatumomab to date are disorders of the nervous system, both peripheral and central, and systemic cytokine release syndrome (CRS). Both categories of events are more likely to occur within the first week of treatment with blinatumomab, and both categories of events are usually reversible and able to be managed with attentive supportive care.

AEs related to blinatumomab that require treatment interruption (according to table below) and do not resolve to CTCAE ≤ Grade 1 within 14 days will require permanent discontinuation of blinatumomab treatment. If the patient is eligible to continue protocol therapy (chemotherapy and/or HSCT), then the patient may, at the discretion of the investigator and family, continue to receive protocol therapy. Otherwise, the patient will be off protocol therapy.

In the case that the AE(s) **DO resolve within 14 days**, blinatumomab treatment may resume at a **reduced dose of 5 mcg/m^2/day** to complete the 28-day course (not counting the duration of treatment interruption).

NOTE: For Grade 4 Nervous System/Psychiatric, Grade 3 or 4 Central Nervous System: Seizure and Grade 4 thromboembolic AEs, blinatumomab must be permanently discontinued.

For patients who had experienced a ≥ Grade 2 Neurologic Systems and Psychiatric AE related to blinatumomab, **no dose escalation beyond 5 mcg/m^2/day will be permitted**.

A **second AE** that requires interruption **will require permanent discontinuation** of treatment and the patient will be off protocol therapy.

The resumption of the infusion at the reduced dose should be accompanied by **dexamethasone premedication** (5 mg/m^2/dose, (maximum dose 20 mg) given prior to restarting infusion), should be performed in the hospital under supervision of the investigator and patients should be observed for at least 72 hours after the start of the next infusion before considering discharge to the outpatient setting.

Table 1: Dose Modifications for Adverse Events (AE) Occurring During Blinatumomab Administration

Category: AE (CTCAE v4.03)	AE Grade	Stop Infusion?	Supportive Care (in addition to institutional guidelines)*	Restart allowed (with dex premeds) if Gr 1 within 14 days?	Restarting dose (mcg/m^2/day)	Escalation to 15 mcg/m^2/day after 7 days (with dex premed) in subsequent cycle allowed?
Nervous system/ Psychiatric[1]: (Confusion, Hallucination, Delirium, Psychosis), Dysarthria, Tremor	1	N	CNS	-	-	-
	2	N	CNS	-	-	-
	3	Y	CNS, DEX	Y	5	N
	4	Y	CNS, DEX	N	-	-
Central Nervous System: Seizure[2]	1, 2	Y	SZ, CNS, DEX	Y	5	N
	3, 4	Y	SZ, CNS, DEX	N	-	-
Immune system: Cytokine release syndrome- NOTE that it is NOT recommended to use the CTCAE grading[3]	1	N	-	-	-	-
	2, 3	Y	DEX to be given as first line. TOCI only if patient experiences refractory CRS despite interruption of infusion/supportive care and use of steroids	Y	5	Y
	4	Y	DEX	N	-	-
Blood and lymphatic system[4] Disseminated intravascular coagulation, hemolysis, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura	1, 2	N	-	-	-	-
	3, 4	Y	-	Y	5	Y
Blood and lymphatic system[5]: All others (lymphopenia, neutropenia, anemia, thrombocytopenia, etc.)	1, 2, 3, 4	N	-	-	-	-
Vascular: Thromboembolic event	1	N	-	-	-	-
	2, 3	Y	-	Y	5	Y
	4	Y	-	N	-	-
Investigations[6,7] Metabolism and Nutrition: All (if not considered clinically relevant or responding to routine medical management)	1, 2, 3, 4	N	-	-	-	-

Category: AE (CTCAE v4.03)	AE Grade	Stop Infusion?	Supportive Care (in addition to institutional guidelines)*	Restart allowed (with dex premeds) if Gr 1 within 14 days?	Restarting dose (mcg/m^2/day)	Escalation to 15 mcg/m^2/day after 7 days (with dex premed) in subsequent cycle allowed?
Investigations[6,7] Metabolism and Nutrition: All (if clinically relevant and not responding to routine medical management)	1, 2	N	-	-	-	-
	3, 4	Y	-	Y	5	Y
All other AE	1, 2	N	-	-	-	-
	3, 4	Y	-	Y	5	Y

Table Footnotes

1. Most AEs in the psychiatric disorders category are unlikely to be caused by blinatumomab and generally require supportive care rather than dose modification or discontinuation of blinatumomab (e.g., Insomnia, Depression, Anxiety). Psychiatric AEs that may reflect underlying central nervous system toxicity (e.g., Confusion, Delirium, Hallucinations, Psychosis) are of greater interest, particularly if accompanied by other AEs in the nervous system disorders category.
2. In the event of more than one seizure, regardless of grade, blinatumomab should be permanently discontinued.
3. Grading of cytokine release syndrome (CRS) severity should be performed according to that of Lee et al (see below table). As many of the symptoms of CRS overlap with those of other medical complications such as infection, attribution should be carefully considered. Accurate application of this grading system requires clinical judgment to confirm that the symptoms are most likely due to CRS rather than to another medical condition. In all grades of CRS, aggressive supportive care is required. In Grade 2 or 3 CRS, careful monitoring of cardiac function is strongly suggested.

Grade 1	Symptoms are not life threatening and require symptomatic treatment only, e.g. fever, nausea, fatigue, headache
Grade 2	Symptoms require and respond to moderate intervention Oxygen requirement < 40%, or Hypotension responsive to fluids or low dose of one vasopressor, or Grade 2 organ toxicity
Grade 3	Symptoms require and respond to aggressive intervention Oxygen requirement >=40%, or Hypotension requiring high dose of one vasopressor or multiple vasopressors, or Grade 3 organ toxicity or grade 4 transaminitis
Grade 4	Life-threatening symptoms Requirement for ventilator support, or Grade 4 organ toxicity (excluding transaminitis)

4. In the first days of treatment, transient DIC-like pictures may develop. Because patients are at risk for capillary leak syndrome and cytokine release syndrome, appropriate supportive care with dexamethasone (described above), blood products and factors (packed red cells, platelets, cryoprecipitate, fresh frozen plasma), vitamin K, and/or albumin should be considered according to institutional standards of care. Particularly in the first week of infusion, when the risk of capillary leak and cytokine release is more prominent, appropriate use of blood products and factors is preferred if laboratory indications suggest the need for replacement, as large volumes of crystalloid fluids tend to exacerbate the capillary leak.
5. In the first days of treatment, a rapid transient drop in platelets, neutrophils and/or hemoglobin may be observed. These effects are not necessarily cytokine-mediated. Counts typically recover to baseline during treatment, and usually within two weeks of starting blinatumomab. Transfusion of blood and platelets should be performed according to appropriate institutional standards.
6. In the first days of treatment, transient increases in transaminases up to over 1000 U/L may develop. These have generally returned to baseline in the 1st week of treatment.
7. Decrease in serum immunoglobulins have been observed in patients treated with blinatumomab. Intravenous immunoglobulin should be administered according to institutional standards but is recommended for any patient with a total IgG level below 400. Immunoglobulin must not be administered through the line through which blinatumomab is actively being infused.

   * Definitions of supportive care abbreviations:

   DEX: Dexamethasone should be administered at a total daily dose of at least 0.2 - 0.4 mg/kg/day (maximum 24 mg per day) administered preferably intravenous divided 3 - 4 times daily for at least 1 day but no more than 4 days. The dose should then be stopped or tapered as clinically indicated.

   SZ: Appropriate imaging should be performed to evaluate for possible hemorrhage or thrombosis, and other diagnostic procedures should be performed as clinically appropriate. Prophylactic anticonvulsant treatment with a therapeutic dose of institutional standard agents (e.g., lorazepam, phenytoin, levetiracetam) should be administered if seizures develop, and continued throughout the blinatumomab infusion. Anti-convulsant therapy should be considered starting at least 24 - 48 hours prior to any subsequent blinatumomab infusions and continuing for the remainder of those treatment cycles. Diagnostic measures to exclude potential infectious causes should be conducted once the patient has stabilized (i.e., a lumbar puncture to evaluate for bacterial, viral or fungal sources should be performed). Any identified pathology should be treated as clinically appropriate.

   CNS: A daily finger-nose-finger or writing sample test is recommended according to age-appropriate activities for patients. In adults treated with blinatumomab, it has been found that a daily handwriting sample can often predict future nervous system toxicity before the clinical toxicity develops. In case of a change in finger-nose-finger or handwriting test it is recommended to start dexamethasone on the schedule above to prevent possible deterioration of nervous system toxicity. Patients who experience nervous system toxicity in the first cycle typically do not experience it again in subsequent cycles, although it is possible.

7.2 Dose Limiting Toxicity: HCT

Dose limiting toxicity criteria are all Grade 3 and 4 non-hematologic toxicities (CTCAE version 5.0) with the exception of the following expected adverse events as exhibited in Table below. Moreover, an expected adverse event will be considered a DLT if the severity of the event results in a higher than expected grade toxicity in the context of a haploidentical HSCT. All Grade 3 and 4 hematologic toxicities are expected in the setting of hematopoietic stem cell transplant.

Expected Adverse Events ≥ Grade 3

Expected Adverse Event	Expected Grade
Non-Hematologic	
Mucositis	3, 4
Infection	3, 4
Febrile neutropenia	3, 4
Fatigue	3
Anorexia	3, 4
Electrolyte disturbance:	3, 4
Hypocalcemia	3, 4
Hypomagnesemia	3, 4
Hypophosphatemia	3, 4
Hypokalemia	3, 4
Hypertension	3
Pneumonitis	3, 4
Pericardial effusion	3, 4
Dyspnea	3
Hyperbilirubinemia	3
Elevated Creatinine	3
Nausea and vomiting	3
Hematologic	
Anemia	3, 4
Bone Marrow hypocellularity	3, 4
CD4 lymphocyte count decreased	3, 4
Lymphocyte count decreased	3, 4
Neutrophil count decreased	3, 4
Platelet count decreased	3, 4
White blood cell count decreased	3, 4

7.3 Known AEs List

7.3.1 Risks of Stem Cell Transplant

7.3.1.1 **Risks Specific to αβ Depletion:** Possible increased risk of graft rejection. Graft rejection is more common in patients who did not receive prior chemotherapy, and in T-depleted transplants compared with transplants where the T cells are not removed, but this risk may

be decreased due to the presence of a low number of T cells in the infused product for patients and the high number of CD34+ cells.

7.3.1.2 **General Risks:** Infection: Bacterial, fungal, viral. These can be life threatening. Aggressive antibiotic use, including antifungal and antiviral therapy, will be initiated as needed.

7.3.1.3 **Graft vs. Host Disease (GVHD):** This can be acute or chronic, mild or severe. Severe acute GVHD (Grades 3 and 4) are associated with an increased risk of mortality from infectious complications. Chronic GVHD occurs most commonly in patients who have had acute GVHD but may occur in patients who did not have any acute symptoms. It usually develops after the third month post-transplant. It has been more common in patients who have received peripheral stem cells or marrow that have not had T cells removed. Patients can have problems with skin, liver, intestine, joints, mucous membranes, eyes, or other organs. Scarring may result. Medicines can help but may not completely eliminate all the symptoms. Chronic GVHD may have lingering symptoms for years or may go away completely. Infection is a major risk for patients with chronic GVHD, as the immune systems often do not return to normal.

7.3.1.4 **Graft Rejection:** This occurs when the patient's body does not accept the transfused donor stem cells.

7.3.1.5 Bleeding due to thrombocytopenia.

7.3.1.6 **Veno-Occlusive Disease (VOD):** This can occur as a result of chemotherapy, radiation therapy, or both. Symptoms include jaundice, with liver dysfunction, weight gain, and extra fluid in the abdominal cavity. It may often be managed successfully, and completely resolve. However, complications can arise that can be fatal.

7.3.1.7 **Mucositis and Diarrhea:** The conditioning therapy causes mucositis. This can result in painful mouth sores and diarrhea. Narcotic pain medicine is generally required for mucositis, which resolves upon engraftment.

7.3.1.8 **Capillary Leak Syndrome:** This may occur as a result of chemotherapy and radiation therapy. The blood vessels may become "leaky" and fluid enters the abdominal cavity and tissues. Edema or anasarca may result, and this may result in or worsen renal failure. Pulmonary capillary leak may cause respiratory failure or death.

7.3.1.9 **Unexpected Organ Damage:** This includes unpredictable life-threatening heart, lung, kidney, or liver damage may occur as a result of conditioning and other factors that occur post SCT. Multisystem organ failure usually results in death despite intensive care treatment.

7.3.1.10 **Late Effects From Conditioning and/or Prior Therapy:** Growth hormone deficiency, hypothyroidism, sterility, cataracts, decreased renal function, decreased heart and lung function. Secondary malignancies may also occur as a result of chemotherapy and/or radiation therapy. The risks of conditioning and prior therapy upon the developing brain are unknown.

7.3.1.11 **Risks of Stem Cell Processing:** The blood cells that have been collected will be brought to the Stem Cell Laboratory and processed under sterile conditions. The risks to this procedure include contamination with bacteria and other agents and malfunction of equipment with a loss or decrease of stem cells. These risks are extremely small.

8.0 ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS

8.1 Definitions

8.1.1 Adverse Event (AE): Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or condition temporally associated with the use of any study procedure or treatment, regardless of whether it is considered related to the study procedure or treatment. Any worsening of a pre-existing condition or illness will be considered an adverse event. The investigator will evaluate all adverse experiences as to their severity and relationship to the blinatumomab as well as the regimen as a whole.

8.1.2 Attribution: An assessment of the relationship between the AE and the medical intervention. CTCAE does not define an AE as necessarily "*caused by a therapeutic intervention"*. After naming and grading the event, the clinical investigator must assign an attribution to the AE using the following attribution categories:

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

8.1.2.1 Relationship Assessment: In-Depth Definitions

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

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

- **Probably Related:** There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result,

occurs within a reasonable time sequence to administration of the drug, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfill this definition.

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

- **Unlikely:** A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).

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

8.1.3 Expectedness: An AE is considered unexpected if the specificity or severity of the AE is not consistent with the protocol drug toxicity tables found in Section 8.0, or with available product information, or with the general investigational plan, or is unexpected in the professional opinion of the treating investigator.

8.1.4 Expedited Reporting: Serious adverse events (SAE) reporting to the coordinating site within **five (5) calendar days** of learning of the event

8.1.5 Onset and Resolution of Adverse Events: If an adverse event occurs more than once in a course (cycle) of therapy only the most severe grade of the event should be reported.

- If an adverse event progresses through several grades during one course of therapy, only the most severe grade should be reported.
- The duration of the AE is defined as the duration of the highest (most severe) grade of the Adverse Effects.
- The resolution date of the AE is defined as the date at which the AE returns to baseline or less than Grade 1, whichever level is higher (note that the resolution date may therefore be different from the date at which the grade of the AE decreased from its highest grade). If the AE does not return to baseline the resolution date should be recorded as "ongoing."
- An adverse event that persists from one course to another should only be reported once unless the grade becomes more severe in a subsequent course. An adverse event which resolves and then recurs during a different course, must be reported each course it recurs.

8.1.6 Protocol Therapy: Any of the study interventions (drugs) administered as part of this study.

8.1.7 Serious Adverse Event (SAE): An adverse event that results in any of the following outcomes:

Death of Patient: An event that results in the death of a patient.

Life-Threatening: An event that, in the opinion of the investigator, would have resulted in immediate fatality if medical intervention had not been taken. This does not include an event that would have been fatal if it had occurred in a more severe form.

Hospitalization: An event that results in an admission to the hospital for any length of time. This does NOT include planned hospitalizations per protocol, an emergency room visit or admission to an outpatient facility.

Prolongation of Hospitalization: An event that occurs while the study patient is hospitalized and prolongs the patient's hospital stay.

Congenital Anomaly: An anomaly detected at or after birth, or any anomaly that results in fetal loss.

Persistent or Significant Disability/Incapacity: An event that results in a condition that substantially interferes with the activities of daily living of a study patient. Disability is not intended to include experiences of relatively minor medical significance such as headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (e.g., sprained ankle).

Important Medical Event Requiring Medical or Surgical Intervention to Prevent Serious Outcome: An important medical event that may not be immediately life-threatening or result in death or hospitalization, but based on medical judgment may jeopardize the patient and may require medical or surgical intervention to prevent any of the outcomes listed above (i.e., death of patient, life-threatening, hospitalization, prolongation of hospitalization, congenital anomaly, or persistent or significant disability/incapacity). Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.1.8 Routine Reporting: AE reporting to the coordinating site at the time of the regularly scheduled time points listed in the Case Report Forms (CRFs).

8.2 AE and SAE Reporting on Protocol

8.2.1 Adverse Event Grading

Grade	Description
0	No AE (or within normal limits).
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade	Description
2	Moderate; minimal, local or noninvasive intervention (e.g., packing cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL).
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
4	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE

8.3 What & When to Report

Adverse events and Serious Adverse Events that meet the below criteria are to be collected. Use the CTCAE version 5.0 to code and grade AEs. These AEs are to be documented from the time the patient initiates protocol therapy until the patient meets off study criteria. The time frame for reporting of AEs depends on the grade, expectedness, attribution, and date of event in relation to the date of the patient's first and last protocol treatment dose. Expected Grades 1, 2, and 3 AEs do not need to be recorded or routine reported. **Unexpected Grade 3** AEs **do need** to be recorded in OnCore and will be routine reported to the IRB at time of Continuing Review.

