CLINICAL STUDY PROTOCOL

Phase 1/1b Study of Redirected Autologous T Cells Engineered to Contain an Anti CD19 and Anti CD20 scFv Coupled to CD3ζ and 4-1BB Signaling Domains in Patients with Relapsed and/or Refractory CD19 or CD20 Positive B Cell Malignancies

Short Protocol Title

Phase 1/1b Study of CAR-20/19-T Cells in Patients with Relapsed Refractory B Cell Malignancies

IND Number 17518

Clinical Trials.gov Number: NCT03019055

Principal Investigator

Nirav Shah, MD MSHP

Version 5
6/18/2019

MCW Protocol No.: PRO00028724.

FDA IND No.: *17518*

Principal Investigator (PI)
Nirav Shah, MD MS
Assistant Professor of Medicine
Medical College of Wisconsin
9200 W. Wisconsin Ave
Milwaukee, WI 53226
Phone: 414-805-4600
Fax: 414-806-6815
Email address: nishah@mcw.edu

Principal Lab Investigator
Bryon D. Johnson, PhD
Professor of Medicine, Hem/Onc
Director MCW BMT Laboratories
Medical College of Wisconsin
8700 Watertown Plank Road
Milwaukee, WI, 53226
Phone 414-805-6145
Email: bjohnson@mcw.edu

Co-Principal Investigator
Parameswaran Hari, MD MS
Professor
Hematology/Oncology
Medical College of Wisconsin
9200 W. Wisconsin Ave
Milwaukee, WI 53226
Phone: 414-805-4600
Fax: 414-806-6815
Email: phari@mcw.edu

Co-Lab Investigator
Fenlu Zhu, PhD
Assistant Professor of Medicine
Medical College of Wisconsin
8700 Watertown Plank Road
Milwaukee, WI, 53226
Phone 414-805-6145
fzhu@mcw.edu

Biostatistician:
Mei-Jie Zhang, PhD
Professor
Division of Biostatistics
Medical College of Wisconsin
9200 W. Wisconsin Ave
Milwaukee, WI 53226
Email: meijie@mcw.edu

Co-Investigator
Mehdi Hamadani MD
Professor of Medicine
Medical College of Wisconsin
9200 W. Wisconsin Ave
Milwaukee, WI 53226
Phone: 414-805-4600
Fax: 414-806-6815
mhamdani@mcw.edu

Co-Investigator
Timothy S. Fenske MD
Professor of Medicine
Medical College of Wisconsin
9200 W. Wisconsin Ave
Milwaukee, WI 53226
Phone: 414-805-4600
Fax: 414-806-6815
tfenske@mcw.edu

PROTOCOL SUMMARY

Title	Phase 1/1b Study of Redirected Autologous T Cells Engineered to Contain an Anti CD19 and Anti CD20 scFv Coupled to CD3ζ and 4-1BB Signaling Domains in Patients with Relapsed and/or Refractory CD19 or CD20 Positive B Cell Malignancies
Protocol Number	PRO00028724
IND #	*17518*
Principal Investigator	Nirav Shah, MD MSHP Assistant Professor, Department of Medicine
Study Sites	Froedtert Hospital & Medical College of Wisconsin Cancer Center
Clinical Trial Phase	Phase 1/1b
Study Disease	CD19 or CD20 Positive B Cell Malignancies including CLL/SLL and B-cell NHL
Inclusion Criteria	1. Diagnosis of B-cell NHL or CLL/SLL: Patients must be aged≥18 years with relapsed, refractory disease and no available curative options that meet clinical criteria to initiate treatment. 2. Patients with B-cell NHL or CLL/SLL must have either CD19 or CD20 positive disease on most recent biopsy performed (a repeat biopsy is not mandatory for this study except as noted below). A minimum of 5% CD19 or CD20 positivity by immunohistochemistry or flow cytometry on prior or repeat biopsy is required. 3. Absolute CD3+ T cell count ≥50/mm^3 4. MRI brain and Lumbar Puncture with CSF analysis by cytology and flow cytometry without evidence of CNS involvement ONLY in patients with history of CNS involvement 5. Measurable disease must have been documented within 4 weeks of the time of consent defined as the following by disease specific subtype: a. B-cell NHL: Active disease defined as nodal lesions greater than 20 mm in the long axis or extranodal lesions >10 mm in long and short axis or bone marrow involvement that is biopsy proven b. CLL/SLL: Active disease by either bone marrow, peripheral flow cytometry, or CT and/or PET imaging with nodal disease 6. Patients should have failed at least two lines of a standard treatment and meet disease specific criteria detailed below: a. CLL/SLL: measurable disease as defined above that has relapsed after at least one line of chemo-immunotherapy and progressed or intolerant to ibrutinib monotherapy b. CD19 or CD20 positive B cell NHL limited to the following histologies: Advanced Stage III or IV Follicular Lymphoma, Diffuse Large B cell Lymphoma and associated subtypes (e.g. aggressive B-cell lymphoma, T-cell/histocyte rich B-cell

	lymphoma, primary mediastinal B-cell lymphoma, EBV+ diffuse large B-cell lymphoma, transformed lymphoma such as transformed follicular or marginal zone lymphoma, and Richter's transformation) and Mantle cell lymphoma. Specific criteria include: - Patients must have active, measurable disease after two lines of cytotoxic chemotherapy of which one must be anthracycline containing. - Must have received Rituximab or another CD20 antibody and at minimum two chemotherapy regimens appropriate for their disease. - Either failed autologous transplant or ineligible to receive autologous transplant 7. Karnofsky performance score ≥70. See Appendix A for scales. 8. Normal Baseline Neurological Evaluation: Mini-Mental Status Exam Score 24-30 (Appendix B) 9. Adequate hepatic function, defined as AST and ALT <5 x upper limit of normal (ULN); serum bilirubin and alkaline phosphatase <5 x ULN, or considered not clinically significant as per the clinical PIs discretion (e.g. Gilbert's or indirect hyperbilirubinemia) or felt to be due to underlying disease. 10. Adequate renal function, defined as creatinine clearance>60 ml/min 11. Able to provide written informed consent 12. Agree to practice birth control during the study 13. Adequate cardiac function as indicated by New York Heart Association (NYHA) classification I or II AND left ventricular ejection fraction of ≥35% (by cardiac ECHO or MUGA) and adequate pulmonary function as indicated by room air oxygen saturation of ≥92%. 14. Expected survival >12 weeks 15. Negative urine or serum pregnancy test in females of child bearing potential at study entry and again within 48 hours' prior lymphodepleting chemotherapy. 16. Patients with prior blinatumomab treatment require repeat biopsy post-blinatumomab treatment that demonstrates CD19 or CD20 positive disease. 17. Meet criteria for regarding fertility and contraception detailed in section 4.4 18. Central line access will be required for CAR-20/19-T cell infusion.
Exclusion Criteria	1. Positive beta-HCG in female of child-bearing potential defined as per table 1. 2. Patients with known systemic allergy to bovine or murine products. 3. Known prior positive serology for human anti-mouse antibody (HAMA). 4. Confirmed active human immunodeficiency virus (HIV), Hepatitis B or C infection. 5. History of significant autoimmune disease OR active, uncontrolled autoimmune phenomenon: such as systemic lupus erythematous,

	Wegner's glomerulonephritis, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura (AIHA, ITP) requiring steroid therapy defined as >20 mg of prednisone or equivalent daily. 6. Presence of ≥grade 3 non-hematologic toxicities as per CTCAE version 5.0 from any previous treatment unless it is felt to be due to underlying disease. 7. Concurrent use of investigational therapeutic agents or enrollment on another therapeutic clinical trial at any institution. Minimum of ≥4 weeks required from administration of any other investigational agents on other clinical trials prior to enrollment on this CAR-T protocol. 8. Refusal to participate in the long-term follow-up protocol 9. Patients with active CNS involvement by malignancy on MRI or by lumbar puncture. a. Patients with prior CNS disease that has been effectively treated will be eligible providing treatment was >4 weeks before enrollment and a remission documented within 8 weeks of planned CAR-T cell infusion by MRI brain and CSF analysis. 10. Previous recipients of allogeneic hematopoietic stem cell transplantation (AHCT) are excluded if they are <100 days' post-transplant, have evidence of active graft-versus-host-disease (GVHD) of any grade, or are currently on immunosuppression. 11. Previous CAR-T cell therapy directed at either CD19 or CD20 within 100 days of planned CAR-20/19-T cell infusion (does not include re-enrollment) a. Patients with prior CAR-T treatment against CD19 or CD20 must have repeat biopsy confirming a minimum of 5% CD19 or CD20 positivity by immunohistochemistry or flow cytometry 12. Anti-CD20 antibody treatment within 4 weeks of cell infusion 13. Anti-CD19 antibody treatment within 4 weeks of cell infusion 14. Cytotoxic chemotherapy other than lymphodepletion within 14 days of CAR-T cell infusion 15. Cytotoxic chemotherapy treatment within 14 days or steroid treatment (other than replacement dose steroids) within 7 days prior to apheresis collection for CAR-T cells 16. Patients post solid organ transplant who develop high grade lymphomas or leukemias 17. Concurrent active malignancy other than basal or squamous cell carcinomas of the skin
Study Rationale	The study is divided into two phases. Phase 1 and Phase 1b. **Phase 1:** To evaluate a novel closed processing method for the production of the first dual CD20 and CD19 CAR-T cell and determine the safety of administration of these cells as part of a first in human study consisting of 2 cohorts; dose escalation and dose expansion. The CAR-T cells were given over 2 days.

	Phase 1b: To determine the safety of single dose (unfractionated CAR-T cell infusion at 2.5 x 10^6 cells/kg
Primary Objectives	1. Determine the safety (dose level) of CAR-20/19-T cell administration in relapsed refractory CLL/SLL and B-cell NHL 2. Determine the feasibility to manufacture CAR-20/19-T cells locally from patient apheresis products using the CliniMACS Prodigy Cell processing device
Secondary Objectives	1. Determine the anti-tumor responses as measured by response rates. 2. Describe the duration of response for responding patients 3. Determine persistence of CAR-20/19-T cells 4. Determine the effects of CAR-20/19-T infusion on non-neoplastic CD19 & CD20 B cells in vivo 5. Determine if cellular or humoral host immunity develops *against* CAR-20/19-T cells 6. Evaluate MRD using molecular technologies
Endpoints	1. Ability to manufacture CAR-20/19-T cells from patient apheresis products using the CliniMACS Prodigy processing device (Miltenyi Biotec). The number T cell lines that do not meet criteria for T cell purity, total transduced T cell content, viability, and sterility will be determined. 2. Occurrence of adverse events, defined as either CRS related Grade 3/4 toxicity or other NCI CTCAE version 5.0 non-hematologic ≥ grade 3 signs/symptoms, laboratory toxicities and clinical events that are possibly, probably or definitely related to study treatment at any time from the infusion until day +28 post CAR-T infusion
Study Design	This is a phase 1/1b, interventional, single arm, open label, treatment study designed to evaluate the safety and feasibility of infusion of CAR-20/19-T in patients with B cell malignancies that have relapsed after prior therapies and are not candidates for curative intent standard therapy. **The study is divided into two phases. Phase 1 and Phase 1b**. **Phase 1:** consists of 2 cohorts; dose escalation and dose expansion. The CAR-T cells were given over 2 days. In the phase 1 portion, there will be a dose escalation cohort to determine the safe CAR-20/19-T cell dose in patients with CLL/SLL and NHL. Once the desired dose has been identified there will be a 6 patient dose expansion phase at the specified dose level. **Phase 1b:** CAR-T infusion given as a single dose. In the Phase 1b portion of the study, we will test the safety of unfractionated CAR-T cells utilizing the safe dose identified in the phase 1 portion (2.5 x 10^6 cells/kg).
Study Agent Description	CAR-20/19-T cells will be administered either fresh or thawed after cryopreservation by IV injection. Patients will receive one of four dose levels of CAR-20/19-T cells based on our dose escalation design (Section 6). Cells will be given over 2 days, 30% on Day 0

	and 70% on Day 1 in the Phase 1 portion and in Phase 1b as a single infusion on Day 0. Cell dose will be maxed at 80 kg. **<u>Phase 1</u>** **Dose Level -1:** 1 x 10^5 CAR-20/19-T cells/kg **Dose Level 0:** 2.5 x10^5 CAR-20/19-T cells/kg *(starting dose level)* **Dose Level 1:** 7.5 x10^5 CAR-20/19-T cells/kg **Dose Level 2**: 2.5 x10^6 CAR-20/19-T cells/kg *(goal cell dose)* **<u>Phase 1b</u>** **Expansion Dose Level:** 2.5 x 10^6 cells/kg (single infusion)
Number of Subjects	A maximum of 24 patients will be enrolled on this Phase 1/1b study.
Subject Participation Duration	Subjects will be followed for up to 2 years post infusion of the CAR-20/19-T cells
Duration of Follow up	Patients will be followed for up to 2 years post infusion of CAR-20/19-T cells infusion as part of this protocol and then placed on a long-term follow-up protocol that will follow patients for up to a total of 15 years post CAR-T cell infusion
Estimated Time to Complete Enrollment:	18-24 months
Statistical Methodology:	This is a phase 1/1b, interventional single arm, open label, treatment study designed to evaluate the safety and feasibility of infusion of CAR-20/19-T cells. In the phase 1 portion the study will utilize a 3+3 dose escalation design in NHL & CLL/SLL followed by a 6 patient expansion cohort. In the Phase 1b portion, 9 patients will be treated at the safe dose from the Phase 1 portion (2.5 x 10^6 cells/kg).and will receive a single infusion rather than fractionated dose.
Safety Assessments	Safety will be determined by assessments of cytokine release syndrome (CRS) using the Lee et al. grading scale [1] and adverse events via NCI CTCAE version 5.0. Dose limiting toxicities (DLT) will be monitored for the first 28 days after CAR-20/19-T cell infusion as per Table 1. DLT will be defined a grade 3-4 non-hematologic toxicity possibly, probably, or definitely related to the infusion of CAR-20/19-T cells. All deaths (grade 5 toxicity) in the first 28 days felt to be either possibly, probably, or definitely related to CAR-20/19-T cell therapy will be considered a DLT. CRS related DLTs and hematologic DLT are defined in the protocol (section 3.4)
Efficacy Assessments	<u>CLL/SLL & B-cell NHL at Day +28 (+/- 3 days)</u> PET/CT or CT neck/chest/abd/pelvis + BM Aspirate and Biopsy

Unique Aspects of this Study	This is the first phase 1/1b study to evaluate the safety and efficacy of a CAR-T cell therapy directed against two B cell antigens and produced under GMP conditions using the closed system CliniMACS Prodigy device in B cell malignancies.