Reporting Requirements for AEs that occur between enrollment until the patient meets off-study criteria.

Attribution	SAE				AE		
	Grade 1, 2 & 3		Grade 4 and 5		Grade 3	Grade 4	
	Expected	Unexpected	Expected	Unexpected	Unexpected	Expected	Unexpected
Unrelated Unlikely	IRB[1] and DSMC[2]- Routine Review[3] Sponsor	IRB[1] and DSMC[2]- Routine Review[3]	IRB[1]- Routine Review[3] DSMC[2]- Within 5 calendar days Sponsor	IRB[1]- Routine Review[3] DSMC[2]- Within 5 calendar days	DSMC[2]- Routine Review[3]	DSMC[2]- Heme: Routine review[4] Non-Heme: Within 5 calendar days	DSMC[2]- Within 5 calendar days
Possible Probable Definite		IRB[1] and DSMC[2]- Within 5 calendar days FDA[5] Sponsor		IRB[1] and DSMC[2]- Within 5 calendar days FDA[5] Sponsor			

1. Guidance on Adverse Event Reporting to the IRB is available online through the IRBNet.
2. For expedited DSMC reporting, study coordinator/research nurse must notify the DSMC via email including the subject ID, date of event, grade, relatedness, expectedness, and a short narrative. DSMC will review data entered into OnCore®.
3. For routine reporting, the events will be reported to IRB as part of the annual continuing progress report and the DSMC will review data entered into OnCore® at the time of scheduled monitoring.
4. Expected hematological grade 4 adverse events will be routine reported.
5. Fatal or life-threatening SAEs meeting the criteria indicated in the above table will be reported to FDA no later than seven calendar days after study staff's initial awareness of the event. If the SAE is not fatal or life-threatening and meets the above criteria, the timeline for submitting an IND safety report to FDA is no later than 15 calendar days after study staff's initial awareness of the event.

How to Report:

- **Routine Reporting** – Complete the AE CRF and submit according to the schedule in the CRF instructions to crconc@mcw.edu

- **Expedited Reporting** – Complete the SAE CRF and email **within 5 calendar days** to crconc@mcw.edu
- **Food and Drug Administration**
  - An IND safety report will be submitted for any adverse event that **meets all three** definitions:
    - possibly related to the study drug,
    - unexpected, and
    - serious
  - If the adverse event does not meet one of the above definitions, it should not be submitted as an expedited IND safety report.
  - Any relevant additional information that pertains to a previously submitted IND safety report will be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)).
    - ***Suggested Reporting Form:***
      US FDA MedWatch 3500A:
      http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

Coordinating Site's Responsibilities:

- **Routine Reporting** – To collect all data from participating sites and to submit data into OnCore for DSMC review at regular intervals.
- **Expedited Reporting** – To collect all data from participating sites, follow-up with participating sites for additional information as needed, and to submit data into OnCore for DSMC review within 5 calendar days. The coordinating site will submit reports to the CHW IRB according to policy. All participating sites should submit any events to the local IRB per local policy.

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

The investigator and his or her team will follow the CHW IRB policies related to unanticipated problems involving risks to subjects or others.

8.5 Time Period and Grade of AE Capture

The period of AE capture will include from the beginning of any study procedures through Day +365 post-HCT. The criteria for grading toxicities and criteria for dose modifications will be according to CTCAE version 5.0.

8.6 Monitoring and Recording an Adverse Event

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

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

earlier or later than planned).

8.6.3 Pretreatment Events Following Signed Informed Consent: For serious pretreatment events, the investigator must determine both the intensity of the event and the relationship of the event to study procedures.

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

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

8.7 Follow-Up of Adverse Events

All adverse events will be followed with appropriate medical management 30 days following the last dose of the study drug or treatment or until they are resolved, if they are related to the study treatment.

8.8 Subject Complaints

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

If the subject has questions about his or her rights as a study subject, wants to report any problems or complaints, obtain information about the study or offer input, the subject can contact the Children's Hospital of Wisconsin Institutional Review Board, whose purpose is to see that the rights and welfare of research participants are adequately protected, and that risks are balanced by potential benefits. A member of this committee is available to speak to you if you have any questions or complaints at 414-337-7133. This information is provided to the subject in their consent.

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

8.9 Routine Reporting Procedures for AEs

Reporting to the Data and Safety Monitoring Committee

Review all unexpected Grade 3, and all Grade 4, and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol. Non-hematological Grade 4 and all Grade 5 events must be reported to the DSMC within 5 calendar days of study staff's knowledge.

Hematological Grade 4 events can be routine reported. For all Grade 5 events during the blinatumomab infusion post-HCT, study enrollment will be paused until a full safety review by the DSMC has been completed.

Report Method: The investigator will use email to report SAEs to the DSMC. The SAE report must include event term(s), serious criteria, and the sponsor-investigator's or sub-investigator's determination of both the intensity of the event(s) and the relationship of the event(s) to study drug administration. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE (Version 5.0) as a guideline whenever possible.

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

Reporting to CHW Institutional Review Board

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

Reporting to Amgen

All Suspected Unexpected Serious Adverse Reactions (SUSARs) are to be reported to Amgen within 24 hours of reporting to a regulatory authority. A line-listing of SAEs needs to be provided to Amgen safety every 6 months.

Please see the following table which outlines reporting information.

Event Type	Report Recipients			
	PI/Study Chair/ Coordinating Center	Institutional Review Board	DSMC	CTO Regulatory Office
Serious Adverse Event	Within 2 days of learning of the event	5 days (or annual CPR)	5 days	Within 2 days of learning of the event
Unanticipated Problems Involving Risks to Subjects of Others (UPIRSO)	Within 2 days of learning of the event	5 days (or annual CPR)	5 days	Within 2 days of learning of the event
Evidence of Causal Relationship between Drug and AE	Within 2 days of learning of the event	5 days (or annual CPR)	5 days	Within 2 days of learning of the event
Dose-Limiting Toxicity (DLT)	Within 2 days of learning of the event	5 days (or annual CPR)	5 days	Within 2 days of learning of the event
CPR, continuing progress report				

9.0 DRUG FORMULATION AND PROCUREMENT

9.1 Cyclophosphamide

Source and Pharmacology: Cyclophosphamide is a nitrogen mustard derivative. It acts as an alkylating agent that causes cross-linking of DNA strands by binding with nucleic acids and other intracellular structures, thus interfering with the normal function of DNA. It is cell cycle, phase non-specific. Cyclophosphamide is well absorbed from the GI tract with a bioavailability of >75%. It is a prodrug that requires activation. It is metabolized by mixed function oxidases in the liver to 4-hydroxycyclo-phosphamide, which is in equilibrium with aldophosfamide. Aldofosfamide spontaneously splits into nitrogen mustard, which is considered to be the major active metabolite, and acrolein. In addition, 4-hydroxycy-clophosphamide may be enzymatically metabolized to 4-ketocyclophosphamide and aldophosfamide may be enzymatically metabolized to carboxyphosphamide that is generally considered inactive. Cyclophosphamide and its metabolites are excreted mainly in the urine. Dose adjustments should be made in patients with a creatinine clearance of < 50 mL/min.

Formulation and Stability: Cyclophosphamide is available in vials containing 100, 200, 500, 1000 and 2000 mg of lyophilized drug and 75 mg mannitol per 100 mg of cyclophosphamide. Both forms of the drug can be stored at room temperature. The vials are reconstituted with 5, 10, 25, 50 or 100 mL of sterile water for injection, respectively, to yield a final concentration of 20 mg/mL. Reconstituted solutions may be further diluted in either 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically stable for 24 hours at room temperature and 6 days if refrigerated, but contain no preservative, so it is recommended that they be used within 24 hours of preparation.

Supplier: Commercially available

CYCLOPHOSPHAMIDE (CTX, Cytoxan):

	Common Happens to 21-100 children out of every 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Loss of appetite (L), nausea (L), vomiting (L)	Metallic taste (L), abnormal hormone function affecting levels of salt in the blood and urine, causing too much or too little urine[1]	Temporary blurred vision[1], heart damage with abnormal heart rhythms[1], decay of muscle tissue in the heart[2]
Prompt: Within 2-3 weeks, prior to next course	Decrease in the number of red and white blood cells and platelets made in the bone marrow, hair loss	Bleeding and inflammation of the urinary bladder (L)	
Delayed: Any time later during therapy, excluding the above conditions	Decreased ability of the body to fight infection or disease, absence of sperm or stopped monthly periods, inability to have children(L)		Damage/scarring of lung tissue[3] (L)
Late: Any time after completion of treatment			A new cancer or leukemia resulting from this treatment, damage/scarring of bladder tissue

[1] *Less common with lower doses.*
[2] *Only with very high doses.*
[3] *Risk increased in someone who has had chest radiation.*
(L) Toxicity may also occur later.

Cyclophosphamide Dosage: 60 mg/kg/dose for 2 days

Administered with Mesna per institutional standard

9.2 Mesna

Source and Pharmacology: Mesna is a synthetic sulfhydryl (thiol) compound. Mesna contains free sulfhydryl groups that interact chemically with urotoxic metabolites of oxaza-phosphorine derivatives such as cyclophosphamide and ifosfamide. Oral bioavailability is 50%. Upon injection into the blood, mesna is oxidized to mesna disulfide, a totally inert compound. Following glomerular filtration, mesna disulfide is rapidly reduced in the renal tubules back to mesna, the active form of the drug. Mesna and mesna disulfide are excreted primarily via the urine.

Formulation and Stability: Mesna is available in 2 mL, 4 mL and 100 mL amps containing 100 mg/mL of mesna solution. The intact vials can be stored at room temperature. Mesna may be further diluted in 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically and chemically stable for at least 24 hours under refrigeration.

Supplier: Commercially available

Toxicity: Mesna is generally well tolerated. Nausea and vomiting, headache, diarrhea, rash, transient hypotension and allergic reactions have been reported. Patients may complain of a bitter taste in their mouth during administration. Mesna may cause false positive urine dipstick readings for ketones.

Dosage and Administration: Mesna administration is optional and will be given according to

local standard of care. It is generally dosed at approximately 25% of the cyclophosphamide dose. It is normally ordered to be given intravenously prior to and again at 3, 6 and 9 hours following each dose of cyclophosphamide. Continuous infusion Mesna according to local standard of care is also allowed.

9.3 Rituximab

Other name(s): Rituxan®, Biogen-IDEC-C2B8

Classification and Mode of Action: Rituximab is a genetically engineered chimeric murine/human monoclonal antibody, which binds specifically to the antigen CD20 (human B lymphocyte restricted differentiation antigen, Bp35) located on the surface of pre-B and mature B lymphocytes of both normal and malignant cells. The antibody is an IgG_1 kappa immunoglobulin containing murine light- and heavy-chain variable region sequences and human constant region sequences. Rituximab is composed of two heavy chains of 451 amino acids and two light chains of 213 amino acids and has an approximate molecular weight of 145 kD. It is produced in mammalian cell (Chinese Hamster Ovary) culture.

CD20 regulates an early step(s) in the activation process for cell cycle initiation and differentiation, and possibly functions as a calcium ion channel. Rituximab binds to the CD20 antigen on B lymphocytes and recruits immune effector functions to mediate B- cell lysis. Possible mechanisms of cell lysis include complement-dependent cytotoxicity and antibody-dependent cell mediated cytotoxicity. The antibody has been shown to induce apoptosis in the DHL-4 human B-cell lymphoma line.

Absorption, Distribution, Fate, and Excretion: Serum levels and half-life are proportional to the dose and have ranged from 31.5 to hours after the first infusion and 83.9 to 407 hours after the fourth weekly infusion of 375 mg/m^2. The wide range of half-lives may reflect the variable tumor burden among patients and the changes in CD20-positive (normal and malignant) B-cell populations upon repeated administrations.

In 83% of patients, circulating B cells were depleted within the first three doses of rituximab with sustained depletion for up to 6 to 9 months post-treatment.

Preparation, Storage and Stability: Rituximab is a sterile, clear, colorless, preservative free liquid concentrate for intravenous administration. Rituximab is supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single use vials. The product contains 9 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and SWFI. The pH is adjusted to 6.5. Store refrigerated at temperatures of 2°- 8°C (36°-46°F). Protect from direct sunlight.

Dilute to a final concentration of 1 to 4 mg/mL in NS or D5W. Rituximab solutions for infusion may be stored at 2-8°C (36-46°F) for 24 hours and have been shown to be stable for an additional 24 hours at room temperature.

DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.

Pre-medications to be given per institutional standard.

Supplier/Availability: Commercially available

Route of Administration: Intravenous: **Do not administer as bolus or IV push.**

Infusion Rate: Per institutional standard.

Dose Specifics: 375 mg/m^2 on Day 1 only if the patient is EBV positive

Toxicity/Side Effects: See drug monograph below

Toxicities associated with Rituximab

	Common Occurs in 21-100 people out of every 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in < 5 people in every 100
Immediate: Within 1-2 days of receiving drug	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,	Anxiety, flushing, syncope, anaphylactic reactions, fatal infusion reaction complex (including hypoxia, pulmonary infiltrates, ARDS, MI, V fib or cardiogenic shock), fatal cardiovascular events (in RA 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: Within 2-3 weeks	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: Anytime later during therapy			Bowel obstruction and/or perforation

	Common Occurs in 21-100 people out of every 100	Less Frequent Occurs in 5-20 people out of every 100	Uncommon Occurs in < 5 people in every 100
Late: Any time after completion of treatment			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
Unknown Frequency and Timing	Fetal toxicities and teratogenic effects of rituximab are unknown; however, human IgG is known to pass the placental barrier. It is unknown whether the drug is excreted in breast milk; however, human IgG is excreted in human milk.		

Drug Incompatibilities: N/A

Nursing Implications:

- Rituximab solutions for infusion may be stored at 2°-8°C (36-46°F) for 24 hours and have been shown to be stable for an additional 24 hours at room temperature.
- Pre-medications should be given per institutional standards

9.4 Fludarabine

Other name(s): Fludara®, fludarabine phosphate, 2-fluoro-ara-AMP

Classification and Mode of Action: Fludarabine phosphate is a synthetic purine nucleoside. It differs from the physiologic nucleosides, adenosine, in that the sugar moiety is arabinose instead of ribose, and by the addition of a fluorine atom to the purine base adenine. Fludarabine is also a fluorinated nucleotide analog the antiviral agent vidarabine, (ara-A). The addition of fluorine results in increased aqueous solubility and resistance to enzymatic degradation by adenosine deaminase. Fludarabine (2-fluoro-ara-A) is commercially available as the monophosphate salt (2-fluoro-ara-AMP). The monophosphorylation increases the drug's aqueous solubility while

maintaining pharmacologic activity. The chemical name for fludarabine phosphate is 9H- Purin-6-amine, 2-fluoro-9-(5-0-phosphono β-D-arabino- furanosyl) (2-fluoro-ara-AMP) and the molecular weight is 365.2.

Fludarabine is a purine antagonist antimetabolite. In vivo, fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then it is phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

Absorption, Distribution, Fate, and Excretion: Phase 1 studies in humans have demonstrated that within several minutes after intravenous infusion, fludarabine phosphate is converted to the active metabolite, 2- fluoro-ara-A and becomes undetectable. Therefore, pharmacokinetics studies have focused on 2-fluoro-ara-A. Fludarabine phosphate 25 mg/m infused intravenously over 30 minutes to adult cancer patients, showed a moderate accumulation of 2-fluoro-ara-A. During a 5-day treatment schedule, 2-fluoro-ara-A plasma trough levels increased by a factor of about 2.

Fludarabine is widely distributed. The volume of distribution at steady state (V_{SS}) reported after daily administration of 25 mg/m^2 for 5 days to adults averaged at 96-98 L/m^2. Tissue distribution studies in animals indicate that the highest concentrations of the drug are in liver, kidney, and spleen. Although the extent to which fludarabine and/or its metabolites distribute into the CNS in humans has not been determined to date, severe neurologic toxicity (e.g., blindness, coma) has been reported in patients receiving the drug, particularly in high dosages. There is evidence from animal studies that fludarabine distributes into the CNS and that a toxic metabolite (2-fluoroadenine, possibly formed by bacteria in the GI tract), can be absorbed systematically via enterohepatic circulation and distributed into CSF. According to in vitro data, about 19-29% of fludarabine is bound to plasma proteins.

Following IV administration, fludarabine phosphate is dephosphorylated rapidly to fludarabine. Plasma concentrations of fludarabine decline in a linear, dose-independent manner. The elimination profile of fludarabine also has been reported to be either biphasic or triphasic; however, reported terminal elimination half-lives have been similar. In adult cancer patients receiving fludarabine 25 mg/m^2 as a 30-minute IV infusion daily for 5 days, a terminal half-life of about 20 hours was reported. In a limited number of pediatric patients, the plasma concentration profile of fludarabine exhibited both monoexponential and biexponential decay, with a mean $t_{1/2}$ of 10.5 hours in patients with monoexponential elimination and a $t_{1/2}$ of 1.2-1.4 and 12.4-19 hours, respectively, in patients with biexponential elimination.

Renal clearance accounts for about 40% of the total body clearance of fludarabine. Renal elimination appears to become more important at high dosages of the drug. The dose of fludarabine needs to be adjusted in patients with moderate renal impairment.