FIGURE 1: STUDY SCHEMA

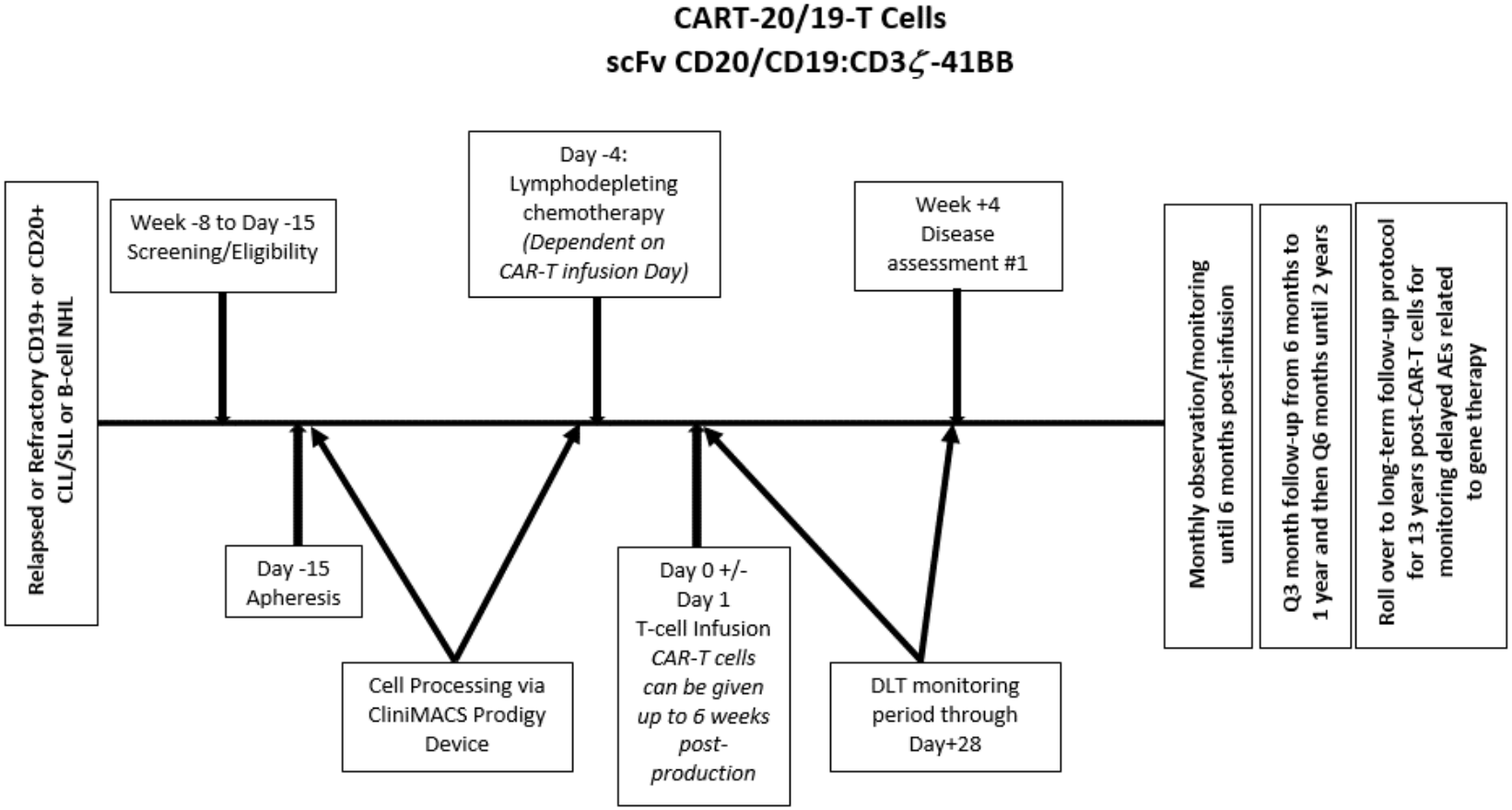


TABLE 1: SCHEDULE OF EVENTS

	Week -8 to Day -15	Day -15	ϕ2 to 4 days prior to day -4	♦Day -4 LDP	§Day 0 +6 weeks	Day +1	**#Day** +2 to Day +7	Day +10	Day +14	Day +21	Day +28	Day +60	Day +90	Day +120	Day +150	Day +180	Day +270	Day +365	$Follow up (Year 1-2)
Visit Window	Screening/ Enrollment	Apheresis	+/- 1 day	+/- 5 days	CAR-20/19-T cells infusion			+/- 1 day	+/- 2 days	+/- 2 days	+/- 3 days	+/- 7 days	+/- 7 days	+/- 7 days	+/- 7 days	+/- 14 days	+/- 14 days	+/- 1 month	+/- 1 month
Signed Inform Consent	x																		
Apheresis		x																	
CAR-20/19-T cells infusion[1]					x	x[1]													
Confirmation of Diagnosis of CD19 or CD20 positive B-cell NHL or CLL/SLL[2]	x																		
Recent History and Physical Examination [3]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Karnofsky performance status	x				x						x							x	
Vitals[4]	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Concomitant Medication & Evaluation of Adverse Events	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Bone Marrow Transplant Markers [5]	x				x						x		x			x		x	x
Tumor Measurements by PET/CT, CT Scans[6]	x		x[6]								x					x		x	x
ECG[7]	x				x						x								
ECHO/MUGA	x																		
Lumber Puncture [8]	x		x[8]																
MRI Brain[9]	x		x[9]																
Bone Marrow Biopsy & Aspirate [10-11]	x										x					x		x	x
CBC and Differential	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Comprehensive Metabolic Panel[12]	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
LDH/Uric Acid	x	x		x	x	x	x	x	x	x	x		x			x		x	x
Quantitative Immunoglobulins	x				x						x	x	x	x	x	x	x	x	x

	Week -8 to Day -15	Day -15	ϕ2 to 4 days prior to day -4	♦Day -4 LDP	§Day 0 +6 weeks	Day +1	**#Day** +2 to Day +7	Day +10	Day +14	Day +21	Day +28	Day +60	Day +90	Day +120	Day +150	Day +180	Day +270	Day +365	$Follow up (Year 1-2)
Visit Window	Screening/ Enrollment	Apheresis	+/- 1 day	+/- 5 days	CAR-20/19-T cells infusion			+/- 1 day	+/- 2 days	+/- 2 days	+/- 3 days	+/- 7 days	+/- 7 days	+/- 7 days	+/- 7 days	+/- 14 days	+/- 14 days	+/- 1 month	+/- 1 month
Ferritin/CRP/ESR	x				x	x	x	x	x	x	x								
PT/INR/PTT/D-Dimer/Fibrinogen	x				x	x	x	x	x	x	x								
TSH/Free T4	x										x							x	
Lipid Panel	x										x								
Urinalysis	x		x								x								
Beta-2-Microglobulin	x										x								
Autoimmune Screen (ANA)	x																		
Pregnancy Test (serum or urine) [13]	x		x																
Infectious Disease Markers [14]	x																		
Respiratory Virus Panel [15]			x																
Creatinine Clearance [16]	x																		
Central Line[17]			x																
Research Studies																			
T Cell Persistent Studies [18]					x	x	x	x	x	x	x		x			x		x	x
Q-PCR for integrated vector DNA[19]					x						x		x			x		x	x
Presence of replication competent virus by Q-PCR for VSV-g[20]					x						x		x			x		x	x
Cytokine Studies) [21]					x	x	x	x	x	x	x								
Neurological evaluations[22]	x					x	x				x								
T-cell repertoire Diversity Studies[23]		x		x			x				x								
PaxGene RNA Tubes[25]		x							x		x		x						
Tryptophan Metabolites[25]		x							x		x		x						
Quality of Life Studies[26]		x							x		x		x			x		x	x

Footnotes:

- ♦ Start of Lymphodepleting Chemotherapy (LDP) Theses assessments should be completed daily during each day of LDP unless otherwise specified. Start date of LDP chemotherapy will be determined on the date of CAR-T cell infusion.
- ϕ Pre-lymphodepletion visit will be based on date of CAR-T cell administration and hence can move forward by up to 6 weeks

§ CAR-20/19-T cells may be given fresh after manufacturing without cryopreservation or can be cryopreserved and given up to 6 weeks after production
# Daily from Days +1 through Day +7 unless otherwise specified in Section 5.4.6
$ Clinical Visits every 6 months (+/- 1 month) from Year 1 to Year 2
1- Split-dosing of CAR-T cells through Phase 1 escalation/expansion. Single dosing of CAR-T cells in Phase 1b
2- Patients will need documentation of relapsed or refractory B cell malignancies. CD19 or CD20 positivity ≥5% by IHC or Flow cytometry required on one biopsy. Please see eligibility criteria for specific requirements for each patient.
3- History and height only required at screening. Physical exam, weight at all other time points
4- Vitals should include Blood Pressure, Temperature, Respiratory rate, Pulse & Pulse Ox. On the days of CAR-20/19-T cell infusion, vitals should be done prior to infusion, at the end of infusion, and every 15 minutes thereafter for a minimum of one-hour post infusion.
5- Absolute numbers of CD3+ T cells, CD4+ T cells and CD8+ T cells should be assessed before scheduling for apheresis collection to determine protocol eligibility. Minimum of ≥50 CD3+ T cells/mm^3 required, with a mixture of both CD4+ and CD8+ T cells.
6- Diagnostic imaging is required at baseline (within 8 weeks of CAR-20/19-T cell infusion) and at 1 month post-cell infusion (PET/CT preferred). Diagnostic CT scans of the neck/chest/abdomen/pelvis must be performed if PET/CT scans cannot be obtained at baseline due to timing constraints, insurance or reimbursement issues, or other reasons. Either PET/CT or diagnostic CT scans are required to document response. If >8 weeks' elapse from date of imaging and planned T-cell infusion, repeat imaging will be required. For follow-up patients with get either PET/CT or diagnostic CT scans at Day +28, 6 months, and 1 year. For year 1-2 scans will be done yearly.
7- ECG to be performed prior to CAR-20/19-T cell infusion on Day 0
8- Lumbar Puncture with CSF analysis by cytology and flow cytometry is required at screening/eligibility for all patients with prior history of CNS disease. If >8 weeks elapse from last LP and planned CAR-20/19-T cell infusion, a repeat LP will be required in patients with a history of CNS disease and who exhibit CNS symptoms.
9- MRI Brain will be performed only for patients with history of prior CNS disease or signs/symptoms of active CNS disease. MRI will be repeated if >8 weeks elapse between previous MRI and scheduled CAR-20/19-T cell infusion.
10- Patients should have a staging bone marrow biopsy and aspirate ≤ 8 weeks prior to CAR-20/19-T cell infusion. If >8 weeks' elapse, a repeat biopsy will be indicated.
11- All patients will have a repeat marrow at Day +28 post-CAR-T cell infusion. Beyond Day +28, for patients with B-cell NHL ongoing marrow biopsy core and aspirate at 6 months, 1, year, and 2 year is indicated only if positive at baseline. Patients with CLL/SLL are required to have BM biopsy at all specified timepoints (6 months, 1 year, 2 year). Bone marrow biopsy and aspirate should also be obtained at time of suspected relapse. If baseline marrow is positive only by flow cytometry, the aspirate and flow cytometry should be repeated.
12- Comprehensive Metabolic Panel: Albumin, Alkaline Phosphatase, AST, Bicarb, Bili T, Calcium (total), Chloride, Creatinine, Glucose, Potassium (K), Sodium (NA), Protein Total, BUN, ALT.
13- Within 48 hours prior to the start of lymphodepleting chemotherapy. Definition of female of childbearing potential(FCBP): A FCBP is a sexually mature female regardless of sexual orientation or whether she has undergone a tubal ligation who: 1) has not undergone a hysterectomy or bilateral oophorectomy or 2) has not been naturally postmenopausal (amenorrhea following cancer therapy does not rule out childbearing potential) for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).
14- Patients must be tested for HIV, Hepatitis B surface antigen/antibody and core antibody, and HCV within 8 weeks prior to CAR T cell infusion. If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed. Repeat testing is NOT required.
15- Viral Respiratory NAAT will be done 2-4 days prior to initiation of lymphodepleting (LDP) chemotherapy if patients exhibit symptoms concerning for viral infection (cough, cold, fever). In the setting of a positive result, initiation of LPD chemotherapy and CAR-T treatment will be delayed until resolution of symptoms.
16- Calculate Creatinine (by Cockcroft-Gault) at screening.
17- Central Line is required prior to initiation of LDP chemotherapy.
18- T-cell persistent studies: baseline sample to be drawn prior to CAR-20/19-T cell infusion on Day 0 followed by Day +1, +7, +10, +14, +21, +28 and then per calendar above. If there are two consecutive negative tests—**this will not need to be repeated.** Additional samples (blood, CSF, ascites, tissue, etc.) for T-cell persistence can be performed per the investigators discretion as felt to be clinically indicated.
19- Q-PCR for Integrated Vector DNA: Will be done prior to infusion then at Day +28, 3 months, 6 months, 1 year, 1.5 years, 2 years. If there are two consecutive negative tests—**this will not need to be repeated.**

20- Replication competent lentivirus (RCL) will be monitor by Q-PCR for VSV-G (Vesicular stomatitis virus-G) which is a surrogate for RCL. Will be done prior to infusion then at Day +28, 3 months, 6 months, 1 year, 1.5 years, 2 years. Testing will continue until Q-PCR for integrated vector DNA is negative for two consecutive timepoints.
21- At minimum, will include assays for the following cytokines: IFN-Ɣ, IL10, IL6 and TNFα. Additional cytokines will be monitored at discretion of investigators Cytokines will be checked on Day 0, +1, Day +7, Day+10, Day+14, Day+21, Day+28. Additional samples may be taken if indicated based on clinical presentation.
22- Given concern for neurotoxicity with CAR-T cells, neurological evaluation will be done at baseline and on Day+1, Day+7 and every third day beyond if the patient develops neurological toxicity. For patients without neurological toxicity after CAR-T therapy, re-evaluation will occur on Day+28. Neurological Assessment Tool is found in Appendix B.
23- T-cell receptor diversity studies. Sample 1 at Week -4 will be from apheresis product. Sample 2 will be from the CAR-T cell product. Sample 3 and 4 will be a peripheral blood sample (10 ml) on Day +7 and Day +28
24- Pax Gene tubes will be collected and stored until sent for analysis as described in Section 11.2 until disease progression
25- Red top tube will be collected at the noted time points and delivered to Dr. Hillard's MCW lab for analysis until disease progression
26- Quality of Life studies until disease progression

VISITS REQUIRED AT MCW: All visits through Day+28 will be required to occur at MCW. For responding patients, beyond Day +28, patients will have required visits at MCW on Day +90 (+/- 7 days), Day+180 (+/- 14 days), Day 365 (+/- 1 month), +1.5 years (+/- 1 month) and 2 years (+/- 1 month). For timepoints not falling on those dates, local oncology evaluation will suffice, and records will be obtained accordingly.

For patients who progress, follow-up will be as per Section 5.4.7

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AAHRPP	Association for the Accreditation of Human Research Protection Programs
AE	Adverse Event
ALC	Absolute Lymphocyte Count
ALL	Acute Lymphoblastic Leukemia
ALP	Alkaline Phosphatase
ANA	Anti-Nuclear Antibody
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical (Classification System)
BCW	Blood Center of Wisconsin
BUN	Blood Urea Nitrogen
CAR	Chimeric Antigen Receptor
CBC	Complete Blood Cell (count)
Cl	Chloride
CLL	Chronic Lymphocytic Leukemia
Cr	Creatinine
CR	Complete Response
CRC	Clinical Research Coordinator
CRF	Case Report Form
CRP	C-Reactive Protein
CRS	Cytokine Release Syndrome
CSF	Cerebral Spinal Fluid
CT	Computerized Tomography
CTCAE	Common Terminology Criteria for Adverse Events

CTEP	Cancer Therapy Evaluation Program
CTMS	Clinical Trial Management System
DFS	Disease-Free Survival
DIC	Disseminated Intravascular Coagulation
DLT	Dose Limiting Toxicity
DLBCL	Diffuse Large B cell Lymphoma
DSMB	Data and Safety Monitoring Board
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
EBV	Epstein-Barr Virus
ECG	Electrocardiography
ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
ESR	Erythrocyte Sedimentation Rate
FACT	Foundation for the Accreditation of Cellular Therapy
FCBP	Female of Childbearing Potential
FDA	Food and Drug Administration
FH	Froedtert Hospital
FT4	Free Thyroxine
GCP	Good Clinical Practice
HAMA	Human Anti-Mouse Antibody
HBeAg	Hepatitis B “e” Antigen
HBV	Hepatitis B Virus
HCT	Hematocrit
HCV	Hepatitis C Virus
HGB	Hemoglobin

HIV	Human Immunodeficiency Virus
HLH	Hemophagocytic Lymphohistiocytosis
ICH	International Conference on Harmonization
IHC	Immunohistochemistry
IND	Investigational New Drug Application
IP	Investigational Product
IRB	Institutional Review Board
iwCLL	International Workshop on Chronic Lymphocytic Leukemia
IV	Intravenous
IVIG	Intravenous Immunoglobulin
K	Potassium
LDH	Lactate Dehydrogenase
LDP	Lymphodepleting
LP	Lumbar Puncture
LPL	Lymphocyte Propagation Laboratory
LFT	Liver Function Test
MAS	Macrophage Activation Syndrome
MCWCC	Medical College of Wisconsin Cancer Center
MedDRA	Medical Dictionary for Regulatory Activities
MMSE	Mini-mental Status Exam
MNC	Mononuclear cells
MRI	Magnetic Resonance Imaging
MUGA	Multigated Acquisition Scan
Na	Sodium
NAAT	Nucleic Acid Amplification Test
NCI	National Cancer Institute

NHL	Non-Hodgkin's Lymphoma
ORR	Overall Response Rate
PET/CT	Positron Emission Tomography-Computed Tomography
PD	Progressive Disease
PO	Per os (by mouth, orally)
PR	Partial Response
QOL	Quality of Life
Q-PCR	Quantitative Polymerase Chain Reaction
RBC	Red Blood Cell (count)
RCL	Replication Competent Lentivirus
SAE	Serious Adverse Event
SD	Stable Disease
SLL	Small Lymphocytic Lymphoma
SRC	Scientific Review Committee
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal
UP	Unanticipated Problem
UPIRSO	Unanticipated Problems Involving Risks to Subjects or Others
VSV-G	Vesicular Stomatitis Virus-G
WBC	White Blood Cell (count)
WM	Waldenstroms Macroglobulinemia

1 INTRODUCTION

1.1 Background

CD19 and CD20 are antigens expressed on both healthy B cells and a multitude of B cell-derived hematological malignancies including non-Hodgkin's lymphomas (NHL), acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL). An estimated 162,000 new cases of hematological malignancies were expected to be diagnosed in 2015.[2]

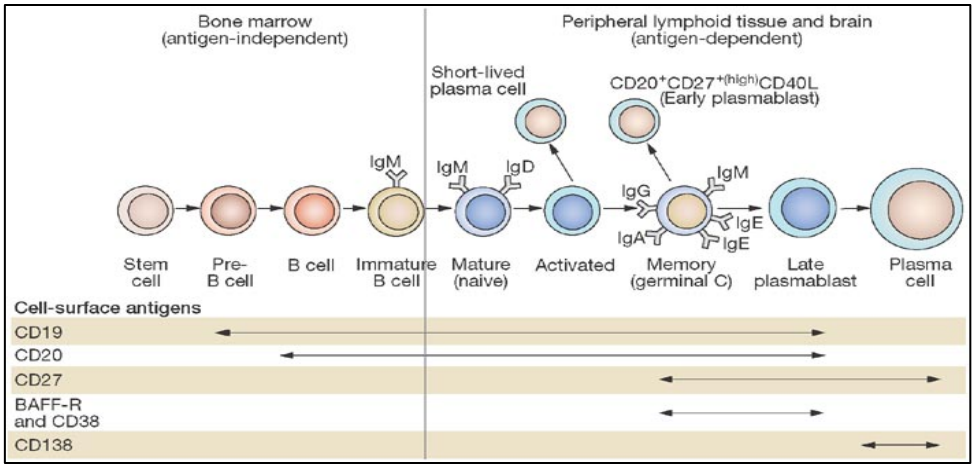
CD19 and CD20 are both highly attractive targets for B cell neoplasm immunotherapy. CD19 is expressed by most B cell malignancies including NHL, ALL, CLL, hairy cell leukemia, and a subset of acute myelogenous leukemia.[3] CD19 is a 95kDa glycoprotein present on the B cell surface from early development until differentiation into plasma cells. It is a member of the immunoglobulin superfamily and a component of a cell surface signal transduction complex that regulates signal transduction through the B cell receptor. CD19 expression is restricted to B lineage cells and is not expressed by pluripotent blood stem cells or on most other normal tissues.[4, 5] Several clinical trials demonstrated effectiveness of CD19 chimeric antigen receptor (CAR) modified T cells in patients with B-cell ALL, CLL/SLL, or B-cell NHL. [6-10]

CD20 is a non-glycosylated 33 to 37 kDa phosphoprotein that is expressed on both normal and malignant B cells. It normally functions as a component of signal transduction in growth regulation of B-lymphocytes. CD20, like CD19, is a cell surface receptor restricted to B cell precursors and mature B cells but is lost following plasma cell differentiation.[11, 12] Monoclonal antibodies against CD20 are effective in treating B cell malignancies. Unlike CD19 CAR, CD20 CAR-T therapies are in early stages of development but have shown significant promise in some clinical trials.[13, 14]

The primary advantage of adoptive immunotherapy using CAR-T cells compared to alternative modalities lies in the ability of T cells to expand and persist upon antigen binding of the target.[15] Persistence of CAR-T cells in patients with refractory NHL and ALL has correlated with sustained progression free survival likely due to the long-term surveillance these cells provide against recurrent malignancy.[6] However, despite encouraging results, not all patients respond to this treatment and even those who have achieved remission may relapse. An important mechanism of relapse to both targeted monoclonal antibody treatment and CAR-T therapy was shown to be downregulation of the targeted receptor allowing tumor cells to escape destruction.[7, 16] Given the overlap of CD19 and CD20 expression on B cells **(Figure 2)** we speculate that simultaneous targeting of these two separate B cell receptors may result in a more complete B cell ablation and a reduced risk of tumor cell escape.

In this Phase 1/1b study we first hope to demonstrate the feasibility of manufacturing CAR-T cells expressing scFv specific for CD20-CD19 (CAR-20/19-T) in a completely closed system using the CliniMACS Prodigy device and then determine the safety of this dual targeted CAR in a first-in-human study of patients with relapsed and refractory B cell malignancies. Secondary outcomes will include response rates, and observed toxicities of the treatment, specifically the development of cytokine release syndrome (CRS)[7], an inflammatory storm that has been seen with previous CAR-T therapies.

Figure 2: B-cell maturation and markers of differentiation



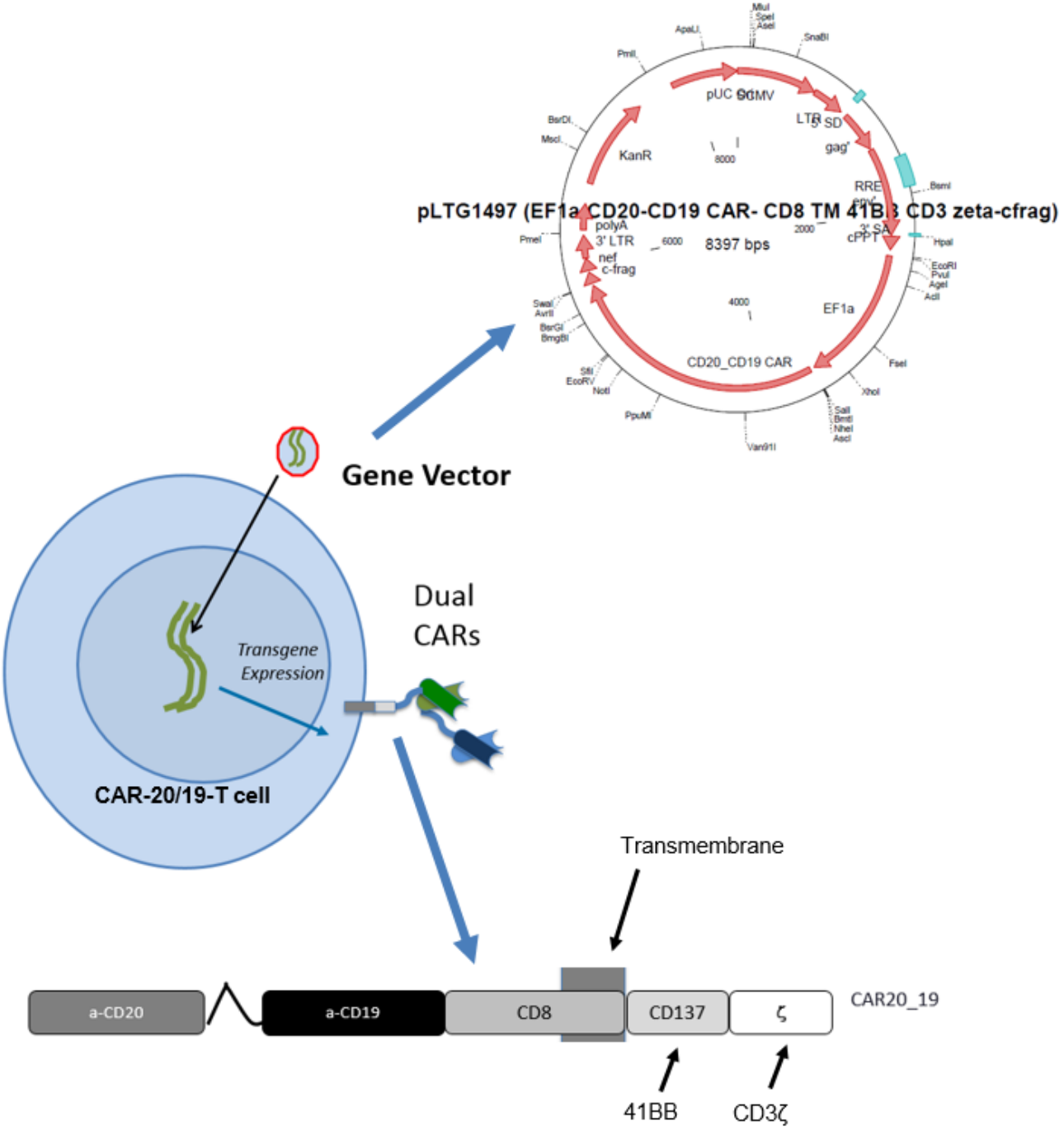
1.2 Investigational Agent(s)