Preparation, Storage and Stability: Fludarabine phosphate injection is available as sterile lyophilized powder and in solution. Each single- dose vial of powder contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. After reconstitution, the pH range for the final product is 7.2-8.2. The single-dose solution vial

contains 25 mg/mL, 2 mL of fludarabine phosphate. It may contain mannitol and is preservative-free.

Fludarabine phosphate vials should be stored refrigerated at 2-8°C (36-46°F). Prior to administration fludarabine phosphate powder should be reconstituted with Sterile Water for Injection and further diluted in D5W or NS to a concentration of 10 - 25 mg/mL. Fludarabine 25 mg/mL solution should be further diluted in the same manner.

Fludarabine phosphate powder should be reconstituted with 2 mL of Sterile Water for Injection. The solid cake should fully dissolve in 15 seconds or less. The resulting concentration is 25 mg/mL. When reconstituted to a final concentration of 25 mg/mL, the drug is stable for at least 16 days at room temperature and normal light conditions. The manufacturer recommends that the solution be used within 8 hours after reconstitution.

Prior to administration, fludarabine 25 mg/mL solution or the reconstituted 25 mg/mL solution should be further diluted in 100 mL or 125 mL of D5W or NS. When diluted to a final concentration of 1 mg/mL, fludarabine is stable for at least 16 days at room temperature and normal light conditions. The manufacturer recommends that the diluted solution be used within 8 hours after preparation. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

Supplier/Availability: Commercially available from various manufacturers

Route of Administration: Intravenous Infusion

Dose Specifics: 40 mg/m^2

Toxicities/Side effects: See following drug monograph for Fludarabine

Toxicities Associated with Fludarabine			
	Common Occurs in 21-100 people out of every 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people in every 100
Immediate: Within 1-2 days of receiving drug	Fever, fatigue, weakness, pain, nausea, vomiting, anorexia, cough, dyspnea	Edema including peripheral edema, chills, rash, diarrhea, rhinitis, diaphoresis, malaise, abdominal pain, headache, back pain, myalgia, stomatitis, flu-like syndrome	Anaphylaxis, tumor lysis syndrome, dehydration
Prompt: Within 2-3 weeks	Myelosuppression (anemia, neutropenia,	Weight loss, gastrointestinal	Sinusitis, dysuria, opportunistic infections and reactivation of latent

Toxicities Associated with Fludarabine			
	Common Occurs in 21-100 people out of every 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people in every 100
	thrombocytopenia), infection (urinary tract infection, herpes simplex infection, pneumonia, upper respiratory)	bleeding, hemoptysis, paresthesia, allergic pneumonitis, bronchitis, pharyngitis, visual disturbance, hearing loss, hyperglycemia	viral infections like Epstein-Barr virus (EBV), herpes zoster and John Cunningham (JC) virus (progressive multifocal leukoencephalopathy [PML])L, EBV associated lymphoproliferative disorder, pancytopenia (can be prolonged), pulmonary hypersensitivity, (dyspnea, cough, hypoxia, interstitial pulmonary infiltrate), pulmonary toxicity (acute respiratory distress syndrome [ARDS], pulmonary fibrosis, pulmonary hemorrhage, respiratory distress, respiratory failure), pericardial effusion, skin toxicity (erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, pemphigus), liver failure, renal failure, hemorrhage, transfusion- associated graft-versus-host disease has occurred following transfusion of nonirradiated blood products, phlebitis*, sleep disorder*, cerebellar syndrome*, depression*, mentation impaired*, alopecia*, pruritus*, seborrhea*, esophagitis*, constipation*, mucositis*, dysphagia*, hesitancy*, cholelithiasis*, abnormal liver function tests *, osteoporosis*, arthralgia*, abnormal renal function test*, proteinuria*, epistaxis*, hemorrhagic cystitis*, eosinophilia*
Delayed: Anytime later during therapy			Neurotoxicity (increased with high doses): seizures, agitation, confusion, weakness, visual disturbances, optic neuritis, optic neuropathy, photophobia, blindness,

Toxicities Associated with Fludarabine			
	Common Occurs in 21-100 people out of every 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people in every 100
			paralysis, coma, death, peripheral neuropathy); autoimmune phenomena: thrombocytopenia/ thrombocytopenic purpura (ITP), Evans syndrome, hemolytic anemia, acquired hemophilia
Late: Any time after completion of treatment			Myelodysplastic syndrome/acute myeloid leukemia (mainly associated with prior or concomitant or subsequent treatment with other anticancer treatments), skin cancer (new onset or exacerbation)
Unknown Frequency and Timing	Pregnancy Category D Based on its mechanism of action, fludarabine phosphate can cause fetal harm when administered to a pregnant woman. Fludarabine phosphate was embryo lethal and teratogenic in both rats and rabbits.		

(L) Toxicity may also occur later.
* Reported in ≤ 3% of subjects. Since these are not considered life-threatening they are not included in the consent.
[a] These effects were not reported in children.

Drug Incompatibilities: Use of fludarabine in combination with pentostatin is not recommended due to the risk of severe pulmonary toxicity.

Nursing Implications: N/A

9.5 Thiotepa

Other Name(s): Tepadina, Tspa, Thiophosphamide, Triethylenethiophosphoramide

Classification and Mode of Action: Thiotepa is a cytotoxic agent of the polyfunctional type, related chemically and pharmacologically to nitrogen mustard. The radiomimetic action of thiotepa is believed to occur through the release of ethylenimine radicals which, like irradiation, disrupt the bonds of DNA. One of the principal bond disruptions is initiated by alkylation of guanine at the N-7 position, which severs the linkage between the purine base and the sugar and liberates alkylated guanines. Thiotepa is desulfurated by cytochrome P-450 enzymes such as 2B1 and 2C11, which catalyze the conversion of thiotepa to tepa. Tepa is less toxic than thiotepa and has been demonstrated to produce alkali-labile sites in DNA, rather than cross-links. These findings indicate that thiotepa reacts differently from thiotepa and produces monofunctional alkylation of DNA. A second metabolite of thiotepa, a mercapturic acid conjugate, is formed via glutathione conjugation.

Monochloro tepa is the third metabolite found in the urine.

Following short intravenous infusion (less than 5 minutes), peak concentrations of thiotepa were measured within 5 minutes. At steady state, the volume of distribution was independent of dose and ranged from 0.3 to 1.6 liters per kilogram (L/kg). Approximately 4.2% of the original dose is eliminated in the urine within 24 hours as tepa. The elimination half-life of thiotepa ranges from 2.3 to 2.4 hours. The half-life of tepa ranged from 3 to 21.1 hours in one study.

Absorption, Distribution, Fate, and Excretion: Following short intravenous infusion (less than 5 minutes), peak concentrations of thiotepa were measured within 5 minutes. At steady state, the volume of distribution was independent of dose and ranged from 0.3 to 1.6 liters per kilogram (L/kg). Approximately 4.2% of the original dose is eliminated in the urine within 24 hours as thiotepa. The elimination half-life of thiotepa ranges from 2.3 to 2.4 hours. The half-life of thiotepa ranged from 3 to 21.1 hours in one study.

Preparation, Storage and Stability: Thiotepa for Injection USP, for single use only, is available in vials containing 15 mg of nonpyrogenic, sterile lyophilized powder. Store in a refrigerator at 2°-8°C (36°-46°F).

Protect from light at all times.

Reconstitute thiotepa for Injection with 1.5 mL of Sterile Water for Injection resulting in a drug concentration of approximately 10 mg/mL (As per manufacturer's information: Actual content per vial 15.6 mg; withdrawable amount 14.7 mg/1.4 mL; approximate reconstituted concentration: 10.4 mg/mL). When reconstituted with Sterile Water for Injection, solutions of thiotepa should be stored at refrigerated temperatures 2°-8°C (36°- 46°F) protected from light and use within 8 hours. The reconstituted solution is hypotonic and should be further diluted with Sodium Chloride Injection (0.9% NaCl) prior to use. Thiotepa at a concentration of 1-5 mg/mL in 0.9% NaCl is stable for 24 hours at room temperature. At concentration of 0.5 mg/mL it is stable for only one hour and stability decreases significantly at concentrations of less than 0.5 mg/mL Therefore, solutions diluted to 0.5 mg/mL should be used immediately.

Supplier/Availability: Commercially available

Route of Administration: Intravenous Infusion

Dose Specifics: 5 mg/kg/dose

Toxicities/Side Effects: See following drug monograph for Thiotepa

Toxicities Associated with Thiotepa			
	Common Occurs in 21-100 people out of every 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people in every 100
Immediate: Within 1-2 days of	Nausea, vomiting, anorexia,	Pain at the injection site, dizziness, headache,	Anaphylaxis, laryngeal

Toxicities Associated with Thiotepa			
	Common Occurs in 21-100 people out of every 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people in every 100
receiving drug	fatigue, weakness	blurred vision, abdominal pain, contact dermatitis, rash	edema, wheezing, hives
Prompt: Within 2-3 weeks	Myelosuppression; at higher doses in conditioning regimens for BMT: mucositis, esophagitis	At higher doses in conditioning regimens for BMT: encephalopathy (inappropriate behavior, confusion, somnolence), increased liver transaminases, increased bilirubin, hyperpigmentation of the skin (bronzing effect)	Febrile reaction, conjunctivitis, dysuria, urinary retention
Delayed: Anytime later during therapy	Gonadal dysfunction/infertility, azoospermia, amenorrhea		Alopecia, secondary malignancy
Unknown Frequency and Timing	Fetal and teratogenic toxicities: Carcinogenic and teratogenic effects of thiotepa have been noted in animal models at doses ≤ to those used in humans. It is not known if thiotepa is excreted into human breast milk.		

Drug Incompatibilities: NA

Nursing Implications

- Protect from light at all times.
- When thiotepa is given in bone marrow transplant doses, bath the patient and change linen frequently, per institutional standards, to avoid the contact dermatitis and discoloration of the skin that is seen with high dose.

9.6 Melphalan

Other name(s): L-phenylalanine mustard, phenylalanine mustard, L-PAM, L- sarcolysin, Alderan®

Classification and Mode of Action: Melphalan, a phenylalanine derivative of nitrogen mustard, is a bifunctional alkylating agent. Melphalan forms covalent cross-links with DNA or DNA protein complexes thereby resulting in cytotoxic, mutagenic, and carcinogenic effects. The end result of the alkylation process results in the misreading of the DNA code and the inhibition of DNA, RNA, and protein synthesis in rapidly proliferating tumor cells. It is cell cycle non- specific.

Absorption, Distribution, Fate, and Excretion: After IV administration, melphalan plasma concentrations decline rapidly in a bi- exponential manner with distribution phase and terminal

elimination phase half-lives of approximately 10 and 75 minutes, respectively. Plasma melphalan levels are highly variable after oral dosing, both with respect to the time of the first appearance of melphalan in plasma (range approximately 0 to 6 hours) and to the peak plasma concentration achieved. These results may be due to incomplete intestinal absorption, a variable "first pass" hepatic metabolism, or to rapid hydrolysis. The oral dose averages 61% ± 26% of that following IV administration. The terminal elimination plasma half-life of oral melphalan is 1.5 ± 0.83 hours. The steady-state volume of distribution of melphalan is 0.5 L/kg. The extent of melphalan binding to plasma proteins ranges from 60-90%. Melphalan is eliminated from plasma primarily by chemical hydrolysis to monohydroxy melphalan and dihydroxy melphalan. The 24-hour urinary excretion of parent drug is approximately 10% suggesting that renal clearance is not a major route of elimination of parent drug. Penetration into CSF is low. Despite the fact that the contribution of renal elimination to melphalan clearance appears to be low, one pharmacokinetic study suggests dosage may need to be reduced in patients with renal impairment.

Preparation, Storage and Stability: Melphalan is available as a 2 mg scored tablet. Inactive ingredients include colloidal silicon dioxide, crospovidone, hypromellose, macrogol/PEG 400, magnesium stearate, microcrystalline cellulose, and titanium dioxide. Store in a refrigerator, 2°-8°C (36°- 46°F). Protect from light.

Melphalan for Injection is supplied as a sterile, nonpyrogenic, freeze-dried powder. Each single-use vial contains melphalan hydrochloride equivalent to 50 mg melphalan and 20 mg povidone. Melphalan for Injection is reconstituted using the sterile diluent provided. Each vial of sterile diluent contains sodium citrate 0.2 g, propylene glycol 6.0 mL, ethanol (96%) 0.52 mL, and SWFI to a total of 10 mL. Store at controlled room temperature 15°-30°C (59°-86°F) and protect from light.

Reconstitute to a concentration of 5 mg/mL by rapidly injecting 10 mL of the supplied diluent directly into the vial of lyophilized powder using a sterile needle (20-gauge or larger needle diameter) and syringe. Immediately shake vial vigorously until a clear solution is obtained. Rapid addition of the diluent followed by immediate vigorous shaking is important for proper dissolution.

Immediately dilute the dose to be administered in NS to a final concentration not to exceed 2 mg/mL for IV central line administration or ≤ 0.45 mg/mL for peripheral IV administration.

A precipitate will form if the reconstituted solution is stored at 5°C. Do not refrigerate the reconstituted product. (The time between re-administration of melphalan should be kept to a minimum because reconstituted and diluted solutions of melphalan are unstable. Over as short a time as 30 minutes, a citrate derivative of melphalan has been detected in reconstituted material from the reaction of melphalan with the sterile diluent for melphalan. Upon further dilution with saline, nearly 1% label strength of melphalan hydrolyzes every 10 minutes.)

Supplier/Availability: Commercially available

Route of Administration: Intravenous

Dose Specifics: 70 mg/m^2/dose

Toxicity/Side Effects: See following drug monograph for Melphalan

Toxicities Associated With Melphalan			
	Common Occurs in 21-100 people out of every 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people in every 100
Immediate: Within 1-2 days of receiving drug	Anorexia, nausea, vomiting, hyponatremia (high dose)		Anaphylaxis, hypotension diaphoresis pruritus atrial fibrillation (high dose), extravasation (rare) but if occurs = local ulceration, SIADH, Seizures
Prompt: Within 2-3 weeks	Myelosuppression (L), mucositis, diarrhea, alopecia		Abnormal liver function tests, jaundice, hepatitis
Delayed: Anytime later during therapy		Amenorrhea, testicular suppression	Bone marrow failure, hemolytic anemia, pulmonary fibrosis, interstitial pneumonitis
Late: Any time after completion of treatment		Sterility, primary ovarian failure	Secondary malignancy
Unknown Frequency and Timing	Melphalan was embryo lethal and teratogenic in rats following oral (6 to 18 mg/m^2/day for 10 days) and intraperitoneal (18 mg/m) administration. Malformations resulting from melphalan included alterations of the brain (underdevelopment, deformation, meningocele, and encephalocele) and eye (anophthalmia and microphthalmos), reduction of the mandible and tail, as well as hepatocele (exomphaly). It is unknown whether the drug is excreted in breast milk.		

(L) Toxicity may also occur later

Drug Incompatibilities: N/A

9.7 Anti-Thymocyte Globulin (Rabbit)

Other Name(s): Rabbit AT, RATG-Rabbit, Antithymocyte Globulin, Thymoglobulin®
Classification and Mode of Action: Anti-thymocyte globulin (rabbit) is a purified, pasteurized, gamma immune globulin, obtained by immunization of rabbits with human thymocytes. This immunosuppressive product contains cytotoxic antibodies directed against antigens expressed on human T- lymphocytes. The mechanism of action by which polyclonal anti-lymphocyte preparations suppress immune responses is not fully understood. Possible mechanisms by which anti-thymocyte globulin (rabbit) may induce immunosuppression in vivo include: T-cell clearance from the circulation and modulation of T-cell activation, homing, and cytotoxic activities. Anti-thymocyte globulin (rabbit) includes antibodies against T-cell markers such as CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, HLA Class I heavy chains, and β2 microglobulin. In vitro, anti- thymocyte globulin (rabbit) (concentrations > 0.1 mg/mL) mediates

T-cell suppressive effects via inhibition of proliferative responses to several mitogens. In patients, T-cell depletion is usually observed within a day from initiating anti-thymocyte globulin (rabbit) therapy.

Absorption, Distribution, Fate, and Excretion: After an intravenous dose of 1.25 to 1.5 mg/kg/day (over 4 hours for 7-11 days) 4-8 hours post-infusion, anti-thymocyte globulin (rabbit) levels were on average 21.5 mcg/mL (10-40 mcg/mL) with a half-life of 2-3 days after the first dose, and 87 mcg/mL (23-170 mcg/mL) after the last dose. During an anti-thymocyte globulin (rabbit) Phase 3 randomized trial, anti-rabbit antibodies developed in 68% of the Thymoglobulin-treated patients (108 of 163 patients evaluated). The volume of distribution is 0.12 L/kg. Approximately 1% of the dose excreted in the urine.

Preparation, Storage and Stability: Each vial contains anti-thymocyte globulin (rabbit) 25 mg and inactive ingredients: glycine (50 mg), mannitol (50mg), sodium chloride (10 mg). The reconstituted preparation contains approximately 5 mg/mL of anti-thymocyte globulin (rabbit), of which > 90% is rabbit gamma immune globulin (IgG). The reconstituted solution has a pH of 7 ± 0.4. Each anti-thymocyte globulin (rabbit) lot is released following potency testing (lymphocytotoxicity and E-rosette inhibition assays), and cross-reactive antibody testing (hemagglutination, platelet agglutination, anti-human serum protein antibody, anti-glomerular basement membrane antibody, and fibroblast toxicity assays) on every fifth lot.

Reconstitute anti-thymocyte globulin (rabbit) with the supplied diluent, SWFI, immediately before use. Anti-thymocyte globulin (rabbit) should be used within 4 hours after reconstitution if kept at room temperature. Allow Thymoglobulin and SWFI vials to reach room temperature before reconstituting the lyophilized product.

- Reconstitute each vial of anti-thymocyte globulin (rabbit) lyophilized powder with 5 mL of SWFI.
- Inject SWFI slowly into the vial containing the lyophilized powder.
- Rotate vial gently until powder is completely dissolved. Each reconstituted vial contains 25 mg or 5 mg/mL of anti-thymocyte globulin (rabbit).
- Inspect solution for particulate matter after reconstitution. Should some particulate matter remain, continue to gently rotate the vial until no particulate matter is visible. If particulate matter persists, discard this vial.

Dilution:

- Transfer the contents of the calculated number of anti-thymocyte globulin (rabbit) vials into the bag of infusion solution (saline or dextrose). Recommended volume: per one vial of anti-thymocyte globulin (rabbit), use 50 mL of infusion solution (total volume usually between 50 to 500 mL).
- Mix the solution by inverting the bag gently only once or twice.

Supplier/Availability: Commercially available from various manufacturers

Route of Administration: Administer by intravenous infusion through a high-flow vein. Anti-thymocyte globulin (rabbit) should be infused over a period of time per institutional standards.

Dose Specifics:

- Dose: 1 mg/kg IV on Day -12
- Dose: 3 mg/kg IV on Day -11, -10, -9

Toxicity/Side Effects: See following drug monograph for Rabbit Anti-Thymocyte Globulin

Toxicities Associated with Rabbit Anti-thymocyte Globulin			
	Common Occurs in 21-100 people out of every 100	**Less Frequent** Occurs in 5-20 people out of every 100	**Uncommon** Occurs in <5 people in every 100
Immediate: Within 1-2 days of receiving drug	Fever, chills, rash, dyspnea with/without bronchoconstriction, hypertension, tachycardia, peripheral edema, nausea, vomiting, diarrhea, abdominal pain, myalgia, headache	Pruritis, dizziness	Anaphylaxis, swelling, redness or phlebitis at the infusion site (peripheral veins)
Prompt: Within 2-3 weeks	Thrombocytopenia, leukopenia, pain, hyperkalemia, asthenia, infection	Malaise	Abnormal liver function test
Delayed: Anytime later during therapy	Antibody development to rabbit ATG (may persist for 1 year)	Serum sickness (L) which can have all or some of the following symptoms: glomerulonephritis, fever, myalgia, arthralgia and periorbital edema (incidence reduced with use of corticosteroid premedication)	Secondary malignancy
Unknown Frequency and Timing	Animal reproduction studies have not been conducted with anti-thymocyte globulin (rabbit). It is not known whether rabbit ATG can cause fetal harm when administered to a pregnant woman or affect reproductive capacity. It is unknown whether the drug is excreted in breast milk.		

(L) Toxicity may also occur later

Drug Incompatibilities: *NA*

Nursing Implications:

- Premedication, per institutional standard, 30-60 minutes prior to the infusion is recommended and may reduce the incidence and intensity of side effects during the

infusion.
- Always keep appropriate resuscitation equipment at the patient's bedside while anti-thymocyte globulin (rabbit) is being administered.
- Observe the patient continuously for possible allergic reactions throughout the infusions.

9.8 Mycophenolate Mofetil

Other names: CellCept®, MMF, RS-61443

Classification and Mode of Action: Mycophenolate (MMF) is the morpholinoethyl ester of mycophenolic acid (MPA), an antibiotic with immunosuppressant properties isolated from Penicillium spp. The chemical name for oral mycophenolate mofetil is 2-morpholinoethyl (E)-6-(1,3-dihydro- 4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate. It has an empirical formula of $C_{23}H_{31}NO_7$ and a molecular weight of 433.50. Mycophenolate mofetil is a white to off-white crystalline powder which is slightly soluble in water (43 µg/mL at pH 7.4); the solubility increases in acidic medium (4.27 mg/mL at pH 31 3.6). The intravenous product is the hydrochloride salt of mycophenolate mofetil. The chemical name for the hydrochloride salt of mycophenolate mofetil is 2-morpholinoethyl (E)-6-(1,3-dihydro-4-hydroxy-6- methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride. It has an empirical formula of $C_{23}H_{31}NO_7$ HCl and a molecular weight of 469.96.

MMF has been used in a variety of solid organ and hematopoietic stem cell transplant settings for the prevention of acute rejection. MMF is a prodrug which, after oral administration, is rapidly and primarily hydrolyzed by the liver to the biologically active metabolite mycophenolic acid. MPA is metabolized principally by glucuronyl transferase to form the pharmacologically inactive phenolic glucuronide of MPA (MPAG). In vivo, MPAG is converted to MPA via enterohepatic recirculation. Mycophenolic acid inhibits nucleic acid synthesis and produces a potent, noncompetitive, and reversible inhibition of inosine monophosphate dehydrogenase (IMPDH), blocking the de novo synthesis of guanosine nucleotides without being incorporated into DNA. Both T and B lymphocytes rely on this de novo pathway for purine synthesis. As a result, the proliferative responses of T and B lymphocytes to both mitogenic and allospecific stimulation are inhibited. Other rapidly dividing cell lines are capable of recycling purine nucleotides via the "salvage" pathway, which is not blocked by mycophenolic acid.

In vitro and in vivo studies have demonstrated the ability of mycophenolic acid to block proliferative responses of T and B lymphocytes, inhibit antibody formation and the generation of cytotoxic T-cells, and suppress antibody formation by B lymphocytes. Mycophenolic acid prevents the glycosylation of lymphocyte and monocyte glycoproteins that are involved in intercellular adhesion of these cells to endothelial cells and may inhibit recruitment of leukocytes into sites of inflammation and graft rejection. Antirejection effects have been attributed to decreased recruitment of activated lymphocytes to the graft site.

Absorption, Distribution, Fate, and Excretion: The mean absolute bioavailability of oral mycophenolate mofetil relative to intravenous mycophenolate mofetil (based on MPA AUC) was 94% in a small sample of healthy, adult volunteers. In this group the mean (± SD) apparent volume of distribution of MPA was approximately 3.6 (± 1.5) and 4.0 (± 1.2) L/kg following intravenous and oral administration, respectively. At clinically relevant concentrations, MPA is 97% bound to

plasma albumin. MPAG is 82% bound to plasma albumin at MPAG concentration ranges that are normally seen in stable renal transplant patients; however, at higher MPAG concentrations (e.g., patients with renal impairment), the binding of MPA may be reduced as a result of competition between MPAG and MPA for protein binding. A negligible amount of the agent (< 1% of dose) is excreted as MPA in the urine. Most of the administered dose (~87%) is excreted in the urine as MPAG. Bile acid sequestrants (e.g., cholestyramine) reduce the AUC of MPA by interfering with the enterohepatic circulation of the drug.

Preparation, Storage and Stability:

Mycophenolate mofetil is available in the following preparations:

- Capsule: 250 mg
- Tablet: 500 mg
- Powder for suspension, oral: 200 mg/mL (following reconstitution)
- Powder for reconstitution, injection: 500 mg per vial

A delayed release tablet (mycophenolic acid, Myfortic) is also commercially available. This preparation is not interchangeable with mycophenolate mofetil (Cellcept®, MMF) due to differences in absorption. This tablet should not be used by patients treated on this protocol.

Inactive ingredients in the 250 mg capsules include the following: croscarmellose sodium, magnesium stearate, povidone (K-90) and pregelatinized starch. The capsule shells contain black iron oxide, FD&C blue #2, gelatin, red iron oxide, silicon dioxide, sodium lauryl sulfate, titanium dioxide, and yellow iron oxide.

Inactive ingredients in the 500 mg tablets include black iron oxide, croscarmellose sodium, FD&C blue #2 aluminum lake, hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, polyethylene glycol 400, povidone (K-90), red iron oxide, talc, and titanium dioxide; may also contain ammonium hydroxide, ethyl alcohol, methyl alcohol, n-butyl alcohol, propylene glycol, and shellac.

Inactive ingredients in the powder for oral suspension include aspartame, citric acid anhydrous, colloidal silicon dioxide, methylparaben, mixed fruit flavor, sodium citrate dihydrate, sorbitol, soybean lecithin, and xanthan gum.

The injectable product is available as a sterile, white to off-white, lyophilized powder in vials containing mycophenolate mofetil hydrochloride for administration by intravenous infusion only. Each vial contains the equivalent of 500 mg mycophenolate mofetil as the hydrochloride salt. Inactive ingredients include polysorbate 80 (25 mg), citric acid (5 mg), and sodium hydroxide to adjust the pH. Reconstitution and dilution with D5W yields a final solution of mycophenolate mofetil that is slightly yellow in color.

To prepare the oral suspension, add 47 mL of water to the bottle and shake well for approximately 1 minute. Add another 47 mL of water to the bottle and shake well for an additional minute. The final concentration is 200 mg/mL of mycophenolate mofetil. Avoid inhalation or direct contact with skin or mucous membranes of the dry powder or the constituted suspension. If such contact occurs, wash thoroughly with soap and water; rinse eyes with water.

To prepare the intravenous injection, reconstitute the contents of each vial with 14 mL of D5W. Dilute the contents of a vial with D5W to a final concentration of 6 mg/mL. Each vial is vacuum-sealed; if a lack of vacuum is noted during preparation, the vial should not be used.

Oral formulations should be stored at 25°C (77°F); excursions are permitted to 15°C to 30°C (59°F to 86°F). Protect from moisture and light. Once reconstituted, the oral solution may be stored at room temperature or under refrigeration. Do not freeze. The mixed suspension is stable for 60 days.

Store intact vials and diluted solutions at 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F). Do not freeze. Begin infusion within 4 hours of reconstitution.

Oral formulations of MMF should be administered on an empty stomach to avoid variability in MPA absorption. The oral solution may be administered via a nasogastric tube (minimum 8 French, 1.7 mm interior diameter). Do not mix the oral suspension with other medications. Some products may contain phenylalanine; refer to the package labeling for additional details.

The intravenous solution should be given over at least 2 hours. Do not administer by rapid or bolus injection.

Supplier/Availability: Commercially available

Route of Administration: Intravenous

Dose Specifics: 20 mg/kg BW/ bid (with a maximum total dose of 2g/day), Days 1 - +30, followed by a rapid taper per recommendation of PI (or PI designee) based on the clinical status of the patient. Administer only if graft exceeds > 25,000 CD3+ TCRαβ+ cells.

Toxicity/Side Effects: Mycophenolate mofetil can cause fetal harm when administered to a pregnant woman. Use of MMF during pregnancy is associated with an increased risk of first trimester pregnancy loss and an increased risk of congenital malformations, especially external ear and other facial abnormalities including cleft lip and palate, and anomalies of the distal limbs, heart, esophagus, and kidney. According to the package labeling, the National Transplantation Pregnancy Registry (NTPR) presents data on 33 MMF- exposed pregnancies in 24 transplant patients. Of these, there were 15 spontaneous abortions and 18 live-born infants. Four of the 18 infants had structural malformations (22%). In post marketing data (collected 1995-2007) of 77 women exposed to systemic MMF during pregnancy, 25 had spontaneous abortions and 14 had a malformed infant or fetus. Six of 14 malformed offspring had ear abnormalities. Because these post marketing data are reported voluntarily, it is not always possible to reliably estimate the frequency of particular adverse outcomes. These malformations seen in offspring were similar to findings in animal reproductive toxicology studies. In animal reproductive toxicology studies, there were increased rates of fetal resorptions and malformations in the absence of maternal toxicity. Female rats and rabbits received MMF doses equivalent to 0.02 to 0.9 times the recommended human dose for renal and cardiac transplant patients, based on body surface area conversions. In rat offspring, malformations included anophthalmia, agnathia, and hydrocephaly. In rabbit offspring, malformations included ectopia cordis, ectopic kidneys, diaphragmatic hernia, and umbilical hernia. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

Women of childbearing potential should have a negative serum or urine pregnancy test with a sensitivity of at least 25 mIU/mL within 1 week prior to beginning therapy (manufacturer's recommendation). MMF should not be initiated until a negative pregnancy test report is obtained. Women of childbearing potential taking MMF must receive contraceptive counseling and use effective contraception. It is recommended by the manufacturer that the patient begin using two chosen methods of contraception 4 weeks prior to starting MMF, unless abstinence is the chosen method. She should continue contraceptive use during therapy and for 6 weeks after stopping MMF. Patients should be aware that MMF reduces blood levels of the hormones in the oral contraceptive pill and could theoretically reduce its effectiveness.

An approved medication guide (www.fda.gov/downloads/Drugs/DrugSafety/UCM170919.pdf) must be dispensed with each refill of mycophenolate mofetil.

Toxicities associated with Mycophenolate Mofetil

Incidence	Toxicities/Side Effects
Common Occurs in > 20 people out of every 100	Hypertension, edema (face, limbs, trunk), rash maculopapular, cholesterol high, hyperglycemia, hyperkalemia, hypocalcemia, hypokalemia, hypomagnesemia, abdominal pain, constipation, diarrhea, nausea, vomiting, anemia, white blood cell decreased, platelet count decreased, back pain, anxiety, generalized muscle weakness, dizziness, headache, insomnia, tremor, creatinine increased, dyspnea, cough, fever, pleural effusion, alanine aminotransferase increased, alkaline phosphatase increased, aspartate aminotransferase increased, blood bilirubin increased, GGT increased, pain
Occasional Occurs in 5-20 people out of every 100	Sepsis, infection, paresthesia, urinary tract pain, urinary frequency
Rare Occurs in < 5 people out of every 100	Phlebitis, Neoplasms benign, malignant and unspecified (including cysts and polyps) - Other, [Malignant epithelial neoplasm of skin, non-melanoma; lymphoma], gastric ulcer, gastrointestinal hemorrhage, gastric perforation, mucositis oral, thromboembolic event, infective endocarditis, renal calculi, pulmonary fibrosis, pneumonitis neutrophil count decreased, leukoencephalopathy

Incidence	Toxicities/Side Effects
Pregnancy & Lactation	Pregnancy Category D Mycophenolate is associated with an increased risk of congenital malformations and spontaneous abortions when used during pregnancy. Adverse events have been reported in animal studies at doses less than the equivalent recommended human dose. Data from the National Transplantation Pregnancy Registry (NTPR) have observed an increase in structural malformations (including ear malformations) in infants born to mothers taking mycophenolate during pregnancy. Spontaneous abortions have also been noted. Females of childbearing potential should have a negative pregnancy test within 1 week prior to beginning therapy. Two reliable forms of contraception should be used beginning 4 weeks prior to, during, and for 6 weeks after therapy. The effectiveness of hormonal contraceptive agents may be affected by mycophenolate. It is unknown if mycophenolate is excreted in human milk. Due to potentially serious adverse reactions, the decision to discontinue the drug or discontinue breast-feeding should be considered. Breast-feeding is not recommended during therapy or for 6 weeks after treatment is complete.

Drug Incompatibilities: Do not mix the oral suspension with other medications.

Nursing Implications

Oral formulations of MMF should be administered on an empty stomach to avoid variability in MPA absorption. The oral solution may be administered via a nasogastric tube (minimum 8 French, 1.7 mm interior diameter). Do not mix the oral suspension with other medications. Some products may contain phenylalanine; refer to the package labeling for additional details.

9.9 Blinatumomab

Source and Pharmacology: Blinatumomab is a fusion protein composed of two single-chain antibodies (scFv), murine anti-CD19 scFv and murine anti-CD3 scFv. Through CD3 binding, blinatumomab recruits and engages T cells for redirected lysis of CD19-positive B cells, including those expressed with B-cell malignancies. T cells are bound by its anti-CD3 moiety, whereas B cells are bound by the anti-CD19 moiety. The subsequent serial lysis of multiple malignant cells by a single blinatumomab-activated T cell closely resembles a natural cytotoxic T cell reaction. Treatment with blinatumomab is associated with a rapid depletion of peripheral B cells, accompanied by T cell activation and a transient increase in cytokines.

Blinatumomab consists of a single chain of 504 amino acids with a molecular weight of approximately 54 kDa. The pharmacokinetics of blinatumomab was assessed over a dose range from 5 to 90 mcg/m^2/day (approximately equivalent to 9-162 mcg/day). Following continuous intravenous infusion, the steady state serum concentration (Css) was achieved within a day and remained stable over time. The estimated mean (SD) volume of distribution based on terminal phase (Vz) was 4.52 (2.89) L. The estimated mean (SD) systemic clearance was 2.92 (2.83) L/hour and the estimated mean (SD) half-life was 2.11(1.42) hours. Negligible amounts of blinatumomab were excreted in the urine at the tested clinical doses. Like other protein therapeutics,

blinatumomab is expected to be degraded into small peptides and amino acids via catabolic pathways. At the clinical doses of 9 mcg/day and 28 mcg/day for the treatment of adult relapsed/refractory ALL, the mean (SD) Css was 211 (258) pg/mL and 621 (502) pg/mL, respectively.

At this time there are no known drug interactions with blinatumomab.