The investigational agent in this protocol is the CAR-20/19-T cells. The CAR-T cells will be produced in the Lymphocyte Propagation Laboratory (LPL) under an IND to be submitted to FDA prior to trial initiation. The Cell lines will be produced as follows: A non-mobilized mononuclear cell (MNC) apheresis product (MNC, Apheresis) will be obtained from the patient approximately 4 weeks before infusion. The MNC, Apheresis product will be washed with buffer to remove platelets and plasma, treated with a combination of antibodies to CD4 and CD8 that have been conjugated to paramagnetic particles and enriched for CD4+ and CD8+ T cells. Minimally 1.0 x 10^8 enriched cells will be activated using Miltenyi TransACT reagent, which consists of nanoparticles conjugated to CD3 and CD28. The cells will be incubated for 24 hours at 37ºC and 5% CO2 prior to transduction. The activated cells will then be incubated with a lenti-virus vector that will introduce a chimeric antigen receptor that will externally express antibody binding regions to CD19 and CD20, with transmembrane signaling domains from 4-1BB and CD3ζ **(Figure 3)**. The cells will be further cultured in medium containing interleukin-2, fed, and expanded within the Prodigy culture chamber to numbers suitable for clinical use. After 13-14 days of expansion in culture the cells will be washed and tested for expression of the CD20/CD19 antibody and for function using cytotoxicity and IFN-Ɣ production assays in response to B cells prior to infusion. The lines will undergo further testing for sterility, viability, average copy number/cell, VSVg qPCR, and for relative subsets include central memory, effector memory, and regulatory T cells. Processing prior to the final preparation for either immediateadministration or cryopreservation will be performed in a closed and sterile system using GMP grade reagents and materials obtained from Miltenyi Biotec within the ISO 7 environment of the LPL. Copy number and VSVg qPCR will be tested by Lentigen Technology.

<u>Closed Processing Using the CliniMACS Prodigy Device</u>

The ability to produce CAR-T cell lines suitable for clinical use requires manufacturing processes that are reproducible, scalable and that maintain sterility. Most CAR-T cell trials have involved complex manual processing procedures that include: enrichment and/or depletion of cellular subsets from starting cells, activation and exposure to viral vectors to introduce the chimeric antigen receptors, and cell expansion steps that may require weeks in culture. There have been attempts to automate individual steps, or to close the system.[17] However, the CliniMACS Prodigy device (Miltenyi Biotec) is the first computer-controlled unit that automates each of the required processing steps while in a completely closed system.[17, 18] The Prodigy device has been approved for use in cell separation procedures and for the selection and culture of antigen reactive T cells.[19, 20] We will use the CliniMACS Prodigy for the automated, closed system processing of the CAR-20/19-T cell line to be used in this study.

1.2.1 Figure 3—Gene Map and Animation of the CD20/CD19 Vector



1.3 Preclinical Data

A wealth of preclinical studies demonstrates the effectiveness of engineered T cells against CD19 and CD20 positive malignancies. While the construction and the vector (gammaretroviral vectors vs lentiviral vectors) used to create CAR T cells has varied, the incorporation of a co-stimulatory signaling domain in 2nd generation CARs with either CD28 or 4-1BB (CD137) increases the potency of these cells.[21, 22] Lentiviral vectors have been favored due to their ability to transduce non-dividing and dividing cells, insert a large genetic segment and allow for long-term transgene expression.[23-25] The pre-clinical data supporting the efficacy of CAR cells in xenograft models is published [26-28] and more recently the feasibility of the production of a dual antigen CAR-20/19-T cell was presented.[29] This preclinical model established the in vitro effectiveness of cytotoxic tumor cell killing by dual expressing CARs and also demonstrated a decreased level of antigen escape by down-regulation when compared to single antigen targeted CAR-T cells.[30]

1.4 MCW Preclinical Data

Clinical Experience at MCW with CliniMACS Devices

The CliniMACS Prodigy device was recently introduced to the North American market as a follow-up to the CliniMACS Cell Separation System. The MCW Cell Processing laboratory has an extensive experience with the CliniMACS Cell Separation systems, having employed them since 2005 for clinical protocols involving graft engineering to deplete and/or enrich specific cellular subsets as detailed below:

Experience with CliniMACS Cell Separation Systems

Trial	Products	Target Purity	Target Recovery	CD34+ Recovery	Log T	Log B
CD34 Enrichment	21	96.7±7.6%	63.4±11.2%	63.4±11.2%	5.1±0.4	NT
CD3 Dplt	5	NA	NA	67.7±24.2%	3.2±0.3	NT
CD3 + CD19 Dplt	21	NA	NA	68.8±9.9%	3.5±0.5	3.12±0.5
CD3 Dplt + CD56 Enr	31	91.3%±6.2%	54.2±8.1%	NA	5.8±0.5	NA
TCRαβ T Dplt + CD19 Dplt	2	NA	NA	64.2±10.0%	4.25±0.3	3.0±0.2

MCW's experience with the CliniMACS Cell Separation system indicates that the device produces reproducible and highly purified target populations, rigorous depletions of unwanted populations, and consistent recoveries of >50% of desired cell subsets. The MCW Cell Therapy Lab has additional experience with use of this device in a multi-center trial conducted by the Blood and Marrow Transplant Clinical Trial Network (CTN), CTN0303 that resulted in the FDA approval of the device for clinical use. Dr. Taylor (previous Lab Dierctor) had primary responsibility for preparing the Standard Operating Procedures for the device's use and analyzing the device's performance in that trial [31] as well as her current participation on the steering committee of the ongoing CTN1301 multi-center trial. The Prodigy device incorporates the same technology as the Cell Separation System but with additional functionalities that include pre-separation steps and post-separation culture steps.

Preclinical Experience using CliniMACS Prodigy Device to produce CAR-T cells

In September 2015, Dr. Fenlu Zhu, Assistant Lab Director, underwent 2 ½ days of intensive training to learn the steps of CAR-T cell production via the Prodigy device at Lentigen Technology, Inc. Eight preclinical experiments using the CliniMACS Prodigy were conducted. Three experiments were performed using a CD19 vector with a design similar to the CD20/CD19 vector to be used clinically, 4 were using a research grade production of the vector

to be used clinically, and the 5th experiment used the GMP grade vector that will be used for the clinical protocol. For all 8 experiments cells were processed through the CD4/CD8 positive cell enrichment phase. There were two failed experiments of these first 8 studies. One failure was due to contamination of the starting cell product caused by a failure of aseptic technique during an open processing step; a step that would not be a factor for the clinical study. The second failure was device-related secondary to a software update that was not properly verified by the service technician from Miltenyi. The error prevented us from moving forward after the cell enrichment phase. The first line that was generated was compromised by the decision to start the experiment even though the source material was 72 hours old due to delays in receiving it into the laboratory. Going forward product processing will begin no longer than 48 hours after collection. The results obtained are summarized below.

CD4/CD8 Enrichment: Data for enrichment were obtained for all 8 lines and are summarized in the table below. Purity and Recovery post CD4/CD8 enrichment (N=8)

Cell Population	% of cells	% recovery
CD4+ plus CD8+	98±4	54±7
CD3+	90±10	54±9
CD3+CD4+	65±8	60±9
CD3+CD8+	26±11	52±16
CD3-CD56+	6±9	27±15

The enriched cells were highly purified for CD4+ and CD8+ cells. While CD4 is limited to T cells, CD8 is also expressed on a subset of NK cells that may or may not be CD3+. An average of 90±10% of the enriched cells were CD3+ and 6±9% were CD3-CD56+ NK cells that express a form of CD8 that has a lower antigen density than CD3+CD8+ T cells. >50% of the starting target population of CD4+ plus CD8+ cells were recovered. Importantly monocytes, which are known to suppress T cell proliferation of patient-derived material, were absent.[32, 33]

Cell Expansion: Cell lines were started using 1.0 x 10^8 starting CD4/CD8 enriched cells and were harvested on day 13. The first experiment using a buffy coat product as the starting material expanded only 5.4-fold from the start, likely due to the age of the cells at the start of processing. One line using the CD19 vector expanded 25.7-fold and the 4 lines using the CD20/CD19 vector expanded 414.5, 33.8, 24.5 and 24.8-fold. Therefore, expansion exceeded the expected results from the Lentigen training experience of approximately 20-fold

Transduction efficiency: The percentage of viable transduced cells was measured by Protein L expression, a protein unique to immunoglobulin kappa light chains.[34] Expression is shown in the table below and flow data from one representative line is shown in **Figure 4**.

Transduction efficiency as measured by Protein L expression

	Vector (MOI)	% of CD3	% of CD4	% of CD8	Copy#/All Cells
#1	CD19 (5)	41.0	42.3	26.4	1.01
#2	CD19 (5)	45.7	52.3	43.7	0.48
#3	CD20/CD19 (5)	21.0	22.7	16.5	0.23
#4	CD20/CD19 (10)	34.0	28.7	35.4	0.53
#5	CD20/CD19 (10)	56.6	57.6	60.3	0.67
#6	CD20/CD19 (10)	25.8	28.7	23.9	nd

Figure 4: Transduction efficiency representative experiment

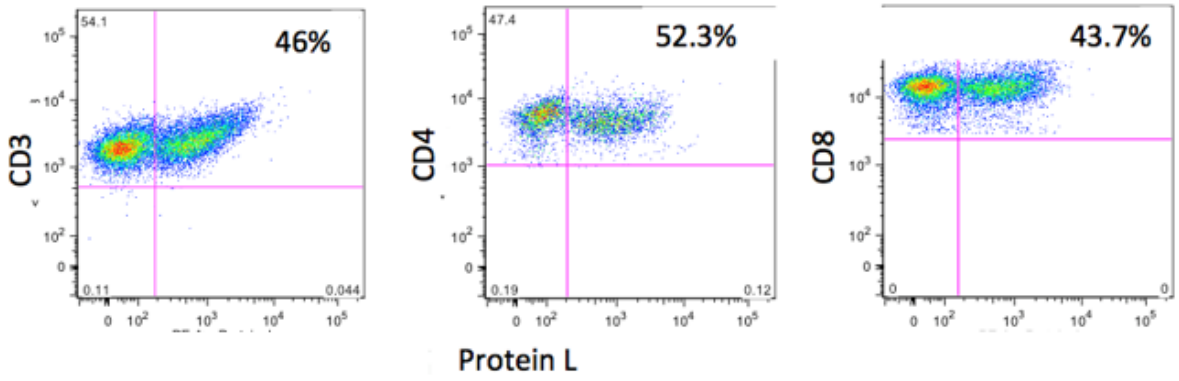


Figure 4: The cells were gated to remove debris, then to include only 7-AAD negative (viable), cells. The data are shown gated on all CD3+ cells (which were >99% of the CAR-T line) in the far left panel, gated on CD4+ T cells in the center panel, or gated on CD8+ T cells in the far right panel.

Post Expansion Phenotype: The phenotype of the expanded population changed from the starting CD4/CD8 enriched cells in that fewer CD3-CD56+ NK cells expanded, as shown most clearly for line #3. The percentage of CD8+ cells increased from the starting cells expect for Line #1 where the line was greatly enriched for CD4+ cells, possibly due to the age of the cells at the start of the culture. The goal is to have both CD4+ and CD8+ cells in the cultures, though cytotoxicity is expected to be mediated predominately by the CD8+ T cells. CD4+ CAR cells are expected to provide a helper function to the CD8+ cells secondarily to activation after exposure to the ligand bearing B cell. CAR-T cell line viability at the time of harvest was 90.1± 6.5%.

Line Designation	%CD3	%CD3+ CD4+	%CD3+ CD8+	%CD3+ CD56+	%CD3- CD56+
Line#1 Pre	99.5%	74.8%	23.9%	0.9%	0.2%
Post	99.3%	93.8%	8.5%	ND	0.6%
Line #2 Pre	97.9%	51.7%	49.5%	4.2%	2.2%
Post	98.8%	29.3%	71.8%	4.3%	1.1%
Line #3 Pre	78.7%	63.2%	16.2%	1.7%	18.4%
Post	99.5%	50.2%	47.1%	1.6%	0.5%
Line #4 Pre	91.7%	64.9%	27.9%	8.9%	1.1%
Post	97.3%	46.5%	50.5%	9.7%	0.5%
Line #5 Pre	90.1%	75.0%	16.3%	2.4%	0.6%
Post	98.5%	42.7%	59.1%	3.4%	0.9%
Line #6 Pre	90.3%	69.4%	22.5%	2.3%	1.7%
Post	99.4%	58.3%	43.7%	1.4%	0.1%

It is important that any method of CAR-T cell production should preserve a population of central memory T cells to ensure longevity in circulation. An advantage of immunotherapy using CAR-T cells is that during transduction the cells are not being expanded through antigen stimulation and thus should not become enriched for T cells with an exhausted phenotype (Terminal Memory T cells). As shown in **figure 5** below for Line#5, a population of Central Memory CD4+ and CD8+ T cells are maintained using the CAR-20/19-T cell vector manufactured both in the CliniMACS Prodigy and using plates followed by expansion in the G-Rex Device (Wilson-Wolf, Inc).

Figure 5: T cell Subset before and after CAR-T cell production in Plate vs. Prodigy

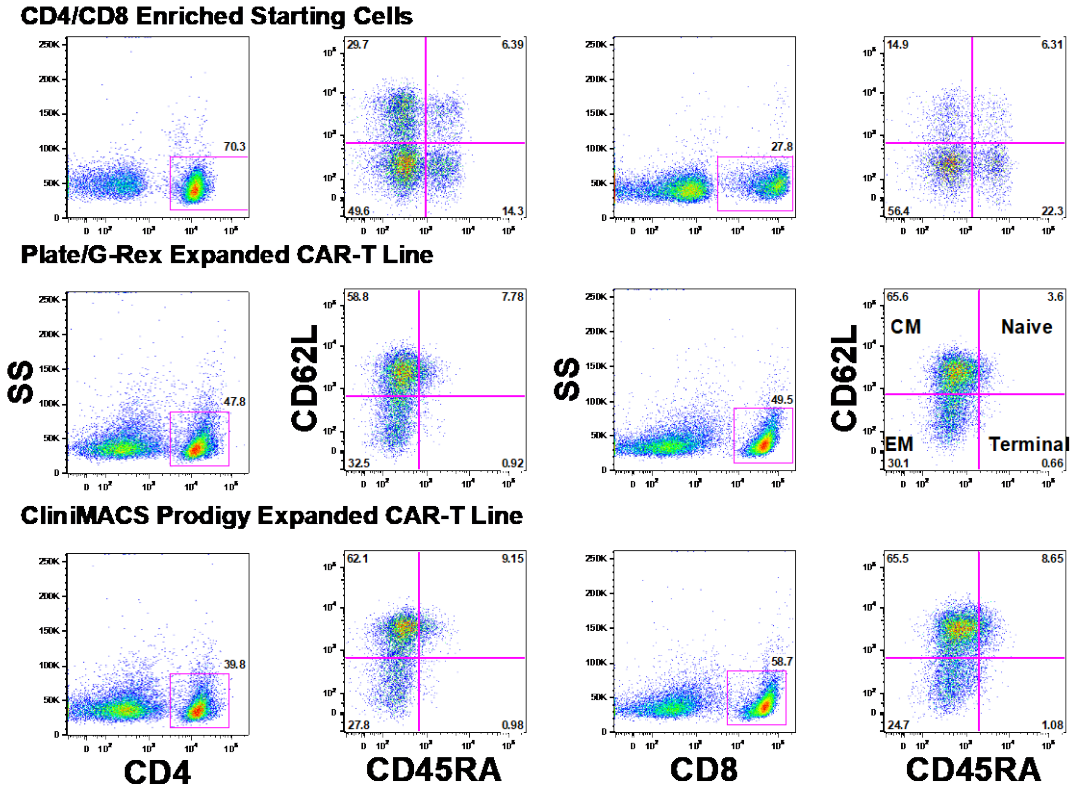


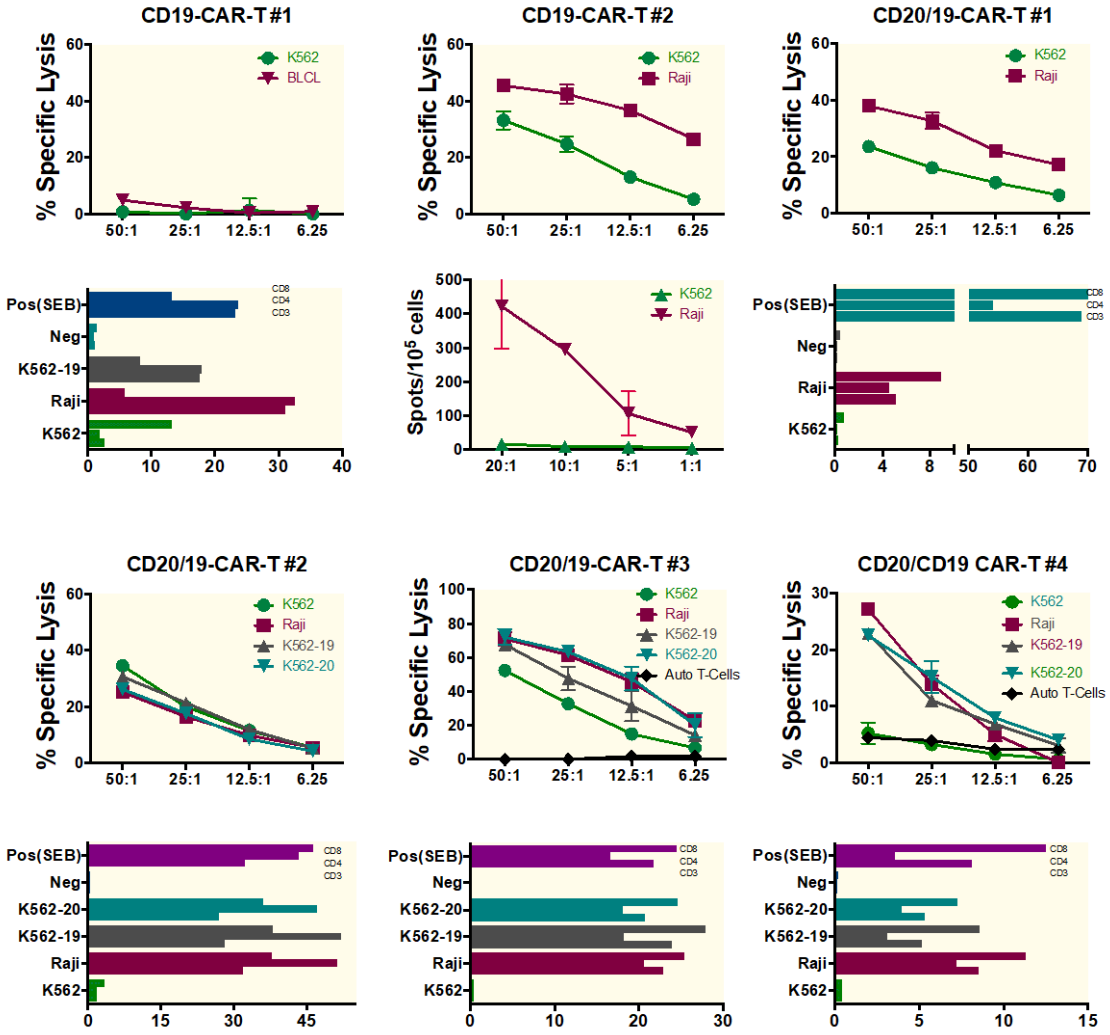
Figure 5: T cell subsets were identified by gating on CD4 or CD8 positive cells and then assessing the percentage of cells expressing CD45RA and CD62Ligand. Naïve T cells are CD45RA+/CD62L+. Central Memory (CM) T cells are CD45RA-/CD62L+, Effector Memory (EM) T cells are CD45RA-/CD62L-, and Terminal (exhausted) CD8+ T cell effectors are CD45RA+/CD62L-. Data shown are from Line #5.

Lines #1, #2, and #3 were tested for the presence of regulatory T cells (Tregs) before and after line generation. Low numbers of Tregs were present in the starting fractions, but were undetectable in the lines post expansion, indicating that our approach does not enrich for populations that might suppress tumor killing.

Effector cell function: Effector cell function was measured both in ^{51}Cr release cytotoxicity assays and by assessment of intracellular gamma interferon production after target cell co-culture. All the lines, except the Line #1 experiment, mediated lytic activity to B cell lines expressing both CD19 and CD2 (Raji or EBV transformed B cells). This was likely due to the failure of the CD8+ transduced cells to expand in this line. However, both the few CD8+ cells and the CD4+ cells produced IFN-γ after co-culture with B cell targets We obtained K562 cells that were separately transduced with CD19 and CD20 after production of Line #4 the second CD20/CD19 transduced line and could demonstrate preferable killing of both transduced targets in addition to Raji cell killing, though this line did contain a higher percentage of CD3+CD56+ cells than the other lines after expansion that resulted in detectable lysis of the parental K562 line. However, an IFN-Y intracellular cytokine assay showed cell activation only after co-culture with the transduced lines, Raji cells, but not parental K562 as shown in **Figure 6**.

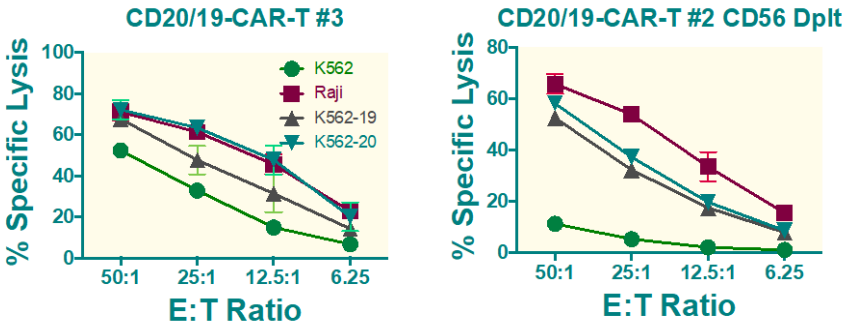
Similar results were obtained with Line #5 where there was lysis of the parental K562 line that was greatly reduced by depletion of CD56+ cells, but failed to stimulate IFN-γ production even without CD56+ cell reduction. For both Line #4 and Line #5 depletion of CD56+ cells had little effect on the killing of CD19 or CD20 expressing target cells.

Figure 6: Effector function of CAR-20/19-T cell line as demonstrated by cytotoxicity and IFN-γ production



Lysis of the K562 parental line was markedly reduced after depletion of CD56+ cells in the cultures, demonstrating that this was NK-mediated lysis, as shown in **Figure 7**.

Figure 7: Effect of CD56+ cell depletion on lysis of parental K562 targets



For all six lines that were generated, we recovered sufficient viable transduced cells to be able to meet the doses required for this phase I study.

1.5 Clinical Data

B-cell ALL

ALL is a fatal lymphoid malignancy that can develop in patients of all ages. Although children with ALL have impressive outcomes with combination chemotherapy, those with high-risk cytogenetics or a Philadelphia (Ph) chromosome do not fare as well despite intensive therapy and allogeneic transplantation. Adults have significantly worse prognosis than children.[35] Therapies for relapsed or refractory ALL are limited, and many patients are unable to tolerate the successive lines of cytotoxic chemotherapy necessary to achieve disease control.[36]

CD19 positivity is almost ubiquitous in ALL and is a marker used in diagnosis of the disease. Consequently, many of the first CD19 CAR-T cell studies involved patients with either relapsed or refractory ALL with remarkable outcomes. In a combined pediatric/adult ALL study, a complete remission (CR) was achieved in 90% of patients with relapsed ALL with sustained remissions in two-thirds of those patients.[6]

B-cell Lymphomas

There are several subtypes of B cell NHL, including Burkitt lymphoma, diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), CLL/SLL (small lymphocytic lymphoma), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), and lymphoplasmacytic lymphoma (LPL)/Waldenstroms Macroglobulinemia (WM). As these are mature B cell malignancies, CD20 expression is almost universal, and treatment with rituximab is standard of care in all subtypes.[37] Standard first line therapy includes the CD20 antibody rituximab in combination with cytotoxic chemotherapy. After relapse, salvage chemotherapy +/- autologous transplant are considerations and can cure a proportion of patients. However, treatment options for those failing second line treatment or refractory to first or second line treatment are quite limited.[38]

Similar to CD20, CD19 expression is also seen in most B cell lymphomas although the level of expression varies with histologic subtype.[39] CD19 CAR-T therapy resulted in dramatic responses in a heavily pretreated population of patients with multiple histologic subtypes of lymphoma. The initial publication for CAR-T cell therapy against CD19 demonstrated significant efficacy in 3 patients with relapsed, refractory CLL[40]. In a single arm study utilizing a CD28, CD3ζ co-stimulatory domain CAR-T cells, 8/15 patients treated with anti-CD19 CARs achieved a CR. Four of seven patients with chemo refractory DLBCL achieved CR; these were durable in 3 patients.[9] In another study, presented at ASH 2015, patients with relapsed, refractory DLBCL, MCL, and FL treated with CD19 CAR-T cells had an overall response rate of 68% with minimal toxicity.[10] More recent data from ASH 2016 from KITE, a large pharmaceutical

company evaluating CD19 CAR-T cells, demonstrated an overall response rate of 76% in patients with relapsed, refractory NHL with 39% of patients having an ongoing response at 3 months.[41] Additionally, from ASH 2016, ibrutinib refractory patients receiving CAR-T cells against CD19 were found to have an overall response rate of 76% with CRs achieved in 5 patients.[42] These above data support the efficacy of CAR-T cells in both NHL and CLL.

CAR-T Therapy

The CAR approach uses genetically programmed, patient-derived lymphocytes transfected with chimeric receptor genes to combine the effector functions of T lymphocytes with the ability of antibodies to recognize predefined surface antigens with high specificity in a non-major histocompatibility complex (MHC) restricted manner.[43] Consequently, CAR-T cells have the ability to recognize intact membrane proteins independent of antigen processing or presentation by the host's immune cells. CARs typically encode a single chain variable fragment (scFv) extracellular domain to bind tumor or virus linked to an intracellular signaling domain that mediates T cell activation. In principle, universal targeting vectors can be constructed because the scFv binds to native cell surface epitopes and bypass the requirement for MHC restriction (interaction with recipient HLA molecules on host immune cells). The scFv antibody, contains the V_H and V_L chains joined by a peptide linker of about 15 residues in length.[44] We intend to use a second generation CAR approach which provides costimulatory signaling domains 4-1BB and CD3ζ together with ***two scFv*** chains recognizing both CD19 and CD20.[15, 45] **(Figure 3)** There are several potential limitations to activity of CAR-T cells: 1) the tumor must express the target antigen on the cell surface; 2) large amounts of shed or soluble antigen could inhibit the CAR-T cells; 3) the chimeric receptor may be immunogenic, resulting in the elimination of the redirected T cells by the host immune system.