Pregnancy and Lactation

Pregnancy Category Unknown: The effect of blinatumomab on fertility has not been evaluated. Blinatumomab is not recommended in pregnant women and in women of childbearing potential not using contraception. It is not known whether blinatumomab or its metabolites are excreted in human milk. Women are not allowed to breastfeed while receiving blinatumomab.

Formulation and Stability: Blinatumomab is available as a 38.5 mcg preservative-free, white to off-white lyophilized powder for injection in 4 mL single-use vial. The agent is formulated with 3.68 mg citric acid monohydrate, 105 mg trehalose dihydrate, and 25.55 mg lysine hydrochloride, and 0.7 mg polysorbate 80, pH 7. The stopper of the vial is latex free.

IV solution stabilizer for blinatumomab is not for reconstitution of blinatumomab; it is a component of the final intravenous product. The stabilizer is available as a 10 mL single-use vial of a preservative-free, clear, colorless-to-slightly yellow liquid solution. Each solution consists of 25 mM citric acid monohydrate, 1.25 M L-lysine hydrochloride, and 0.1% (w/v) polysorbate 80, pH 7. The stopper of the vial is latex free.

Store intact vials of blinatumomab and the IV solution stabilizer of blinatumomab refrigerated at 2° to 8°C (36° to 46°F). Protect from light. Shelf life stability studies of the intact vials of blinatumomab and stabilizer solution are on-going. The stability of the final prepared IV solution is 8 days when stored refrigerated at 2° to 8°C. For storage prior to administration, the prepared infusion solution must be kept at 2° C to 8° C (36°F to 46°F). The total storage and administration time must not exceed 8 days. Once at room temperature, discard the IV bag after 96 hours.

Guidelines for Administration: See Treatment and Dose Modifications sections of the protocol. See Appendix 3 for Blinatumomab preparation instructions and refer to the current USPI for volume calculations.

IV Infusion and Infusion Set Details: Blinatumomab must be administered through a central line at rate of 5 mL/hr IV over 24 hours through an acceptable IV line. Only **PVC non-DEHP lines with a 0.2 µm inline filter are acceptable**. Do not flush the IV line as it will create an IV bolus to be administered into the patient. For outpatient administration, use FDA approved pumps. Only the exact volume should be administered; any remaining overfill should be discarded appropriately.

Infusion Pump Requirements: Use a programmable pump that is approved by the appropriate regulatory authority for the country in which the subject is undergoing treatment. The pump alarm must be visual and auditory. The pump must be lockable. **Elastomeric pumps are NOT allowed.**

CADD pumps are allowed; however, the cassettes used in CADD pumps are not compatible with blinatumomab and thus, **not** allowed.

Record all Infusion Interruptions. Technical or logistical interruption must be as minimal as possible and re-start the infusion as soon as possible. If an interruption is longer than four hours, the re-start of the infusion must take place in the hospital under supervision of the investigator. Monitor patients for potential adverse events as described in the protocol and the Investigator Brochure.

Monitor patients for cytokine release syndrome, tumor lysis syndrome, and infusion reaction. Refer to protocol for specific recommendation. Monitor patients for psychiatric events such as confusion, disorientation, and cognitive attention disturbances. Patients should not drive or operate dangerous machinery while receiving blinatumomab.

Risk of Serious Adverse Reactions in Pediatric Patients Due to Benzyl Alcohol Preservative: Serious and fatal adverse reactions including "gasping syndrome" can occur in neonates and infants treated with benzyl alcohol-preserved drugs, including BLINCYTO (with preservative). The "gasping syndrome" is characterized by central nervous system depression, metabolic acidosis, and gasping respirations.

When prescribing BLINCYTO (with preservative) for pediatric patients, consider the combined daily metabolic load of benzyl alcohol from all sources including BLINCYTO (with preservative) (contains 7.4 mg of benzyl alcohol per mL) and other drugs containing benzyl alcohol. The minimum amount of benzyl alcohol at which serious adverse reactions may occur is not known.

Due to the addition of bacteriostatic saline, 7-day infusion bags of Blinatumomab solution contain benzyl alcohol and are not recommended for use in any patients weighing less than 22 kg. Prepare Blinatumomab solution for infusion with preservative-free saline in 24-hour or 48-hour infusion bags for patients weighing less than 22 kg. Please refer to Appendix 3 for infusion instructions for patients weighing less than 22 kg.

Known Blinatumomab AEs List

Known Risks and Side Effects Related to Blinatumomab Include Those Which Are:		
Very Common (In 100 people receiving blinatumomab, more than 10 and up to 100 people may have)	**Common** (In 100 people receiving blinatumomab, between 1 and 10 people may have)	**Uncommon** (In 1000 people receiving blinatumomab, between 1 and 10 people may have)
• Anemia (decreased red blood cells) which may require blood transfusion • Thrombocytopenia (decreased platelets, for clotting blood) • Leukopenia (decreased white blood cells) • Pyrexia (fever) • Infusion related reactions • Weight increased • Hypertension (high blood pressure) • Neutropenia (decreased neutrophils with fever • Increased hepatic enzymes (in the blood, which may be due to inflammation or damage to liver cells) • Tachycardia (rapid heart rate) • Edema (swelling of hands, legs, ankles, feet, face, or trunk) • Back pain • Bone pain • Headache • Insomnia (difficulty falling and/or staying asleep) • Cough • Rash • Hypotension (low blood pressure) • Infections in the blood including bacteria, fungi, viruses or infections in	• Leukopenia, Lymphopenia (decreased types of white blood cells) • Leukocytosis (increased white blood cells) • Lymphadenopathy (swelling in lymph nodes) • Hyperbilirubinemia (high levels of bilirubin in the blood) • Decreased immunoglobulins (in the blood, proteins made by the body's immune system to fight against infections and foreign substances) • Increased alkaline phosphatase (in the blood can be due problems in your liver or in your bones) • Chills • Chest pain • Pain in the arms, legs and hands • Overdose, Accidental overdose • Weight increase • Hypertension (high blood pressure) • Flushing • Dyspnea (difficulty breathing, wheezing or respiratory failure) • Hypersensitivity, allergic reactions to blinatumomab, including hypersensitivity, have been reported. Signs and symptoms of allergic reactions can be very similar to infusion reaction. If you have symptoms of an allergic reaction, you should contact the study doctor or his/her study staff immediately. • Hematophagic histiocytosis can occur with cytokine release	• Speech disorder • Cytokine storm, is a severe form of cytokine release syndrome which is described under the "Very Common" column. • Pancreatitis, inflammation of the pancreas that can be life-threatening or may even lead to death. Symptoms can include severe and persistent stomach pain, with or without nausea and vomiting. • Leukoencephalopathy, a rare, serious disorder of the white matter in the brain that can lead to severe disability and death and for which there is no known prevention, treatment, or cure. Symptoms can include difficulty thinking, loss of balance, changes in speech or walking, weakness on one side of your body, or blurred or lost vision. • Capillary leak syndrome (leakage of fluid from small blood vessels into other

Known Risks and Side Effects Related to Blinatumomab Include Those Which Are:		
Very Common (In 100 people receiving blinatumomab, more than 10 and up to 100 people may have)	**Common** (In 100 people receiving blinatumomab, between 1 and 10 people may have)	**Uncommon** (In 1000 people receiving blinatumomab, between 1 and 10 people may have)
other organs. Serious infections can happen during and after treatment and can lead to death. Serious infections such as sepsis (infection in the bloodstream), and pneumonia (severe lung infection) have been reported in patients treated with blinatumomab. Your doctor may give you antibiotics to treat the infection or stop your treatment with blinatumomab • Infusion related reactions occur during or after the drug is given through the vein. Symptoms of infusion reaction may include headache, rash, itching, flushing, swelling, shortness of breath, nausea and sometimes vomiting. Severe infusion reactions can cause dizziness, severe skin reactions, difficulty breathing or swallowing, a decrease in blood pressure, and could be life threatening. Signs and symptoms of infusion reaction can be very similar to cytokine release syndrome. • Cytokine release syndrome is when your body releases substances	syndrome, described under the "Very Common" column. It is a life- threatening overactivity of your immune system caused by releasing large amounts of inflammatory cytokines. Your doctor may give you medications such as steroids and/or other medications to prevent or treat cytokine release syndrome. • Tumor lysis syndrome (a group of complications from release of large amounts of potassium, phosphate, and nucleic acid caused by the breakdown of tumor cells after cancer treatment). Tumor lysis syndrome may cause kidney failure, abnormal heart rhythm, and can even lead to death. Patients with moderate kidney failure showed an increased rate of tumor lysis syndrome compared with patients with mild kidney failure or normal kidney function. However, this did not lead to permanent discontinuation of treatment with blinatumomab. Your doctor may give you medicines before your treatment to help prevent tumor lysis syndrome. • Nervous system problems such as tremor (shaking), dizziness, seizures, somnolence (changes in alertness), paresthesia (abnormal skin sensation such as burning, prickling, tingling), hypoaesthesia (numbness), aphasia (difficulty speaking or slurred speech), cognitive disorder (difficulty understanding words), encephalopathy (loss of	body spaces that could cause swelling of the trunk, arms and legs)

Known Risks and Side Effects Related to Blinatumomab Include Those Which Are:		
Very Common (In 100 people receiving blinatumomab, more than 10 and up to 100 people may have)	**Common** (In 100 people receiving blinatumomab, between 1 and 10 people may have)	**Uncommon** (In 1000 people receiving blinatumomab, between 1 and 10 people may have)
called cytokines during the blinatumomab infusion. This can cause fever, chills, headache, decreased blood pressure, increased liver enzymes, nausea, and vomiting. Cytokine release syndrome symptoms generally are mild to moderate but occasionally can be serious or life-threatening or may even lead to death. Your doctor may give you medications such as steroids and/or other medications to prevent or treat cytokine release syndrome.	consciousness, brain malfunction), memory impairment (memory loss), confusion and/or disorientation, or loss of balance. These nervous system problems can be serious, or life-threatening or may even lead to death. Patients with a medical history of neurologic signs and symptoms had a higher rate of neurologic events (such as tremor, dizziness, confusion, encephalopathy and poor coordination). Your doctor will be closely monitoring you and may give you medications such as steroids and/or other medications to treat nervous system problems or stop your treatment with blinatumomab.	

Supplier

Blinatumomab and the solution stabilizer for blinatumomab is supplied by Amgen, Inc. and will be distributed by Amgen to Children's Hospital of Wisconsin and American Family Children's Hospital/University of Wisconsin. Do not use commercial supply.

Obtaining the Agent

Agent Ordering: Amgen supplied agent may be requested by the eligible participating investigator (or their authorized designee) at each participating institution (Children's Hospital of Wisconsin and American Family Children's Hospital/University of Wisconsin).
To request shipment for this order: gccs@amgen.com or Fax orders to (805) 376-9807
ATTN: Amgen GCSCM CDCs

Order:	**____ Initial ____ Resupply** **____ Rush (shipment within 72 hours of receipt)** **Provide Justification:**	
Study Title:		
Study Sponsor:		
Sponsor Study #:	**Amgen Reference #:**	
Product Description		
Blinatumomab (AMG 103)		
Quantity Requested		
Blinatumomab (AMG 103)	38.5 µg/vial; 15 vials per box	vials/ boxes
IV Solution Stabilizer for Blinatumomab (AMG 103)	10 mL vial; 6 vials per box	vials/ boxes
Site Comments		
Investigator:	**Date of Request:**	
Amgen Site#:	**Preferred Arrival Date:**	

Shipments are processed on Monday through Thursday 12:00 p.m. PST.

Drug Shipment Requests received on Thursday after 12:00 p.m. PST will be shipped on the following Monday.

9.10 Total Body Irradiation

TOTAL BODY IRRADIATION:

	Common Happens to 21-100 children out of 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to <5 children out of every 100
Immediate: Within 1- 2 days of receiving drug	Slight redness of the skin, nausea	Inflammation of salivary glands	
Prompt: Within 2-3 weeks, prior to the next course	Poor appetite		Inflammation of the lungs with trouble breathing
Delayed: Any time later during therapy, excluding the above conditions	Sleepiness, headache		
Late: Any time after completion of therapy	Poor growth, sterility (inability to have children)	Lung scarring, cataracts, decreased thyroid function	New cancer

Total Body Irradiation Dosage: 200 cGy bid Days -3, -2, -1*

- *TBI may be administered prior to chemotherapy for ease in scheduling unrelated donors.
- Thorax shielding after 800 cGy

- Patients with leukemia or lymphoma and prior central nervous system relapse: 600 cGy in 3 fractions cranial irradiation prior to initiation of conditioning.

9.11 General Description of the CliniMACS™ System

- The CliniMACS device is not licensed by the U.S. Food and Drug Administration (FDA) and therefore is investigational.

- The mechanism of action of the CliniMACS Cell Selection System is based on magnetic-activated cell sorting. The CliniMACS device is a powerful tool for the isolation of many cell types from heterogeneous cell mixtures. For example, apheresis products can be separated in a magnetic field using an immunomagnetic label specific for the cell type of interest, such as CD34+ human hematopoietic cells enrichment, CD3+ T-cell depletion, or CD56+ NK-cell enrichment.

- The cells to be isolated or depleted are specifically labeled with super- paramagnetic particles by an antibody directed toward a cell surface antigen. After magnetic labeling, the cells are separated using a high-gradient magnetic separation. The magnetically labeled cells are retained in the magnetized column while the unlabeled cells flow through. Retained cells are eluted by removing the magnetic field from the column, washing the cells out and collecting them.

- The super-paramagnetic particles are small in size (about 50 nm in diameter) and are composed of iron oxide/hydroxide and dextran conjugated to monoclonal antibodies. These magnetic particles form a stable colloidal solution and do not precipitate or aggregate in magnetic fields. The CliniMACS device incorporates a strong permanent magnet and a separation column with a ferromagnetic matrix to separate the cells labeled with the magnetic particles. The high-gradient system allows the application of strong magnetic forces and a rapid demagnetization. Small ferromagnetic structures, such as the column matrix, placed in a magnetic field, concentrate this homogenous field and thereby produce high magnetic gradients. In their immediate proximity, the ferromagnetic structures generate magnetic forces 10,000-fold greater than in the absence of those structures enabling the retention of magnetically labeled cells. After removing the column from the magnet, the rapid demagnetization of the column matrix allows the release of retained cells.

- The CliniMACS device is comprised of a computer-controlled instrument incorporating a strong permanent magnet, a closed-system sterile tubing set containing columns with a coated ferromagnetic matrix and a paramagnetic, cell specific, labeling reagent. The instrument will separate the cells in a fully automated process.

10.0 REPORTING AND DOCUMENTING RESULTS (MEASUREMENT OF EFFECT)

10.1 Evaluation of Efficacy (or Activity)

10.1.1 Definitions

Evaluable for Toxicity: All subjects will be evaluable for toxicity from the time of their first

exposure to blinatumomab.

Evaluable for Objective Response: Subjects surviving to Day 100 who have had their disease re-evaluated and completed at least 1 day of blinatumomab therapy (24 hours) will be considered in the final study cohort and be considered evaluable for response. These patients will have their response classified according to the definitions stated below.

10.2 Bone Marrow Response Criteria

Complete Remission, MRD Negative (CR MRD-)

- A bone marrow with < 5% blasts by morphology; and
- MRD < 0.01% by flow or molecular testing (e.g. PCR); and
- No evidence of circulating blasts or extramedullary disease; and
- Recovery of peripheral counts (ANC > 500/µL and PLT count > 50,000 µL).
- Qualifying marrow and peripheral counts should be performed within 1 week of each other

Complete Remission (CR)

- A bone marrow with < 5% blasts by morphology; and
- No evidence of circulating blasts or extramedullary disease; and
- Recovery of peripheral counts (ANC > 500/µL and PLT count > 50,000 µL).
- Qualifying marrow and peripheral counts should be performed within 1 week of each other.

Complete Response with Incomplete Count Recovery (CRi)

- A bone marrow with < 5% blasts by morphology; and
- No evidence of circulating blasts or extramedullary disease; and
- Insufficient recovery of absolute neutrophil counts (ANC <500/µL), and or insufficient recovery of platelets (PLT counts < 50,000/uL),

Partial Remission (PR)

- Complete disappearance of circulating blasts and one of the following:
- A decrease of at least 50% of blasts in the bone marrow with > 5% and ≤ 20% blasts by morphology with recovery of peripheral counts (ANC > 500/µL and PLT count > 50,000 µL)

Note: only patients who entered the study with ≥ 20% blasts in the marrow may be assessed as PR

Stable Disease (SD)

Patient does not satisfy the criterion for either CR, CRi, PR or disease progression.

Progressive Disease (PD)

An increase of at least 25% in the absolute number of bone marrow or circulating leukemic cells, development of new sites of extramedullary disease, or other laboratory or clinical evidence of progression of disease.

Not Evaluable (NE)

Aplastic or severely hypoplastic marrow with any blast percentage. Bone marrow aplasia/hypoplasia is defined as overall marrow cellularity less than 10-20%. In this instance, marrow evaluation should be repeated every 1-2 weeks until response determination can be made, or patient meets criteria for a DLT of prolonged myelosuppression.

Relapse

After documentation of remission, one bone marrow aspirate and/or biopsy showing ≥ 5% leukemic blasts or pathological evidence of extramedullary disease.

10.3 Evaluation of Safety

Analyses will be performed for all patients having received at least 1 day of blinatumomab therapy (24 hours). The study will use the CTCAE version 5.0 for reporting of nonhematologic adverse events and modified criteria for hematologic adverse events. See Section 8.