1.6 Rationale for Lymphodepletion and CAR-T cell dosing

Lymphodepletion

It is increasingly clear that adoptive immunotherapy strategies are more effective in the context of homeostatic T cell proliferation – the proliferation of naive T cells and their differentiation into memory-like T cells when total numbers of naive T cells are reduced by lymphodepleting chemotherapy. Lymphodepletion also eliminates regulatory T cells and other competing elements of the immune system that act as "cytokine sinks", enhancing the availability of cytokines such as IL-7 and IL-15.[46, 47] In this protocol we propose to transfer CAR-20/19-T cells into subjects that are rendered lymphopenic as a result of cytotoxic chemotherapy.

The optimal regimen for lymphodepletion remains unknown. Various CAR trials have utilized a combination of fludarabine, cyclophosphamide, pentostatin, or disease specific chemotherapeutic agents.[7, 9, 10, 40, 48, 49] Given the heterogeneity of previous CAR trials and using the previously published literature, we will, like most other institutions, utilize a combination of a purine analog (fludarabine) with cyclophosphamide, which is the most common lymphodepletion regimen used.[48, 49] The regimens for this trial is listed in section 6.1.

Dose Rationale

Previous literature has demonstrated a response to CAR-T cells at a variety of dose levels from 1.0-5.0 $x10^6$ cells/kg to 1.0-5.0$x10^9$ CAR-T cells as a flat, weight independent dose.[9, 10, 40] Although an optimal dose has not been determined, based on prior studies, our goal dose level is 2.5 x 10^6 cells/kg. However, due to concern for increased toxicity with a dual targeted CAR-T cell, we will start at a dose level of 2.5 x 10^5 cells/kg and dose escalate per our study design (Section 6.4) to our target dose. Based on FDA recommendations we will dose per kg for uniformity and the dose will be maxed at a weight of 80 kilograms. Recent data regarding cell

dose optimization presented at the American Society of Clinical Oncology meeting in 2016 demonstrated that administration in split-dose fashion may improve safety without altering efficacy and that a flat cell dose of 10^8 was the optimal dose level. Higher doses led to more CRS related deaths. [50, 51] Based on that data we will split our infusion during the phase 1 portion over 2 days, 30% on Day 0 and 70% on Day 1. If there is no significant toxicity during the phase 1 portion, we will transition to single day dosing in our Phase 1b portion of the study. Phase 1b will be open to enrollment once a subject has consented for the final slot on the Phase 1 portion. Our target dose was chosen on the basis of efficacy and safety data from CD19 directed CAR-T cells with similar 4-1BB and CD3ζ co-stimulatory domains.[48, 49] Given that CAR-T cell expansion occurs in vivo post infusion, there has not been clear evidence of dose response related to efficacy or toxicity.

1.7 Known and Potential Risks and Benefits

Potential Risks
Patients enrolled on this study will be exposed to a genetically engineered autologous T cell. Potential risks of the CAR-20/19-T cells include the following:

- *B cell ablation and consequent increased risk of infection*: As both healthy and malignant B cells express CD19 and CD20 antigens, B cell ablation with consequent elimination of immunoglobulin production is an expected side effect of administration of the CAR-20/19-T cells. This may be transient or permanent depending on the longevity of the CAR-T cells; it generally resolves when the CAR-20/19-T cells are cleared. Patients with persistent low levels of immunoglobulins can be given intravenous immune globulin if felt to be indicated by their treating physician.

- *Immune Reaction*: Although we are utilizing autologous T cells, it is possible that the processing will make these cells immunogenic and that patients will have an immune response directed against the scFv. If such a response occurs, it is possible that the cells will be rejected or a serious allergic reaction will occur. Thus far, such allergic reactions have not been reported in clinical studies of CAR-T cells. [6, 8, 9, 40] Additionally patients can get exacerbation of pre-existing auto-immune conditions or development of new auto-immune or neurologic conditions.

- *Transformation:* Gene transfer may result in new tumors derived from genetically modified cells, due to incorporation of viral DNA, which may dysregulate genes controlling cell apoptosis and proliferation leading to an uncontrolled malignant clone or new hematologic malignancy. Again, this is not yet reported in clinical studies of CAR-T cells in human patients, which now number greater than 100. [6, 8, 9, 40]

- *Graft-Versus-Host Disease (GVHD)*: In patients who have underwent previous allogeneic transplant and then relapse with disease and receive CAR-T therapy with allogeneic cells, one potential risk is re-activation of GVHD. This was not seen in one recent prospective study of patients receiving allogeneic CAR-T cells from their donor. [52]

- *Risk of tumor lysis syndrome (TLS) related to lymphodepleting chemotherapy or CAR-T cells:* The risk of TLS is dependent on the burden of disease and rapidity of response. There are reports of patients treated with CD19 CAR-T cells developing tumor lysis syndrome. Therefore, all patients will be closely monitored both before and after

chemotherapy and CAR-20/19-T cell infusions including blood tests for potassium, uric acid, and calcium. All subjects will receive allopurinol prophylactically. TLS resulting in renal insufficiency, or rapidly rising uric acid, or evidence of organ dysfunction will be managed with intravenous fluids and rasburicase as needed and determined by the treating physicians. Appropriate clinical therapy will be administered should any significant TLS occur.

- *Cytokine Release Syndrome (CRS)*: CRS is an inflammatory syndrome that occurs as a result of T cell activation and proliferation associated with engagement of the targeted antigen. It manifests when large number of lymphocytes become activated and release inflammatory cytokines. Its symptoms (**Figure 8**) can vary from mild flu-like complaints with fevers, anorexia, nausea/vomiting, headache, and rash to a severe inflammatory storm that can result in hypotension, multi-system organ failure, shock, and even death despite treatment with anti-cytokine therapy and steroids. [1, 53, 54] It is associated with markedly increased production of acute phase reactants such as C-reactive protein (CRP) and ferritin as well as increases in the erythrocyte sedimentation rate (ESR). The presentation of CRS is most similar with MAS (macrophage activation syndrome) or HLH (hemophagocytic lymphohistiocytosis) and is felt to be a reaction to the immune activation that occurs with antigen binding by the CAR-T cells. Diagnosis of MAS or HLH is based on the 2004 HLH criteria (Appendix C). [55]

  It is unclear if the development of CRS predicts clinical response. Initial reports of CRS in CAR-T cell clinical trials revealed dramatically increased levels of IL-6 that peak during maximal T cell proliferation. Inhibition of IL-6 binding with the IL-6 receptor antibody, tocilizumab results in a rapid and dramatic improvement in the life-threatening symptoms associated with CAR-T therapies. It is unclear if this inhibition dampens the efficacy of the modified T cells.[53, 56] Given the unique presentation of CRS associated with CAR-T cell therapy, a revised grading system has been recommended for appropriate classification of degree of toxicity. We will employ this revised grading system when evaluating patients with CRS in this study.[1]

Figure 8
Clinical Symptoms associated with CRS [1]

Organ system	Symptoms
Constitutional	Fever ± rigors, malaise, fatigue, anorexia, myalgias, arthalgias, nausea, vomiting, headache
Skin	Rash
Gastrointestinal	Nausea, vomiting, diarrhea
Respiratory	Tachypnea, hypoxemia
Cardiovascular	Tachycardia, widened pulse pressure, hypotension, increased cardiac output (early), potentially diminished cardiac output (late)
Coagulation	Elevated D-dimer, hypofibrinogenemia ± bleeding
Renal	Azotemia
Hepatic	Transaminitis, hyperbilirubinemia
Neurologic	Headache, mental status changes, confusion, delirium, word finding difficulty or frank aphasia, hallucinations, tremor, dymetria, altered gait, seizures

- *CRS related Neurological Toxicity:* Neurological toxicity is a specific risk that has been observed with anti-CD19 CAR-T cell therapy. This has manifested with symptoms such as hallucinations, delirium, aphasia, and confusion. These toxicities range from mild to severe (requiring ventilator support for airway protection).[1, 53, 57, 58] Neurological toxicity generally emerges within the first week after anti-CD19 CAR-T cell infusion, and

resolves within a few days. Similar neurological changes are observed with blinatumomab administration and CD28 mAb ligation, for which it has been speculated that neurological toxicity arises from generalized T cell mediated inflammation rather than direct toxicity mediated by T cells on CNS tissue.[56] The neurological toxicities reported with anti-CD19 CAR-T cell therapy to date have generally been medically manageable and reversible. However, recently Juno Therapeutics reported 3 deaths due to CNS toxicity as a result of their anti-CD19 CAR-T cell trial.[59] Their CAR utilizes a different signaling domain than the one proposed in this study. In addition, we will require prophylactic levetiracetam in all patients at the time of lymphodepletion chemotherapy to prevent potential neurotoxicity.

- *Infection*: Patients who receive fresh CAR-T cells are at risk of developing an infection as results of complete sterility testing will not be available until after infusion. To decrease this risk, in process testing (Appendix D) will be performed prior to start of lymphodepletion chemotherapy. Should the cultures return positive post-infusion appropriate antibiotics and infectious disease work-up will be performed.

Potential risks associated with lymphodepleting chemotherapy
A combination of fludarabine with cyclophosphamide will be utilized for lymphodepletion in all patients undergoing treatment with CAR-20/19-T cells. Potential risks associated with this combination chemotherapy include the following

- *Cytopenias*: Cytotoxic chemotherapy can result in drug-induced pancytopenia placing the patient at risks of several complications including bleeding related to thrombocytopenia, infection related to neutropenia, or fatigue/hypotension associated with anemia. Patients will be allowed to receive prophylactic transfusions as indicated in **Appendix E**. In the event of neutropenic fever, patients will be appropriately managed with broad spectrum antibiotics.

- *Tumor Lysis Syndrome:* As defined above, patients will be monitored for TLS post treatment with both lymphodepleting and CAR-20/19-T cell treatment.

- *Organ Toxicity:* Any cytotoxic chemotherapy regimen can result in organ damage as defined as hypoxia (requiring O2), an elevated creatinine, neurological toxicity, or elevations in liver function tests. These parameters will be monitored per our follow-up schedule in Table 1.

Unknown Risks
In the setting of genetic therapy and a first-in-human study there are potential unknown risks. The degree of CRS with dual antigen binding has not been previously tested; it is possible that this will lead to a more robust immune response, and therefore more severe CRS, than previously seen. Additionally, as there is limited long-term data in patients treated with CAR-T cell therapies, it is unknown if there will be treatment-related secondary malignancies or other unexpected toxicities in the future.

Potential Benefits
Patients with relapsed leukemia or lymphoma as based on the inclusion criteria have very poor outcomes with standard treatments options. Allogeneic transplant can be offered to those patients in remission but this comes with high treatment related mortality and many patients are not eligible due to active or progressive disease. Recent data published and presented at

national conferences establishes the efficacy of CAR-T cells in the treatment of relapsed, refractory leukemia and lymphoma [6, 8-10, 40] Although clinical responses are plausible, given this is a phase 1/1b study the clinical benefit of this specific CAR-20/19-T cell infusion is unknown.

2 HYPOTHESIS AND OBJECTIVES

2.1 Hypothesis

Single antigen targeted monoclonal antibodies and CAR-T therapies have significant efficacy in the treatment of patients with lymphoma and leukemia. However, not all patients respond, and those that do respond can still experience progressive disease. In the setting of monoclonal antibodies such as rituximab (CD20 targeted antibody), one method of resistance has been the downregulation of the CD20 receptor leading to tumor cell escape.[16] Similarly, the proposed mechanism of relapse in CD19 positive ALL treated with CD19 CAR-T cells has been downregulation of the CD19 receptor and development of a clonal CD19 negative population.[7] Preclinical studies have demonstrated the decreased downregulation of tumor cell antigens when treated with combined CD20/CD19 CAR-T cells.[29] We hypothesize that a dual targeted CD20/CD19 CAR-T cell treatment will be more efficacious than single antigen targeting and will decreased the risk of progression or relapse after treatment.

2.2 Primary Objectives

1. Determine the safety (dose level) of CAR-20/19-T cell administration in relapsed refractory CLL/SLL and B-cell NHL
2. Determine the feasibility of manufacturing CAR-20/19-T cells locally from patient apheresis products using the CliniMACS Prodigy Cell processing device

2.3 Secondary Objectives

1. Determine the anti-tumor responses as measured by response rates.
2. Describe the duration of response for responding patients
3. Determine persistence of CAR-20/19-T cells
4. Determine the effects of CAR-20/19-T infusion on non-neoplastic CD19 & CD20 B cells in vivo
5. Determine if cellular or humoral host immunity develops *against* CAR-20/19-T cells
6. Evaluate MRD using molecular technologies

2.4 Rationale for the Outcome Measures Selection

As a first-in-human and Phase 1 study, our primary outcome is to demonstrate both the safety and feasibility of CAR-20/19-T cell production and administration to patients. Production feasibility will be measured by our ability to perform apheresis and produce gene modified CAR-20/19-T cells that can be administered to enrolled patients. Safety will be determined by assessments of CRS using the Lee et al. grading scale [1] and adverse events via NCI CTCAE version 5.0. Response assessment will vary by specific disease subtype and will occur initially at Day +28 post-treatment (+/- 3 days) with CAR-20/19-T cells. Ongoing response assessment will continue until progression and/or death up to 2 years (see Table 1), after which patients will be transitioned to a long-term follow-up protocol.

At a minimum, the Day +28 (+/- 3 days) response assessment will include the following:

Bone Marrow Aspirate and Biopsy and either PET/CT or CT neck/chest/abdomen/pelvis

3 STUDY DESIGN

3.1 General Description

This is a phase 1/1b, interventional single arm, open label, treatment study designed to evaluate the safety and feasibility of infusion of CAR-20/19-T cells in adult patients with B cell malignancies that have failed prior therapies. The primary objective is safety of infusion and feasibility of manufacturing CAR-20/19-T cells at MCW-FH. We will monitor and describe safety, tolerability and engraftment potential of CAR-20/19-T cells in these patients. The general protocol schema is shown in **Figure 1**.

Subjects who have adequate T cells *as* per eligibility criteria will be leukopheresed to obtain a MNC apheresis product for CAR-20/19-T manufacturing. The apheresis product will be washed free of platelets and plasma and the CD4+ and CD8+ T cells will be purified from the MNC, activated through the CD3 and CD28 antigens, then transduced with the CD19 and CD20 scFv regions coupled to CD3ζ/4-1BB signaling domains using a lentiviral vector. The cells will be expanded *in vitro* washed from culture, removed from the device, and either administered to the patient within 48 hours of production or cryopreserved for later administration. In process and end of production release testing will be performed (**Appendix D** for schema) The entire manufacturing process up until removal from culture will be performed using a computer controlled program within the closed system of the CliniMACS Prodigy device.

Patients will receive CAR-20/19-T cells through a central venous catheter and then will be monitored closely with physical examinations and blood tests to assess safety, engraftment, and persistence of the CAR-20/19-T cells at regular intervals as outlined (Table 1). Response assessments will also be performed for secondary endpoint analysis as described in Section 3.5. Patients will be followed daily from Day 0 to Day +7. Then seen on Day +10 (+/- 1 day), Day +14 (+/- 2 days), Day +21 (+/- 2 days) and then on Day 28 (+/- 3 days). After Day +28 patients will be seen monthly up to 6 months, every 3 months up to year 1, and every 6 months up to year 2. Details of further follow-up are in listed in the study calendar. Following completion of this study, subjects will be enrolled onto a roll-over long-term follow-up protocol for up to an additional thirteen years to assess the diagnosis of long-term health problems, such as development of new malignancies and persistent of CAR-T cells.

3.2 Number of Subjects

A maximum of 24 patients will be treated with CAR-20/19-T cells. In the phase 1 portion an initial dose escalation cohort using 3+3 patients at the first dose level in this study. Once a safe dose level has been identified, there will be a 6 patient dose expansion cohort at the selected dose level. Once the final subject has enrolled on the Phase 1 portion of the study, the Phase 1b portion of the study will be open to accrual of 9 patients).

3.3 Primary Endpoint(s)

Primary feasibility endpoints assessed include:

Ability to manufacture CAR-20/19-T cells from patient apheresis products using the CliniMACS Prodigy processing device (Miltenyi Biotec). The number of T cell lines that do not meet criteria for T cell purity, total transduced T cell content, viability, and sterility will be determined.

Primary safety endpoints assessed include:

Occurrence of adverse events, defined as either CRS related Grade 3/4 toxicity or other NCI CTCAE version 5.0 non-hematologic ≥ grade 3 signs/symptoms, laboratory toxicities and clinical events that are possibly, probably or definitely related to study treatment at any time from the infusion until week 4. The revised CRS grading system will be used for patients who develop CRS **(Appendix G)**.[1] This will include infusion toxicity and any toxicity possibly related to the CAR-20/19-T cells including but not limited to:

a. Fevers
b. Rash
c. Hepatic dysfunction
d. Pulmonary infiltrates or other pulmonary toxicity (unrelated to concomitant infections)
e. CRS/MAS
f. Neurologic toxicity

3.4 Dose Limiting Toxicity

Dose-limiting toxicity (DLT) will be defined as a grade 3-4 non-hematologic toxicity possibly, probably, or definitely related to the infusion of CAR-20/19-T cells. All deaths (grade 5 toxicity) in the first 28 days felt to be either possibly, probably, or definitely related to CAR-20/19-T cell therapy will be considered a DLT. All Grade 4 non-hematologic toxicity felt to be either possibly, probably, or definitely related to CAR-20/19-T cell infusion will be considered a DLT. If multiple toxicities are seen, the presence of a DLT will be based on the most severe toxicity experienced. The DLT will be based on the tolerability observed during the first 28 days of treatment/observation.

CRS and neurotoxicity are possible DLTs that can occur as a result of administration of CAR-T cells. For this protocol, any grade 4 CRS related toxicity per revised CRS grading system **(Appendix G)** will be considered a DLT. In addition, patients with Grade 3 CRS toxicity that does not improve to grade 1 or 2 despite treatment with cytokine inhibition and/or immunosuppression within 7 days will also be considered a DLT. All Grade 4 neurotoxicity will also be considered a DLT and similarly any Grade 3 neurotoxicity which does not improve to grade 1 or 2 despite treatment within 7 days will also be considered a DLT. All infusional reactions ≥grade 2 lasting more than 24 hours will be considered a DLT. Lastly, while hematologic toxicity is an expected side effect of lymphodepletion, persistent Grade≥3 neutropenia that does not resolve to Grade≤2 neutropenia by Day +28 will be considered a DLT. Refractory thrombocytopenia and anemia are known potential complications among heavily pre-treated patients with hematological malignancies and lymphodepletion chemotherapy and will be managed per supportive care guidelines. While grade and toxicity will be recorded for this clinical trial the degree of thrombocytopenia/anemia will not count towards our DLT evaluation.

The first 28 days were chosen for the DLT monitoring period given the variability in the presentation of CRS, which can occur within hours to weeks after infusion [1]. Additionally, as more severe CRS tends to occur earlier after infusion compared to non-severe CRS, 28 days was felt to be an appropriate monitoring period.[6] The attribution of adverse events with respect to their relationship to the infusion of CAR-20/19-T cells will be the responsibility of the site PI.

3.5 Secondary Endpoint(s)

Secondary endpoints include assessment of the following:

1. The number of patients who have CAR-20/19-T cells manufactured but not infused due to failure to meet release criteria.
2. Disease assessment will include the following:
    - Number of patients with anti-tumor responses after CAR-20/19-T cell infusion. Since patients with a variety of histologies will be treated – response will be assessed using disease appropriate imaging/marrow and international consensus criteria most applicable to the disease (Section 10).
    - Repeat bone marrow biopsy and aspirate with evaluation of minimal residual disease *when indicated* (i.e., patients with baseline disease on bone marrow biopsy). These will be analyzed by multiparameter flow cytometry (MFC) and/or molecular analysis of immunoglobulin rearrangements, as available.
    - Duration of response in responders
    - Relapse rates and time to relapse
    - Cause of death in treated patients
    - Overall survival and progression free survival of individual patients

Exploratory Endpoints

1. Characterize CAR-20/19-T cells with respect to their expansion, persistence, phenotype, and function. This will be assessed by flow cytometry, Q-PCR, RNA sequence, and in vitro functional assays.
2. Determine if host immunity develops against the CAR-20/19-T cell construct or other elements of the transgene or vector and assess correlation with loss of detectable CAR-20/19-T cells (loss of engraftment).
3. Evaluate the effect of CAR-20/19-T cells on B cell and plasma cell compartments assessed by MFC to measure both benign and malignant B cell populations and plasma cell populations.
4. Describe the incidence of new-onset secondary malignancies if any.
5. Determine the utility of a T-cell diversity assay in predicting outcomes with CAR-T cell therapy

3.6 Study Timeline

Preclinical lines of CD19 and CD20/CD19 CAR T-cells have been generated in support of the IND application. These studies have demonstrated that the MCW lymphocyte propagation laboratory can produce CAR-T cell lines using the Miltenyi CliniMACS Prodigy device.

1) Submission of IND (1-month review time for FDA)
2) Submission of clinical protocol through IRB, DSMC at local institution
3) Clinical accrual after FDA and IRB approved protocol along with regulatory approval from local institutions (MCW and FH).

3.7 Primary Completion

The study is expected to reach primary completion 18-24 months from the time the study opens to accrual.

3.8 Study Completion

Initial data on the safety, feasibility and short-term toxicity of CAR-20/19-T cells will be complete and available 4 months after completion of accrual. Thus, preliminary toxicity and efficacy data will reach completion 24 months from the time the study opens to accrual.

4 PATIENT SELECTION

4.1 Eligibility Criteria

Patients must meet all the below inclusion and exclusion criteria prior to apheresis. After consent patients can receive additional bridging chemotherapy except during listed time periods in inclusion/exclusion criteria prior to apheresis and prior to CAR-20/19-T cell infusion.

4.2 Inclusion Criteria

1. Diagnosis of B-cell NHL or CLL/SLL: Patients must be aged≥18 years with relapsed, refractory disease and no available curative options that meet clinical criteria to initiate treatment.
2. Patients with B-cell NHL or CLL/SLL must have either CD19 or CD20 positive disease on most recent biopsy performed (a repeat biopsy is not mandatory for this study except as noted below). A minimum of 5% CD19 or CD20 positivity by immunohistochemistry or flow cytometry on prior or repeat biopsy is required.
3. Absolute CD3+ T cell count ≥50/mm^3
4. MRI brain and Lumbar Puncture with CSF analysis by cytology and flow cytometry without evidence of CNS involvement ONLY in patients with history of CNS involvement
5. Measurable disease must have been documented within 4 weeks of the time of consent defined as the following by disease specific subtype:
    c. B-cell NHL: Active disease defined as nodal lesions greater than 20 mm in the long axis or extranodal lesions >10 mm in long and short axis or bone marrow involvement that is biopsy proven
    d. CLL/SLL: Active disease by either bone marrow, peripheral flow cytometry, or CT and/or PET imaging with nodal disease
6. Patients should have failed at least two lines of a standard treatment and meet disease specific criteria detailed below:
    a. CLL/SLL: measurable disease as defined above that has relapsed after at least one line of chemo-immunotherapy and progressed or intolerant to ibrutinib monotherapy
    b. CD19 or CD20 positive B cell NHL limited to the following histologies: Advanced Stage III or IV Follicular Lymphoma, Diffuse Large B cell Lymphoma and associated subtypes (e.g. aggressive B-cell lymphoma, T-cell/histocyte rich B-cell lymphoma, primary mediastinal B-cell lymphoma, EBV+ diffuse large B-cell lymphoma, transformed lymphoma such as transformed follicular or marginal zone lymphoma, and Richter's transformation) and Mantle cell lymphoma. Specific criteria include:
        - Patients must have active, measurable disease after two lines of cytotoxic chemotherapy of which one must be anthracycline containing.
        - Must have received Rituximab or another CD20 antibody and at minimum two chemotherapy regimens appropriate for their disease.
        - Either failed autologous transplant or ineligible to receive autologous transplant

7. Karnofsky performance score ≥70. See Appendix A for scales.
8. Normal Baseline Neurological Evaluation: Mini-Mental Status Exam Score 24-30 (Appendix B)
9. Adequate hepatic function, defined as AST and ALT <5 x upper limit of normal (ULN); serum bilirubin and alkaline phosphatase <5 x ULN, or considered not clinically significant as per the clinical PIs discretion (e.g. Gilbert's or indirect hyperbilirubinemia) or felt to be due to underlying disease.
10. Adequate renal function, defined as creatinine clearance>60 ml/min
11. Able to provide written informed consent
12. Agree to practice birth control during the study
13. Adequate cardiac function as indicated by New York Heart Association (NYHA) classification I or II AND left ventricular ejection fraction of ≥35% (by cardiac ECHO or MUGA) and adequate pulmonary function as indicated by room air oxygen saturation of ≥92%.
14. Expected survival >12 weeks
15. Negative urine or serum pregnancy test in females of child bearing potential at study entry and again within 48 hours' prior lymphodepleting chemotherapy.
16. Patients with prior blinatumomab treatment require repeat biopsy post-blinatumomab treatment that demonstrates CD19 or CD20 positive disease.
17. Meet criteria for regarding fertility and contraception detailed in section 4.4
18. Central line access will be required for CAR-20/19-T cell infusion.

4.3 Exclusion Criteria

A potential subject who meets any of the following exclusion criteria is ineligible to participate in the study.

1. Positive beta-HCG in female of child-bearing potential defined as per table 1.
2. Patients with known systemic allergy to bovine or murine products.
3. Known prior positive serology for human anti-mouse antibody (HAMA).
4. Confirmed active human immunodeficiency virus (HIV), Hepatitis B or C infection.
5. History of significant autoimmune disease OR active, uncontrolled autoimmune phenomenon: such as systemic lupus erythematous, Wegner's glomerulonephritis, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura (AIHA, ITP) requiring steroid therapy defined as >20 mg of prednisone or equivalent daily.
6. Presence of ≥grade 3 non-hematologic toxicities as per CTCAE version 5.0 from any previous treatment unless it is felt to be due to underlying disease.
7. Concurrent use of investigational therapeutic agents or enrollment on another therapeutic clinical trial at any institution. Minimum of ≥4 weeks required from administration of any other investigational agents on other clinical trials prior to enrollment on this CAR-T protocol.
8. Refusal to participate in the long-term follow-up protocol
9. Patients with active CNS involvement by malignancy on MRI or by lumbar puncture.
    a. Patients with prior CNS disease that has been effectively treated will be eligible providing treatment was >4 weeks before enrollment and a remission documented within 8 weeks of planned CAR-T cell infusion by MRI brain and CSF analysis.
10. Previous recipients of allogeneic hematopoietic stem cell transplantation (AHCT) are excluded if they are <100 days' post-transplant, have evidence of active graft-versus-host-disease (GVHD) of any grade, or are currently on immunosuppression.