11.0 EXPLORATORY STUDIES

This trial contains correlative biology studies designed to objectively assess how treatment with blinatumomab 100-128 days following transplantation with TCR-α/β- and CD19- depleted hematopoietic stem cells impacts immune cell reconstitution and function. Immunophenotyping using flow cytometry will provide a broad analysis of how immune cell subsets are impacted, while functional assays will focus on impact of blinatumomab on T cells (αβ, γδ, NKT) and NK cells. Plasma cytokine analyses will also be performed to examine systemic effects of blinatumomab treatment. **For the analyses, 10-20 mL of blood will be collected in lavender-top tubes**.* The blood will be centrifuged, and plasma collected for cytokine studies. The pelleted cells will be separated further on density gradients to isolate peripheral blood mononuclear cells (PBMC) for the phenotyping and functional analyses.

*The total desired amount is 20 mL with a 10 mL acceptable minimum.

- Please draw one 4 mL tube per test.
- Blood can only be collected Monday through Thursday.
- Please deliver at room temperature to the CPL.

Time points for analysis will include Days +19, +91, +135 and +180 (± 14 days at each time point). Additional time-points may be requested by study investigators if patient health status dictates the need (e.g., development of GVHD).

11.1 Immune Cell Phenotyping *(to be conducted in the MCW Cell Therapy Laboratory in Milwaukee)*

Reconstitution of T, B, and NK cell subsets in the peripheral blood will be analyzed by immune cell phenotyping using flow cytometry. PBMC will be evaluated for expression of cell surface markers to identify the presence and frequency of specific immune cell subsets and expression of lymphocyte activation markers (e.g., CD3, TCRαβ, TCRγδ, CD4, CD8, CD19, CD20, CD16,

CD56, CD25, CD69, PD-1, TIM-3, LAG-3, CTLA-4, 2B4, HLA-DR). In addition, CD3+CD4+ cells will be evaluated for expression of intracellular FoxP3 to detect regulatory T cells. Intracellular staining of cells for the proliferation marker Ki67 will also be done. Monocyte/macrophage markers will also be examined, including CD11b, CD14, CD33, CD68 and PD-L1.

The expression of surface activating receptors (e.g., NKG2D, NCRs, DNAM-1, CD27 and CD69) on NK/NKT cells and γδ T cells will be also analyzed using flow cytometry. Additionally, KIR molecules (both activation and inhibitory receptors) on the surface of the same cells will be assessed.

11.2 Functional Assessment of Lymphocyte Subsets *(to be conducted in the MCW Cell Therapy Laboratory in Milwaukee)*

To assess lymphocyte function (αβ T cells, γδ T cells, NKT & NK cells), PBMC will be co-cultured with the following stimulator cells in the presence or absence of blinatumomab: (a) the classical MHC-deficient NK cell target, K562; (b) K562 cells engineered to express CD19; (c) a NK cell-resistant sarcoma cell line, U2OS; (d) U2OS cells engineered to express CD19. As indicated in more detail below, after co-culture with CD19-positive and negative stimulator cells, the functional activity of αβ T cells, γδ T cells, NKT and NK cells will be examined for CD107a cell surface expression and for accumulation of intracellular IFN-γ and granzyme B using flow cytometry. The value of using flow cytometry for these functional assessments is that we can examine each cell subset separately by staining with lymphocyte subset-specific markers.

- **CD107a Expression:** A surrogate marker of αβ T cell, γ δ T cell, NKT and NK cell functional activity is the quantitation of lysosomal-associated membrane protein-1 (LAMP-1, or CD107a) on the cell surface. LAMP-1 lines the inner membrane of lytic granules of cytotoxic immune cells. Upon degranulation, which involves the incorporation of lytic granule membranes into the extracellular membrane, the LAMP-1 proteins localize to the extracellular surface. CD107a has been shown to be strongly upregulated on the cytotoxic cell surface following stimulation in concordance with the loss of perforin.
- **Intracellular IFN-γ and Granzyme B:** As the PBMC are co-cultured with stimulator cells, Golgi transport is inhibited using Brefeldin A followed by fixation, permeabilization and flow cytometry using standard cell markers (e.g., Granzyme B, CD3, CD56, CD16, TCR αβ and TCR γδ). IFN- γ and Granzyme B expression will be evaluated in αβ T cells, γδ T cells, NKT and NK cells.

11.3 Plasma Multiplex Cytokine Analysis *(to be conducted in the Capitini Laboratory at UW Madison)*

The plasma cytokines Eotaxin, Eotaxin-3, GM-CSF, IFN-γ, IL-10, IL-12p70, IL-12/IL-23p40, IL-13, IL-15, IL-16, IL-17A, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC, TNF-α, TNF-β, VEGF-A, and IL-8 will be measured using a multiplex cytokine analyzer where 10 antibodies specific for the above cytokines are attached to a single well providing cytokine capture and immune specificity. Thus, adding samples to 3 wells will allow us to examine pro and anti-inflammatory cytokines, chemokines and growth factors

produced by donor cells during immune reconstitution and GVHD. Human cytokine 30-plex kits that detect all of these cytokines on a single plate are commercially available.

11.4 Shipping Instructions

Blood samples collected at Children's Hospital of Wisconsin will be transported to the Medical College of Wisconsin (MCW) BMT Cell Therapy Laboratory suite located on the 3rd floor of the Froedtert Pavilion Building (Rooms 302-324). Following processing of the samples, PBMC will be analyzed immediately in the immune correlate assays or cryopreserved for future batch analyses. Fresh or frozen blood plasma samples collected at Children's Hospital of Wisconsin will be shipped by the Cell Processing Lab Monday-Thursday to Dr. Christian Capitini's laboratory at the University of Wisconsin, Madison overnight via Fed Ex. Ship 1 subset of a 1 mL aliquot to the following address:

Christian Capitini, MD
University of Wisconsin
4136 WIMR
1111 Highland Ave
Madison, WI 53705

After shipment, please email a copy of the completed FedEx airbill to the following email address: PhelanBBT-HCT@mcw.edu.

Blood samples collected at the American Family Children's Hospital, University of Wisconsin, will be transported to the laboratory located at the Wisconsin Institute of Medical Research at the University of Wisconsin. Following processing of the samples, plasma samples will be sent internally to Dr. Capitini's laboratory for analysis. PBMC will be shipped fresh (Monday-Thursday; 24-hour delivery via UPS or Fed Ex) to the MCW BMT Cell Therapy Laboratory:

MCW BMT Cell Therapy Laboratory
Attention: Fenlu Zhu, PhD
Froedtert Hospital Pavilion, Room 304
9200 West Wisconsin Avenue
Milwaukee, WI 53226

12.0 STATISTICAL CONSIDERATIONS

This study is designed as a multi-center Pilot study to test the ability of a biologically active therapy in blinatumomab, an anti-CD19/CD3 bispecific T-cell engager, to reduce the risk of leukemia relapse following HCT to improve post-HCT outcomes. This is a single arm study in which participants will be enrolled into one of two strata. The first stratum is restricted to subjects who are FC-MRD negative only and receive myeloablative conditioning (MAC). The second stratum will consist of subjects who are FC-MRD negative **AND** HTS-MRD negative and will receive reduced intensity conditioning (RIC) prior to HCT.

Safety monitoring will occur on a monthly basis for several safety endpoints.

12.1 Study Endpoints

12.1.1 Primary Endpoints

The feasibility of post-HCT blinatumomab therapy will be evaluated by computing the percentage of patients who are able to start blinatumomab at Day +100 after transplantation and complete a minimum of 14 of the 28 planned days of treatment.

12.1.2 Secondary Endpoints

Secondary endpoints will be evaluated separately in the two strata (MAC and RIC) in patients with B-ALL.

Tolerability: As defined by treatment-related adverse events. All adverse events which cause death or lead to discontinuation of the treatment as defined in Section 8 will be counted toward the tolerability endpoint. Adverse events that are considered disease-related (i.e., not suspected to be related to blinatumomab) will not be considered as therapy related adverse events. All adverse events observed up to Day +180 will be counted this endpoint.

Overall Survival (OS): OS is defined as the time interval from the date of transplant to death or to last follow-up.

Disease-Free Survival (DFS): Disease free survival is defined as the time interval from date of transplant to disease relapse, death or to last follow-up.

Persistence of Minimal Residual Disease (MRD) negativity: MRD status (negative/positive) will be evaluated by flow cytometry and high throughput sequencing on Day +28, +100, +180, and Day +365.

Engraftment: Engraftment is defined as first day of ANC > 500 /μL for the first of 3 consecutive days.

Primary Graft Failure: Primary graft failure is defined by the failure to achieve ANC > 500/μL by Day +28.

Secondary Graft Failure: Only patients who achieve engraftment will be considered for the analysis of secondary graft failure. Secondary graft failure is observed in patients who experience initial neutrophil engraftment followed by a decline in ANC < 500/μL that is unresponsive to growth factor therapy.

Treatment Related Mortality: Treatment related mortality is defined as death occurring in a patient from causes other than disease relapse or progression. Disease progression is the competing event for TRM. Patients alive without disease progression at last contact are considered censored for this event.

Relapse: After documentation of remission, one bone marrow aspirate and/or biopsy showing ≥ 5% leukemic blasts or pathological evidence of extramedullary disease indicate a relapse. Time

from HCT to the date of the diagnosis of relapse will be used to calculate a cumulative incidence probability. TRM is the competing event for relapse.

Acute GVHD: The events are the incidences of Grades 2-4 and Grades 3-4 acute GVHD from day of transplant. The first day of acute GVHD onset at a certain grade will be used to calculate a cumulative incidence probability for that acute GVHD grade. Death is considered as a competing risk.

Chronic GVHD: The events are the incidences of chronic GVHD from the day of transplantation. The first day of chronic GVHD onset at a certain grade will be used to calculate a cumulative incidence curve for that chronic GVHD grade. Death is considered as a competing risk.

Patient Reported Outcomes: PROMIS Pediatric/Parent Proxy Profile 25 (either pediatric self-report if age 8-17 or parent proxy if age 5-8, or both if feasible) or PROMIS-29 Profile if age 18 or older at baseline, Day +100, Day +180 and 1 year will be used to assess patient reported outcomes. Questionnaires are provided in paper form to the patient/family at their scheduled clinic visits. Patient/family will be offered an opportunity to say "yes" or "no" to providing their email address so research personnel can email the questionnaire to the patient/family in certain circumstances (i.e. clinic visit was missed, ran out of time during the clinic visit, etc.).

Length of Stay: Length of hospital stay following HCT will be measured as the number of days a patient spends in the hospital between the day of transplantation, Day 0, and Day +180. This will allow us to evaluate the LOS immediately after HCT and in the period starting with Day +100 when patients are receiving blinatumomab.

12.2 Study Design

This is a Pilot study, open label, multicenter trial to evaluate the use of blinatumomab as part of post-HCT treatment for patients diagnosed with B-ALL and undergoing alpha-beta T-cell and B-cell depleted stem cell transplantation. Upon enrollment, patients will be stratified based on the evaluation of MRD prior to HCT and assigned to a conditioning regimen. The myeloablative conditioning stratum will consist of subjects receiving myeloablative conditioning prior to HCT if their pre-HCT bone marrow evaluation for MRD was negative by flow cytometry (FC), but positive by high throughput sequencing (HTS). If subjects are MRD negative by **BOTH** FC and HTS prior to HCT, they will be assigned to the reduced intensity conditioning (RIC) stratum of the study. The target enrollment is around 25 patients, 10 to 15 in each stratum, who undergo HCT and receive blinatumomab for at least 1 day. While we anticipate similar number of subjects in each stratum, we allow for unequal enrollment in each stratum due to uncertainty in the mix of patients receiving blinatumomab as part of their pre-HCT therapy. Analysis of each stratum will be carried out separately.

12.3 Randomization

There is no randomization on this protocol.

12.4 Stratification Factors

Patients will be stratified based on the evaluation of minimal residual disease prior to HCT and subsequently chosen conditioning regimen. Myeloablative conditioning stratum will consist of subjects receiving myeloablative conditioning if prior to HCT their bone marrow evaluation is FC-MRD negative (performed at the University of Washington, Brent Wood, MD), but MRD positive by high throughput sequencing (HTS) (performed at Adaptive Technologies, Seattle, WA). If subjects are MRD negative by **BOTH** flow cytometry and HTS prior to HCT, they will be assigned to the reduced intensity conditioning stratum of the study.

12.5 Determination of Sample Size and Accrual Rate

12.5.1 Sample Size and Power Estimate

The primary objective of this study is to evaluate feasibility of giving blinatumomab after alpha-beta T-cell and B-cell depleted HCT. The percentage of patients who are able to start blinatumomab at Day +100 after transplantation and complete a minimum of 14 of the 28 planned days of treatment will be computed. The total sample size for this study is 25 patients with 10 to 15 subjects in each of the two strata consisting of subjects receiving myeloablative conditioning and reduced intensity conditioning, respectively. Table 1 provides confidence intervals for a variety of underlying proportions based on the binomial distribution. Ninety percent confidence intervals were calculated for a range of proportions based on a sample size of 10 and 15 since estimation will be carried out separately for each stratum. Of particular interest is the proportion of patients for whom such a therapy is feasible. This is expected at 50%, which includes the percentage of patients who will survive to Day +100 without > Grade 1 GVHD and will be able to receive at least 14/28 days of blinatumomab. This estimate is based on the literature[51] as well as local data with the use of alpha/beta T-cell depletion in hematologic malignancies where 6 out of 14 patients treated locally developed GVHD prior to Day +100. If the observed proportion of patients for whom the post-HCT blinatumomab therapy is feasible is 50%, given the sample size of 10 subjects, the half-width of the 90% confidence interval is 26.6%. Given the proportion of 50%, in the stratum with 15 subjects, the half-width of the 90% confidence interval is 22.2%. The tabulated percentages and confidence intervals for observed probabilities above and below 50%are meant to represent other plausible proportions of patients for who the therapy under consideration was feasible. Calculations were carried out using R package *binom* and represent half-length of exact binomial confidence intervals obtained by using Pearson-Klopper method.

Possible 90% confidence interval for various underlying proportions with n=10 and n=15

Adverse event Rate (%)	Half-Width of 90% Confidence Interval, n=10	Half-Width of 90% Confidence Interval, n=15
30	24.7	20.6
40	26.1	21.8
50	26.6	22.2
60	26.1	21.8
70	24.7	20.6

12.5.2 Accrual Estimates

The target sample size is 10 to 15 subjects in each stratum. The estimated accrual period is 3 years.
Patients will be followed up for 1-year post-transplant for primary and secondary endpoints.

12.6 Interim Analyses and Stopping Rules

Toxicity and adverse events will be monitored on continuous basis based on the rules detailed later in this section.

Given that Miltenyi columns used for preparing the graft for transplantation is an investigational device, it is important to monitor patients for adverse effects associated with its usage. Therefore, the first set of stopping rules is established in order to monitor the rates of TRM, Grade 4 acute GVHD, non-engraftment, and Epstein–Barr virus-associated lymphoproliferative diseases (EBV LPD) after HCT prior to administering blinatumomab. We assume the maximum acceptable rate is 15% for Day 100 TRM (based on current CliniMACS studies), is 15% for Grade 4 aGVHD, is 10% for non-engraftment, and is 5% for EBV LPD. Patients who satisfy eligibility criteria, will start blinatumomab therapy on Day +100. After that point, they will be continuously monitored for toxicities, TRM, aGVHD, relapse, and MRD positivity possibly associated with blinatumomab treatment.

The second set of stopping rules is established to ensure that, by Day +180, the maximum acceptable rate for post-blinatumomab TRM rate, toxicities and Grade 3-4 aGVHD is 15%; the maximum acceptable rate for Day +180 relapse and MRD rate is 30%.

The stopping rules are constructed for each type of the event separately. We will evaluate these events continuously starting a minimum of 3 enrolled patients. The stopping rule for each type of event will be triggered if there is significant evidence that the event rate exceeds the maximum acceptable rate.

The Pocock boundary will be used for monitoring the aforementioned adverse events. At each monthly analysis, the number of patients who experienced the event of interest will be noted and compared to the boundary computed based on the number of evaluable patients. If the number of events falls above the boundary, the null hypothesis is rejected by concluding that there are more events than predicted by the number of evaluable patients. The study is suspended at that time pending further evaluation. Otherwise, testing continues until enrollment reaches the maximum of 10 to 15 subjects in each stratum. Only instances of the events that occur within prespecified time interval are counted.

The usual measures of performance of such testing are the error probabilities α and β of rejecting H_0: $p = p_0$ when the probability of the adverse event of interest $p=p_0$ and of accepting H_1 when $p = p_1$, respectively, and the expected sample size. The stopping rules used in this protocol for each of the key safety endpoints are summarized below. The operating characteristics of the test were determined by simulation study and using R package clinfun. [55]

The rate of Grade 4 aGVHD and TRM will be first checked at 100 days post-HCT. The incidence

of both events should not exceed 15%. At each check, the null hypothesis that the Day +100 Grade 4 aGVHD and TRM rate is less than or equal to 15% is tested. Table 2 summarizes the stopping rules for monitoring both of these events with the target accrual of 15 subjects. The actual operating characteristics of the test evaluated via simulation study are shown in Table 3.

Table 2: Stopping Rules for Monitoring the Rate of TRM and Grade 4 aGVHD by Day +100, n=15.

Number of enrolled patients	3-5	6-8	9-12	13-15
Maximum number of observed events	2	3	4	5

Table 3: Operating Characteristics of Testing Procedure for TRM and Grade 4 aGVHD by Day +100, n=15.

True Day +100 Rate of TRM or Grade 4aGVHD	15%	30%	45%
Probability of stopping early	0.05	0.36	0.76
Expected sample size	15	12	9

For example, the testing procedure rejects the null hypothesis in favor of the alternative 5% of the time when the true 100-day rate of TRM is 15%, and 76% of the time when the rate is 45%.

Engraftment will be assessed at Day +100. Percentage of non-engrafted patients should not exceed 10%. Table 4 summarize the stopping rules for monitoring the number of patients who do not engraft by day +100 with the target accrual of 15 subjects. The actual operating characteristics of the test evaluated via simulation study are shown in Table 5.

Table 4: Stopping Rules for Monitoring Failure to Engraft by Day +100, n=15.

Number of enrolled patients	3	4-7	8-12	13-15
Maximum number of observed events	1	2	3	4

Table 5: Operating Characteristics of Testing Procedure for Failure to Engraft by Day +100, n=15.

True Day +100 Rate of Failure to Engraft	10%	25%	40%
Probability of stopping early	0.06	0.43	0.83
Expected sample size	15	11	8

EBV LPD incidence will be also assessed at Day +100. Its rate should not exceed 5%. Table 6 summarize the stopping rules for monitoring incidence of EBV LPD by day +100 with the target accrual of 15 subjects. The actual operating characteristics of the test evaluated via simulation study are shown in Table 7.

Table 6: Stopping Rules for Monitoring EBV LPD by Day +100, n=15.

Number of enrolled patients	3-6	7-14	15
Maximum number of observed events	1	2	3

Table 7: Operating Characteristics of Testing Procedure for EBV LPD by Day +100, n=15.

True Day +100 Rate of EBV LPD	5%	17.5%	30%
Probability of stopping early	0.05	0.50	0.86
Expected sample size	15	11	8

Next, a formal interim analysis for feasibility of administering blinatumomab post HCT is planned once half of the enrollment has been completed (10-12 evaluable patients). At that time, percentage of patients who underwent HCT and were able to start blinatumomab at Day +100 after transplantation completing at least 14/28 days of treatment will be calculated. If, after the first 10 subjects have been enrolled, 2 or fewer subjects are able to receive at least 14/28 days of blinatumomab, treatment feasibility will need to be discussed. The upper 95% one-sided confidence interval around 2/10 is 41% thus, it would be unlikely that the true feasibility rate is 50% or greater.

Once patients complete their 100-day post-HCT observation window and are assessed for early adverse events possibly caused by HCT with a graft processed using the Miltenyi columns, they start blinatumomab therapy. Patients will be monitored for several safety endpoints after blinatumomab treatment is started. Monitoring of key safety endpoints will be conducted monthly. If rates significantly exceed pre-set thresholds, the Medical College of Wisconsin (MCW) Data Safety Monitoring Committee (DSMC) and the sponsor will be notified. The main safety monitoring endpoints for all subjects who receive blinatumomab are as follows:

- Relapse by Day +180
- Toxicity caused by blinatumomab that results in death or meets criteria for discontinuation of the treatment as defined in Protocol Section 7.1.
- In addition, all subjects who proceed to receive at least one day of blinatumomab will be monitored for the following adverse events:
    - Treatment related mortality defined by any cause of death excluding death from disease progression in the first 180 days post-transplant.
    - Acute GVHD Grade 3-4 at Day +180
    - Minimal residual disease detected either by flow cytometry and/or high throughput sequencing by Day +180 (applicable to patients who were FC-MRD and HTS-MRD negative before HCT and receive reduced intensity conditioning)

Relapse rate (up to Day +180) will be monitored and it is not expected to exceed 30%. Each month, the null hypothesis that the 180-day relapse rate is less than or equal to 30% is tested. Table 8 summarize the stopping rules with the target accrual of 15 subjects. The actual operating characteristics of the test evaluated via simulation study are shown in Table 9.

Table 8: Stopping Rules for Monitoring the Rate of Relapse, n=15.

Number of enrolled patients	3-4	5-6	7-9	10-11	12-13	14-15
Maximum number of relapsed patients	3	4	5	6	7	8

Table 9: Operating Characteristics of Testing Procedure for the Rate of Relapse, n=15.

True Day 100 Rate of Relapse	30%	50%	70%
Probability of stopping early	0.04	0.37	0.87
Expected sample size	15	13	9

For example, the testing procedure rejects the null hypothesis in favor of the alternative 4% of the time when the true 180-day rate of relapse is 30%, and 87% of the time when the rate is 70%.

The rate of Grade 3-4 aGVHD, TRM, and therapy-related toxicities leading to death or treatment discontinuation will be assessed at Day +180. This time frame would allow to monitor patients who complete blinatumomab treatment after having temporary interruption in treatment which resumes within two weeks. The expected probability of each -Grade 3-4 aGVHD, TRM and therapy related toxicities - is not to exceed 15%. Each month, the null hypothesis that the 180-day toxicity rate is less than or equal to 15% is tested. Stopping rules described in Table 2 will be applied to monitor these events after blinatumomab therapy has started.

Minimal residual disease status assessed by both flow cytometry and high throughput sequencing up to Day +365 will be monitored. A patient is considered MRD positive if MRD is detected by either FC or HTS or both assessments. Only instances of the events that occur within prespecified time interval are counted. The expected percentage of MRD positive patients by Day +180 should not be more than 30%. Each month, the null hypothesis that the Day +180 MRD rate is less than or equal to 30 % is tested. This type of safety monitoring will be done only for FC-MRD and HTS-MRD negative patients receiving reduced intensity conditioning. The stopping rules developed for monitoring relapse and described in Table 8 will be applied for monitoring the rate of MRD positivity rate in this stratum.

12.7 Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for the patients in both strata. Patient-, disease-, and transplant-related factors will be summarized using descriptive statistics (median and range for continuous variables, frequencies/percent for categorical variables). Characteristics to be examined are: age, gender (donor and recipient), race/ethnicity, performance status, disease, disease status at transplant (including MRD status for all patients and HTS-MRD status for ALL patients), post-transplant HTS and FC-MRD status for ALL patients, time from diagnosis to transplantation, cytogenetics at diagnosis, conditioning regimen, graft source, recipient CMV status.

12.8 Analyses Plans

The primary objective of the study is to assess the feasibility of receiving blinatumomab post-

HCT. Secondary objectives of the study call for the estimation of the adverse event and toxicity rate as well as the evaluation of clinical outcomes and quality of life among patients who receive blinatumomab as part of post-HCT treatment. Both – primary and secondary – objectives will be addressed by estimating the probabilities of interest via appropriate methods outlined in this section. Point estimates as well as appropriate 90% confidence intervals will be produced in each of the two strata of patients. Exploratory objectives will be addressed by using descriptive statistics.

12.8.1 Analysis Population

All patients enrolled in the study and receiving HCT will be included in the analysis of primary outcome of therapy feasibility. Patients receiving HCT and living to Day +100 and receiving at least 1 day (24-hour infusion) of blinatumomab therapy will be used to evaluate the tolerability of blinatumomab given post-HCT. All other outcomes will be evaluated on the patients who received HCT, lived to Day+180 and received at least 14 days of blinatumomab.

12.8.2 Analysis of Primary Endpoints

The primary objective of this pilot study is to estimate the feasibility of receiving post-HCT blinatumomab therapy. All patients receiving at least 14 days day of blinatumomab starting at day +100 following HCT will be considered as events for the primary endpoint. Exact binomial 90% confidence interval for the true population proportion of patients able to start blinatumomab on Day +100 and complete at least 14 days of treatment will be produced.

Percentage of eligible subjects enrolled on this protocol who are able to start blinatumomab on Day +100 after HCT will be calculated. This calculation will be carried out on the population of patients who are enrolled in the study and receive HCT. Reasons preventing patients from receiving blinatumomab will be documented. Characteristics of patients who could not be treated with blinatumomab will be summarized and compared to the characteristics of patients who did start the treatment successfully.

12.8.3 Analysis of Secondary Endpoints

All secondary endpoints will be reported within each stratum. Interval estimation will be done by constructing two-sided 90% confidence intervals for the quantities of interest.

Overall Survival: Kaplan-Meier product limit estimator using Greenwood's formula for the variance will be used for estimating the overall survival probabilities. A point estimate and confidence interval will be provided for the survival probability at various time points including 1-year post HCT.

Disease Free Survival: Kaplan-Meier product limit estimator using Greenwood's formula for the variance will be used for estimating the disease-free survival probabilities. A point estimate and confidence interval will be provided for the survival probability at various time points including 1-year post HCT.

A previous publication indicates that 1-year DFS probability in patients receiving myeloablative

conditioning for high-risk patients in CR2 or very high-risk patients in CR1 is approximately 60%[17]. If this estimate falls within the 90% confidence interval constructed for each of the two strata of this study, we will consider therapy involving alpha-beta T-cell and B-cell depleted HCT followed by blinatumomab to be comparable to the standard myeloablative HCT.

Persistence of Minimal Residual Disease (MRD) negativity: Proportion of patients who are MRD negative by flow cytometry and high throughput sequencing on Day +180, and Day +365 will be calculated. Appropriate 90% confidence interval will be provided. Number of cases where FC and HTS both result in negative, both result in positive, and mismatched findings will be counted.

Engraftment: The incidence of engraftment from transplant will be estimated using the cumulative incidence function, treating death or second transplant as a competing risk. A point estimate and confidence interval will be provided for the cumulative incidences at various time points including 1-year post HCT.

Secondary Graft Failure: The incidence of secondary graft rejection from transplant will be estimated using the cumulative incidence function, treating death as a competing risk. A point estimate and confidence interval will be provided for the cumulative incidence at 1-year post-transplant for secondary graft rejection.

Treatment Related Mortality: To assess the incidence of treatment related mortality after HCT, a cumulative incidence curve will be estimated, treating relapse as a competing risk. A point estimate and confidence interval will be provided for the cumulative incidence at 1-year post HCT.

Relapse: To assess the incidence of disease recurrence after HCT, a cumulative incidence curve will be estimated, treating death as a competing risk. A point estimate and confidence interval will be provided for the cumulative incidence at 1-year post HCT.

Acute GVHD: Rates of acute GVHD will be tabulated. Cumulative incidence of acute Grade 2-4 GVHD will be estimated using the cumulative incidence function, treating death prior to acute GVHD as a competing risk. A point estimate and confidence interval will be provided for the cumulative incidence at Day 100 and Day 180 post-transplant. Time to GVHD will be calculated from transplant date. Cumulative incidence of acute Grade 3-4 GVHD will be estimated using the cumulative incidence function, treating death prior to acute GVHD as a competing risk. A point estimate and confidence interval will be provided for the cumulative incidence at each time point

Chronic GVHD: Rates of chronic GVHD will be tabulated by severity. Cumulative incidence of chronic GVHD will be estimated using the cumulative incidence function, treating death prior to chronic GVHD as a competing risk. A point estimate and confidence interval will be provided for the cumulative incidence at 6 months and one-year post-transplant. Analysis of severe chronic GVHD will be similar. Time to GVHD will be calculated from transplant date.

Patient Reported Outcomes: Patient reported outcomes will be described using the PROMIS. Descriptive statistics and confidence intervals for the change from baseline will be reported for each domain.

Length of Stay: Post HCT length of hospital stay as measured by the number of days spent in the hospital will be summarized by providing means and standard deviations. LOS will be analyzed separately for the initial time period immediately after HCT and starting with Day +100 when patients are receiving blinatumomab.

Tolerability: Tolerability will be defined as the estimate of the incidence of adverse effects when blinatumomab is given as part of post-HCT maintenance therapy. All patients receiving at least one day of blinatumomab will be evaluated for primary endpoint. All types of toxicities observed in this phase of the study will be catalogued, their frequency, timing and resolution will be noted for each stratum. Percentage of patients experiencing adverse events of toxicities listed in Section 8 will be calculated. Exact binomial 90% confidence interval for the true population proportion will be produced.

12.8.4 Other Analyses/Assessments

Descriptive statistics will be used for exploratory outcomes such as evaluation of immune reconstitution and function, evaluation of T cell number and function. The frequencies and percentages of immune reconstitution outcomes, as determined by immune cell phenotyping, spectratyping and FACS analysis, will be summarized. Cell counts will be summarized in terms of means, standard deviations, medians and ranges. T cell activity, cytotoxic and γδ T cell and NK cell activity measures will be summarized using standard descriptive statistics. For continuous measurements, we will use box and whisker plots, to summarize and display the data. Data from the patients in both strata will be combined to explore the use of HTS-MRD following HCT to predict relapse.

12.9 Missing Data

Data collection will be monitored very strictly, and we do not anticipate having a high rate of missing data. In primary endpoint analysis, feasibility will be assessed on all patients who were enrolled in the study and underwent HCT. Those who are lost to follow up by Day +100 will be excluded from the analysis. Patients who receive at least one day course of blinatumomab will contribute data for evaluating the rate of adverse events. In case a patient is not observed for the full 180-day period post HCT but experiences an adverse event his or her data will be used. Sensitivity analysis using patients lost to follow without experiencing an adverse event after starting or completing blinatumomab will be conducted. It should be noted that patients are unlikely to be lost to follow up while blinatumomab is being administered due to the fact that it is given by a continuous infusion monitored by a healthcare provider.

For the analysis of all secondary endpoints, patients will be censored at the time of their last follow up and appropriate survival analysis techniques will be used to analyze the outcomes.

13.0 DATA AND SAFETY MONITORING PLAN (DSMP)

Please refer to the MCWCC DSMC Charter.

13.1 Data and Safety Management Overview

The Medical College of Wisconsin (MCW) Data Safety Monitoring Committee (DSMC) and the MCW or CHW Institutional Review Board (IRB) will approve protocol-specific DSM plans. A local, investigator-initiated trial will be required to be continuously monitored by the principal investigator of the study with twice annual (or more frequently if needed) safety and progress reports submitted to the DSMC.

13.1.1 Study Team

The study team minimally consists of the principal investigator, the clinical research coordinator, regulatory specialist, and the study biostatistician. While subjects are on treatment, the principal investigator will meet regularly with the research coordinator and the study biostatistician to review study status (attendees and time periods should be modified so as to make sense within the context of the study). This review will include but not be limited to reportable SAEs and UPIRSOs and an update of the ongoing study summary that describes study progress in terms of the study schema. The appropriateness of further subject enrollment and the specific intervention for a next subject enrollment is addressed. SAE and any AEs requiring assigning of attribution are discussed and recorded via email or by physician signature on applicable documentation.

Pediatric CTO DVL, Leukemia/Lymphoma, and HCT groups meet weekly to discuss upcoming protocols, SAEs, existing patients, and new patients. Individual SAEs are discussed with the attending provider as needed.

13.1.2 Quality Assurance

This protocol will be reviewed internally every six months by MCW CTSI staff. Approximately 30% of subject files will be selected randomly for review (max 5 subjects at each monitoring timepoint). Consent, eligibility and objective based data will be reviewed for those files selected. One file will be selected randomly for a comprehensive review at each quality assurance review timepoint. Regulatory will also be reviewed each time.

13.1.3 Clinical Trials Office

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

13.1.4 DSMC

The pediatric CTO places the highest priority on ensuring the safety of patients participating in clinical trials. Every cancer interventional trial conducted at MCW or CHW includes a plan for safety and data monitoring.

More information can be found related to the MCWCC Data and Safety Monitoring Plan at the MCWCC website (Data and Safety Monitoring Plan).

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

follows:

- Review the clinical trial for data integrity and safety
- Review all unexpected Grade 3, and all Grade 4 and 5 adverse events, as well as any other requiring expedited reporting as defined in this protocol. Non-hematological Grade 4 and all Grade 5 events must be reported to the DSMC within 5 calendar days of study staff's knowledge. For all grade 5 events during the blinatumomab infusion post-HCT, study enrollment will be paused until a full safety review by the DSMC has been completed.
- Review all DSM reports
- Submit a summary of any recommendations related to study conduct
- Terminate the study if deemed unsafe for patients

A copy of the MCWCC Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study PI twice annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary.

Any available DSMC letters will be submitted to the IRB of record as required.

13.2 Monitoring

The PI at each participating center is responsible for monitoring this study for accuracy of data and protocol compliance at their institution. Patient eligibility will be confirmed by Dr. Rachel Phelan before each patient is enrolled.

The PI, data coordinators, and research nurses are responsible for review and maintenance of all patient records at their individual institution to ensure data integrity and protocol adherence.

The site PI will permit study-related monitoring, audits, and inspections by the MCW/CHW compliance groups, as necessary. The investigator will make available all study related documents (i.e. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (i.e. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

14.0 REGULATORY COMPLIANCE, ETHICS AND STUDY MANAGEMENT

14.1 Ethical Standard

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

14.2 Regulatory Compliance

This study will be conducted in compliance with:

- The protocol
- Federal regulations, as applicable, including: 21 CFR 50 (Protection of Human

Subjects/Informed Consent); 21 CFR 56 (Institutional Review Boards) and §312 (Investigational New Drug Application; and 45 CFR 46 Subparts A (Common Rule), B (Pregnant Women, Human Fetuses and Neonates), C (Prisoners), and D (Children), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

14.3 Pre-Study Documentation

Prior to implementing this protocol at Children's Hospital of Wisconsin within the Pediatric CTO, the protocol, informed consent form, HIPAA authorization, and any other information pertaining to participants must be approved by the CHW IRB.

The clinical investigation will not begin until the following conditions have been met, as applicable:

- The FDA has determined that the study under the Investigational Device Exemption (IDE) is allowed to proceed

OR

- The Investigator has received a letter from FDA stating that the study is exempt from IDE requirements

OR

- The study meets all of the following IDE Exempt requirements (as applicable):
    - The drug product is lawfully marketed in the United States
    - The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication and there is no intent to use it to support any other significant change in the labeling of the drug
    - In the case of a prescription drug, the investigation is not intended to support a significant change in the advertising for the drug
    - The investigation does not involve a route of administration, dose, patient population, or other factor that significantly increases the risk (or decreases the acceptability of the risk) associated with the use of the drug product (21 CFR 312.2(b)(1)(iii))
    - The investigation is conducted in compliance with the requirements for review by an IRB (21 CFR part 56) and with the requirements for informed consent (21 CFR part 50)
    - The investigation is conducted in compliance with the requirements of § 312.7 (i.e., the investigation is not intended to promote or commercialize the drug product).

14.4 Institutional Review Board

The protocol, the proposed informed consent form, and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the CHW Institutional Review Board.

Prior to obtaining CHW approval, the protocol must be approved by the Medical College of Wisconsin Cancer Center Scientific Review Committee. The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

14.5 Informed Consent Process

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

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

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

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

A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. If there are changes to the consent form, all revisions will be reviewed with study subject at the next appropriate opportunity. Subjects that require re-consenting will be defined in the IRB approved amendment submission. The process for obtaining informed consent will again be performed. Study subjects will not be re-consented for continuing reviews. The Pediatric CTO will follow the CHW IRB's policy for subjects who demonstrate limited English proficiency or limited literacy.

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

14.6 Subject Confidentiality and Access to Source Documents/Data

Subject confidentiality must be contained at all material submitted to the key sponsor contact. The following rules are to be applied.

- Subjects will be identified by a unique identification number

- Date of birth or year of birth/age at time of enrollment will be reported according with local laws and regulations

For reporting of serious adverse events, subjects will be identified by their respective subject identification number, initials and data of birth (as per their local reporting requirements for both initials and date of birth).
Per federal regulations and ICH/GCP guidelines, investigators and institutions are required to permit authorization to the sponsor, CRO, IRB/IEC and regulatory agencies to subject's original source documents for verification of study data. The investigator is responsible for informing potential subjects that such individuals will have access to their medical records which includes personal information.

14.7 Protection of Human Subjects

14.7.1 Protection from Unnecessary Harm

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

14.7.2 Protection of Privacy

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

14.8 Changes in the Protocol

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

If the protocol is amended, the investigators agreement with the amendment and the IRB/IEC approval of the amendment must be obtained. Documentation acknowledging approval from both parties are to be submitted to the key sponsor contact.

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

The IRB may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the

approval/favorable opinion of the IRB. The investigator will submit all protocol modifications to the sponsor and the regulatory authority(ies) in accordance with the governing regulations.

Both CHW and the investigator reserve the right to terminate the investigators participation in the study as per the terms of the agreement in the study contract. The investigator is to provide written communication to the IRB/IEC of the trial completion or early termination and provide CHW with a copy of the correspondence.

14.9 Investigator Compliance

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

Onsite Audits/Monitoring

Auditing is essential to ensure that research conducted at the Pediatric CTO is of the highest quality and meets regulatory agency standards.

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

The key sponsor contact, monitors, auditors or regulatory inspectors are responsible for contacting and visiting the investigator for the purpose of inspecting the facilities and verifying source documents and records assuring that subject confidentially is respected.

The monitor is responsible for source document verification of CRF data at regular intervals during the study. Protocol adherence, accuracy and consistency of study conduct and data collection with respect to local regulations will be confirmed. Monitors will have access to subject records as identified in Section 15.4.
By signing the investigator agreement, the investigator agrees to cooperate with the monitor to address and resolve issues identified during monitoring visits.

In accordance with ICH GCP and the audit plan, a site may be chosen for a site audit. A site audit would include, but is not limited to, an inspection of the facility (ies), review of subject and study related records, and compliance with protocol requirements as well as ICH GCP and applicable regulatory policies.

All data will be collected in an electronic CRF system. All entries must be completed in English and concomitant medications should be identified by trade names. For further details surrounding the completion of CRFs, please refer to the CRF completion guidelines.

15.0 DATA HANDLING AND RECORD KEEPING

15.1 Overview

Every effort is made to uphold the integrity of the project, the research, the institution, and the researchers involved. Data collection guidelines and methodologies are carefully developed before the research begins. Investigators focus on the following to ensure data integrity: well-trained data collectors/recorders to ensure consistency and quality, well-designed data collection protocols and ongoing monitoring. In this way, study rigor and validity are maintained. Data is protected from physical damage as well as from tampering, loss or theft. This project's data management is a multidisciplinary activity that includes investigators, research coordinators and nurses, data mangers, support personnel, biostatisticians and database programmers. Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

15.2 Data Management Responsibilities

15.2.1 Principal Investigator

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

15.2.2 Research Coordinator

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

15.2.3 Research Nurse/Medical Staff

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

15.2.4 Biostatistician

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

15.3 Handling and Documentation of Clinical Supplies

The Pediatric CTO Principal Investigator (and each participating site) will maintain complete records showing the receipt, dispensation, return, or other disposition of all investigational drugs.

The date, quantity and batch or code number of the drug, and the identification of patients to whom study drug has been dispensed by patient number and initials will be included. The sponsor-investigator will maintain written records of any disposition of the study drug.

The principal investigator shall not make the investigational drug available to any individuals other than to qualified study patients. Furthermore, the principal investigator will not allow the investigational drug to be used in any manner other than that specified in this protocol.

15.4 Source Documents

The investigator will maintain a list of qualified staff to whom study responsibilities have been delegated. These individuals authorized to fulfil these responsibilities should be outlined and included in the Delegation of Authority Form.

Source documents are original documents, data and records for which the study data are collected and verified. Example of such source documents may include, but are not limited to, hospital records and patient charts, laboratory, pharmacy, radiology and records, subject diaries, microfiches, correspondence and death registries. Case report form entries may be considered as source data if the site of the original data collection is not available. However, use of the CRFs as source documentation as a routine practice is not recommended.

The investigator and study staff are responsible for maintaining a comprehensive and centralize filing system of all subject records that are readily retrieved to be monitored and or audited at any time by the key sponsor contact, regulatory authorities and IRB/IECs. The filing system will include at minimum:

- Subject content including assents/consents and subject identification lists
- Protocols and protocol amendments, investigator brochure, copies of pre-study documentation, and all IRB/IEC and sponsor communication
- Proof of receipt, experimental treatment flow records and experimental product related correspondence.

Original source documents supporting entries into CRFs must be maintained at the site and readily available upon request. No study documents should be discarded without prior written agreement between CHW and the investigator. Should storage no longer be available to archive source documents or must be moved to an alternative location, the research staff should notify the key sponsor contact prior to the shipping the documents.

All source documents will be written following ALCOA standards:

ALCOA Attribute	Definition
Attributable	Clear who has documented the data.
Legible	Readable and signatures identifiable.
Contemporaneous	Documented in the correct time frame along with the flow of events. If a clinical observation cannot be entered when made, chronology should be recorded. Acceptable amount of delay should be defined and justified.
Original	Original, if not original should be exact copy; the first record made by the appropriate person. The investigator should have the original

ALCOA Attribute	Definition
	source document.
Accurate	Accurate, consistent and real representation of facts.
Enduring	Long-lasting and durable.
Available and accessible	Easily available for review by treating physicians and during audits/inspections. The documents should be retrievable in reasonable time.
Complete	Complete until that point in time.
Consistent	Demonstrate the required attributes consistently.
Credible	Based on real and reliable facts.
Corroborated	Data should be backed up by evidence.

15.5 Case Report Forms

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

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

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

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

15.6 Study Record Retention

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

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

informed consent was obtained prior to participation in the study.

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

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study. In accordance with FDA regulations, the investigator shall retain records for a period of two years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until two years after the investigation is discontinued and FDA is notified.

The investigator will retain study records including source data, copies of case report forms, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for 10 years after the study file is closed with the IRB.

In addition, the Pediatric Clinical Trials Office (CTO) will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient. Please contact the CTO before destroying any study related records.

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APPENDIX 1

Performance Status Criteria

Karnofsky Performance Scale For Patients 16 Years of Age and Older	
Percent	**Description**
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead

Lansky Performance Scale For Patients Less Than 16 Years of Age	
Lansky Score	**Play Score**
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both greater restriction of and less time spent in play activity
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Gets dressed but lies around much of the day, no active play but able to participate in all quiet play and activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	No play; does not get out of bed
0	Unresponsive

APPENDIX 2

Article 1: Criteria for Staging and Grading Acute GVHD

Criteria for staging and grading acute GVHD based on the Keystone Consensus Criteria (Przepiorka 1995) and adjusting diarrhea volume for pediatric patients based on BSA. Grading acute GVHD is a two-step process. First, acute GVHD is staged according to organ involvement and then an overall grade is assigned.

Article 2: Staging

Stage	Skin	Liver	Gut	
			Lower GI	Upper GI
0	No rash	Bilirubin < 2.0 mg/dL	Diarrhea < 500 mL/day or < 280 mL/m^2	No protracted nausea or vomiting
1	Maculopapular eruption over < 25% of body surface area	Bilirubin 2.0 - 3.0 mg/dL	Diarrhea 500 -1000 mL/day or 280 – 555 mL/m^2/day	Persistent nausea, vomiting or anorexia plus histologic changes of aGVHD on biopsy of stomach or duodenum
2	Maculopapular eruption over 25 - 50% of body surface area	Bilirubin 3.1 - 6.0 mg/dL	Diarrhea 1001-1500 mL/day or 556 – 833 mL/m^2/day	
3	Generalized erythroderma or rash over > 50% of body surface area	Bilirubin 6.1 - 15 mg/dL	Diarrhea >1500 mL/day or > 833 mL/m^2/day	
4	Generalized erythroderma with bullous formation and/ or with desquamation	Bilirubin > 15 mg/dL	Severe abdominal pain ± ileus or stool with frank blood or melena	

Grading

Grade[a]	Skin stage[c]	Liver Stage	Gut stage
	Use Rule of Nines	Downgrade one stage if additional cause of elevated bilirubin documented	Downgrade one stage if additional cause of diarrhea documented
I	1-2	0	0
II	3 or	1 or	1
III	---	2-3 or	2-4
IV[b]	4 or	4	---

a Criteria for grading given as minimum degree of organ involvement required to confer that grade
b Grade IV may also involve lesser organ involvement but with extreme decrease in clinical performance status
c If no skin disease, the overall grade is the higher single organ grade

Rule of Nines for Quantitating Body Surfaces

Body Area	Percent	Total Percentage
Each Arm	9	18
Each Leg	18	36
Chest & Abdomen	18	18
Back	18	18
Head	9	9
Pubis	1	1

Grading of Chronic GVHD
Grading of chronic GVHD assessment used in the current CIBMTR manuals reflecting grading system published by Sullivan KM (Sullivan 1981))

Grading Chronic GVHD is a 3-Step Process

- Determine maximum grade of chronic GVHD: limited or extensive
- Determine the overall severity of chronic GVHD: mild, moderate, severe
- Determine the severity specific to organ involvement: absent, present or unknown, if present and indicate if proven by biopsy

Determine maximum grade of cGVHD:

Clinically Limited Chronic GVHD	Clinically Extensive Chronic GVHD
• Localized skin involvement resembling localized scleroderma with or without liver involvement • **NO other organ involvement**	One or more of the following attributed to cGVHD: • Generalized skin involvement and/or liver dysfunction • Liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis • Involvement of the eye • Involvement of the salivary glands oral mucous membranes • Involvement of any other target organ

Determine the overall severity of cGVHD

Mild	Moderate	Severe
Signs and Symptoms of chronic GVHD:		
• Do not interfere substantially with function **AND** • Do not progress once appropriately treated with local therapy or standard systemic therapy (e.g., corticosteroid and/or cyclosporine or FK 506)	• Interfere somewhat with function despite appropriate therapy **OR** • Are progressive through first line systemic therapy (corticosteroids and/or cyclosporine or FD 506)	• Limit function substantially despite appropriate therapy **OR** • Progressive through second line therapy

Determine the severity specific to organ involvement: absent, present or unknown, if present

System	Signs and Symptoms of cGVHD
Skin/Hair	Thickening of skin, which may cause loss of suppleness, rash, ulcers pruritus, lichenoid skin changes, dyspigmentation, erythema or vitiligo, alopecia, other skin or hair involvement
Eyes	Xerophthalmia, Abnormal Schirmer's test, corneal erosion/conjunctivitis, abnormal slit lamp, other ocular involvement
Mouth	Lichenoid changes, mucositis/ulcers, erythema, other oral involvement

System	Signs and Symptoms of cGVHD
Lungs	Bronchiolitis obliterans (BO, BOOP), impairment on pulmonary function tests, poor exercise tolerance, shortness of breath, other lung involvement
Gastrointestinal tract	Dysphagia, odynophagia, esophageal webbing, poor motility, chronic nausea/vomiting (at least 25% of days or frequently enough to interfere with functioning and lifestyle), chronic diarrhea (at least 25% of days or frequently enough to interfere with functioning and lifestyle), malabsorption, abdominal pain/cramps, other gastrointestinal tract involvement
Liver	Elevated liver function tests, liver biopsy may show obliteration of bile ducts or cirrhosis
Genitourinary tract	Vaginitis/stricture: pain, ulceration, inflammation, scarring/narrowing of vaginal os
Musculoskeletal	Arthritis, contractures, myositis, myasthenia, other musculoskeletal involvement
Blood	Thrombocytopenia (usually 20,000-100,000/μL), eosinophilia (>5% ULN), autoantibodies, other hematologic involvement,
Other	Serositis, weight loss

APPENDIX 3

Preparation of Blinatumomab

Only trained staff may prepare the blinatumomab IV solution. Blinatumomab must be prepared in an ISO Class 5 containment device, ideally in an ISO Class 7 room as described in USP <797>, but ISO Class 7 is not required. Use aseptic technique and prepare blinatumomab IV solution under a qualified biological safety cabinet.

The label on the IV bag must include the following:

- Patient name and number
- Name of the drug
- Dose (mcg/day and volume/day)
- Infusion rate
- Expiration date and time
- CAUTION: NEW DRUG – Limited by United States law to investigational use.
- Bag number
- Additional information as required by state, local, and country pharmacy regulations.

Blinatumomab must be dispensed in an acceptable IV bag. Acceptable bags include those made of polyolefin/polyethylene, ethylene vinyl acetate (EVA), or PVC non-DEHP.

The final IV solution **must** be prepared in the following sequential order (do not deviate from this order; refer to the table below for volume details):

1. Reconstitute Blinatumomab lyophilized powder

Blinatumomab 38.5 mcg/vial
Add 3 mL of Sterile Water for Injection (SWFI) to the vial to yield 3.08 mL of Blinatumomab at a final concentration of **12.5 mcg/mL.**

a. Rotate the vial to dissolve all powder. Do not shake.
b. The stability of the reconstituted vial is 4 hours at room temperature (22°C to 27°C) or 24 hours refrigerated at 2° to 8°C.

2. Add the appropriate amount of 0.9% NaCl into the IV bag

3. Add the IV solution stabilizer for blinatumomab to the IV bag

a. Gently mix the contents of the bag to avoid foaming. Do not shake.
b. Discard remaining IV solution stabilizer vial.

4. Add the calculated dose (mL) of blinatumomab into the solution in the IV bag

a. Rotate the IV bag to mix the solution thoroughly. Do not shake. Avoid foaming the IV bag.
b. Visually inspect for floating particles or discoloration of the IV solution. If floaters or discoloration is present, do not use the prepared solution.

c. The total volume of blinatumomab IV solution will account for the volume of the IV infusion set for the inpatient or outpatient setting.

Note: Overfill volume depends on the volume of **the IV set** used.

Patients Weighing Under 22 kg

Due to the addition of bacteriostatic saline, 7-day infusion bags of Blinatumomab solution contain benzyl alcohol and are not recommended for use in any patients weighing less than 22 kg.

Prepare Blinatumomab solution for infusion with preservative-free saline in 24-hour or 48-hour infusion bags for patients weighing less than 22 kg.

Please Refer to the Current USPI for Preparation of the Infusion Bags and Volume Calculations

Blinatumomab can be infused over 24 hours (preservative-free), 48 hours (preservative-free), or 7 days (with preservative). The choice between these options for the infusion duration should be made by the treating healthcare provider considering the frequency of the infusion bag changes and the weight of the patient. The administration of Blinatumomab as a 7-day infusion is not recommended for patients weighing less than 22 kg.

Call 1-800-77-AMGEN (1-800-772-6436) if you have questions about the reconstitution and preparation of Blinatumomab.

Blinatumomab Prescribing Information can be found here:
https://www.pi.amgen.com/~/media/amgen/repositorysites/pi-amgen-com/blincyto/blincyto_pi_hcp_english.pdf