11. Previous CAR-T cell therapy directed at either CD19 or CD20 within 100 days of planned CAR-20/19-T cell infusion (does not include re-enrollment)
    a. Patients with prior CAR-T treatment against CD19 or CD20 must have repeat biopsy confirming a minimum of 5% CD19 or CD20 positivity by immunohistochemistry or flow cytometry
12. Anti-CD20 antibody treatment within 4 weeks of cell infusion
13. Anti-CD19 antibody treatment within 4 weeks of cell infusion
14. Cytotoxic chemotherapy other than lymphodepletion within 14 days of CAR-T cell infusion
15. Cytotoxic chemotherapy treatment within 14 days or steroid treatment (other than replacement dose steroids) within 7 days prior to apheresis collection for CAR-T cells
16. Patients post solid organ transplant who develop high grade lymphomas or leukemias
17. Concurrent active malignancy other than basal or squamous cell carcinomas of the skin

4.4 Special Criteria for regarding Fertility and Contraception

Female subjects of reproductive potential (women who have reached menarche or women who have not been post-menopausal for at least 24 consecutive months, i.e., who have had menses within the preceding 24 months, or have not undergone a sterilization procedure [hysterectomy or bilateral oophorectomy]) must have a negative serum or urine pregnancy test performed as part of eligibility criteria and again within 48 hours of initiation of lymphodepleting chemotherapy.

Due to the high-risk level of this study, while enrolled, all subjects must agree not to participate in a conception process (e.g., active attempt to become pregnant or to impregnate, sperm donation, in vitro fertilization). Additionally, if participating in sexual activity that could lead to pregnancy, the study subject must agree to use reliable and double barrier methods of contraception during the follow-up period of the protocol.

Acceptable birth control includes a combination of two of the following methods:

- Condoms* (male or female) with or without a spermicidal agent.
- Diaphragm or cervical cap with spermicide
- Intrauterine device (IUD)
- Hormonal-based contraception

Subjects who are not of reproductive potential (women who are premenarche or have been post-menopausal for at least 24 consecutive months or have undergone hysterectomy tubal ligation, salpingectomy, and/or bilateral oophorectomy or men who have documented azoospermia) are eligible without requiring the use of contraception.

4.5 Subject Recruitment and Screening

Subjects will be identified through the clinical practices of the investigator or sub-investigators and through referrals from outside hospitals and physicians. No direct-to-patient advertising will be performed.

To be eligible, the subjects must have an adequate number of T cells (CD3+ ≥50/mm^3) for CAR-20/19-T cell line production, as determined from a peripheral blood sample before being considerable as eligible to undergo apheresis collection. The purpose of this screening procedure is to exclude subjects from participation who would otherwise undergo a futile

apheresis and restaging. We will additionally determine the % of CD4 and CD8 in circulation to ensure the CAR-20/19-T line contains both helper and effector T cells.

5 STUDY ENTRY AND WITHDRAWAL; STUDY PROCEDURES

5.1 Study Entry Procedures

Required Preregistration Screening Tests and Procedures:

The study-specific assessments are detailed in this section and outlined in Table 1. Screening assessments must be performed within 8 weeks 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 as noted in the study calendar. 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 signed ICF copy will be given to the subject and a copy will be filed in the medical record. The original will be kept on file with the study records.

All patients who are consented will be registered in OnCore®, the MCW Cancer Center Clinical Trial Management System. The system is password protected and meets HIPAA requirements.

Registration Process:
Subjects will be identified through the clinical practices of the investigator or sub-investigators and through referrals from outside hospitals and physicians.

To be eligible, patients need a diagnosis of CLL/SLL or B-cell NHL as specified in Section 4. CD19 or CD20 expression should be confirmed by IHC or flow cytometry (>5% expression of either CD19 or CD20) or MFC on malignant tissue, if it has not previously been documented. In the Phase 1 portion, *at each dose level, the first two patients will be monitored for a full 28 days after CAR-T infusion prior to subsequent patient's treatment with CAR-T cells to allow monitoring of acute and sub-acute toxicities. After that patients will be infused no sooner than at 14-day intervals among the specified dose level or in-between two dose levels. (e.g. 28 days between patient 1 and patient 2, and 28 days between patient 2 and patient 3).*

For the Phase 1b portion, please see section 6.6 for details on dosing. The PI on the study, Dr. Nirav Shah, should be contacted regarding any questions regarding subject eligibility. Contact information for the PI is listed on the front page of the protocol.

5.2 Pretreatment Period

Screening Assessments
The screening procedures and assessments must be completed within ≤8 weeks prior to CAR-20/19-T cells infusion (except as noted otherwise). For patients who fail to get their CAR-T cells within that time frame the studies below will have to be reported unless noted otherwise.

Eligible subjects who have signed an informed consent and have adequate pre-screening evaluation will undergo a routine staging workup including:

- History and Physical Examination including height and weight, Performance Status

Assessment (Karnofsky score), concomitant medications, and review of adverse events.

- Vitals: Blood Pressure, Temperature, Respiratory rate, Pulse & Pulse Ox.
- Radiologic imaging:
    - Diagnostic PET-CT scans or CT of the Neck, Chest, Abdomen, and Pelvis; If >8 weeks' elapses between screening assessment and planned CAR-20/19-T cells infusion, scans will be required to be repeated.
- Complete Blood Count and differential.
- Comprehensive Metabolic Panel: Albumin, Alkaline Phosphatase, AST, Bicarb, Bili T, Calcium (total), Chloride, Creatinine, Glucose, Potassium (K), Sodium (NA), Protein Total, BUN, and ALT.
- Uric Acid
- LDH
- Ferritin, ESR, CRP
- Lipid Panel
- Beta-2-Microgloublin
- Creatinine Clearance
- Coagulation Assessments: PT/INR, PTT, Fibrinogen, and D-Dimer.
- Thyroid Function Tests: TSH, Free T4
- Bone Marrow Transplant Markers/Apheresis Screening (Absolute numbers of CD3+ T cells, CD4+ T cells and CD8+ T cells should be assessed before scheduling for apheresis collection to determine protocol eligibility. Minimum of ≥50 CD3+ T cells/mm3 required, with a mixture of both CD4+ and CD8+ T cells).
- Quantitative Immunoglobulins (IgM, IgG, IgA)
- Autoimmune Screen: ANA
- Urinalysis
- Serum or urine pregnancy test for females of child bearing potential
- Infectious Disease Markers: HIV, Hepatitis B surface antigen, and HCV antibody within 8 weeks prior to CAR-20/19-T cells infusion. If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed. Patients with HIV or Hep B positivity will be excluded from the study. *(Does not need to be repeated)*
- Bone marrow and aspirate evaluation within 8 weeks of CAR-20/19-T cells infusion
- Baseline cardiac ECHO/MUGA *(Does not need to be repeated if no new cardiac symptoms)*
- Electrocardiogram (ECG)
- Lumbar puncture as specified in Table 1 and inclusion criteria *(Does not need to be repeated if no active CNS symptoms)*
- MRI Brain *(Only in patients with history of CNS disease or signs/symptoms of active CNS disease)*
- Baseline neurological assessment

5.3 Enrollment

Documents required:

- Signed informed consent forms and eligibility documentation
- Assignment of subject sequence number.

5.4 Study Procedures Related to Treatment

5.4.1 Study Procedures – Day -15 (Apheresis day)

- Physical Examination including weight, concomitant medications, and review of adverse events.
- Vital signs: Blood Pressure, Temperature, Respiratory rate, Pulse & Pulse Ox.
- CBC with differential
- Comprehensive Metabolic Panel: Albumin, Alkaline Phosphatase, AST, Bicarb, Bili T, Calcium (total), Chloride, Creatinine, Glucose, Potassium (K), Sodium (NA), Protein Total, BUN, and ALT.
- Uric Acid
- LDH
- Apheresis
- T-cell repertoire diversity testing from apheresis sample
- Quality of Life Studies
- Tryptophan Metabolites
- PaxGene RNA gene expression

5.4.2 Mononuclear Cell Apheresis

A 2-5 blood volume apheresis will be targeted if tolerated by the patient, to be collected at Froedtert Hospital, as appropriate for CAR-20/19-T cell production. From a single apheresis, the intention is to harvest 1.0-5.0 x 10^9 MNC cells total to manufacture CAR-T cells. Baseline blood leukocytes for FDA requirements and for research are also obtained and cryopreserved.

5.4.3 Study Procedures Week -1 + 6 weeks (~2 to 4 days prior to lymphodepletion)

Patients will be seen 2-4 days prior to lymphodepleting chemotherapy.

- Physical Examination including weight, concomitant medications, and review of adverse events.
- Vital signs: Blood Pressure, Temperature, Respiratory rate, Pulse & Pulse Ox.
- Urine or serum pregnancy test in females of childbearing potential
- Viral Respiratory NAAT will be performed in patients with signs/symptoms concerning for viral infection (cough, cold). In the setting of a positive result, initiation of LDP chemotherapy will be delayed until resolution of symptoms
- Urinalysis
- Lumbar Puncture: For high risk patients with concern for CNS involvement, at the discretion of the PI, a repeat LP may be done prior to lymphodepleting chemotherapy
- If >8 weeks' elapse from staging bone marrow biopsy and aspirate, a repeat biopsy will be indicated
- Confirm patient has central line access
- Radiologic imaging:
    - Diagnostic PET-CT scans or CT of the Neck, Chest, Abdomen, and Pelvis; If >8 weeks' elapses between screening assessment and planned CAR-20/19-T cells infusion, scans will be required to be repeated.

5.4.4 Start of Lymphodepletion Chemotherapy (Day -4 + 6 weeks)

Patients will then be seen again the day of initiation of Lymphodepletion chemotherapy. The following procedures must be completed prior to initiation of lymphodepleting chemotherapy

- Physical Examination including weight, concomitant medications, and review of adverse events.
- Vital signs: Blood Pressure, Temperature, Respiratory rate, Pulse & Pulse Ox.
- CBC with differential
- Comprehensive Metabolic Panel: Albumin, Alkaline Phosphatase, AST, Bicarb, Bili T, Calcium (total), Chloride, Creatinine, Glucose, Potassium (K), Sodium (NA), Protein Total, BUN, and ALT.
- Uric Acid
- LDH
- T-cell repertoire diversity testing from CAR-T sample

A chemotherapy cycle (3 days of chemotherapy with a day of rest) is planned for all patients to start within approximately one week of CAR-20/19-T cell infusion. Patients will receive a combination of fludarabine/cyclophosphamide lymphodepletion. These chemotherapy agents are all commercially available and will be infused per institutional standards. Antimicrobial prophylaxis and TLS prophylaxis is outlined in **Appendix E.**

The chemotherapy will be planned so that it completes BEFORE the infusion of the first dose of CAR-20/19-T cells. The purpose of the chemotherapy is to induce lymphopenia to facilitate expansion of infused T cells. Although the chemotherapy is not investigational, patients will be required to receive it at Froedtert Hospital (FH) and/or the Medical College of Wisconsin Cancer Center. Patients will be started on allopurinol for tumor lysis prevention.

5.4.5 CAR-20/19-T Cell Infusion (+ 6 weeks)

Infusion begins 1-7 days after the completion of chemotherapy and will be administered over two days (Day 0 and Day +1) in the Phase 1 portion. In the phase 1b portion cells will be administered as a single dose on Day 0. Patients can receive cells fresh, if appropriate or after cryopreservation for up to 6 weeks post-harvest. Patients will be either admitted to FH under the care of the hematology/oncology service for the cell infusion or seen daily in the FH Day Hospital for infusion from Days 0-7. Details of the infusion and hospitalization plan can be found in the treatment plan section 6. On these days, the following evaluations will occur prior to CAR-20/19-T cells infusion unless stated otherwise:

- Physical Examination including weight, Performance Status Assessment (Karnofsky score), concomitant medications, and review of adverse events.
- Vital signs: Blood Pressure, Temperature, Respiratory rate, Pulse & Pulse Ox
- CBC with differential
- Comprehensive Metabolic Panel: Albumin, Alkaline Phosphatase, AST, Bicarb, Bili T, Calcium (total), Chloride, Creatinine, Glucose, Potassium (K), Sodium (NA), Protein Total, BUN, and ALT.
- Uric Acid
- LDH
- Ferritin/ESR/CRP
- Bone Marrow Transplant Markers (Day 0 only)
- Quantitative Immunoglobulins (IgM, IgG, IgA) only on Day 0 prior to infusion
- Electrocardiogram (ECG)

- Coagulation Assessments: PT/INR, PTT, Fibrinogen, and D-Dimer.
- Research Studies
    - T-Cell Persistent Studies: Baseline sample to be drawn prior to CAR-20/19-T cells infusion on Day 0 only.
    - Cytokine Studies: At minimum, will include IFN-Ɣ, IL10, IL6 and TNFα
    - Q-PCR for Integrated Vector DNA baseline sample on Day 0 prior to infusion
    - Detectable RCL by Q-PCR for VSV-G baseline sample on Day 0 prior to infusion

5.4.6 Day +1 through day +7 after cell infusion

Patients will be seen daily through day+7 of infusion. This will occur either in the inpatient setting or with daily visits to the Day Hospital through day+7. At these evaluations, the following will occur:

- Physical Examination including weight, concomitant medications, and review of adverse events.
- Vital signs: Blood Pressure, Temperature, Respiratory rate, Pulse & Pulse Ox.
- CBC with differential
- Comprehensive Metabolic Panel: Albumin, Alkaline Phosphatase, AST, Bicarb, Bili T, Calcium (total), Chloride, Creatinine, Glucose, Potassium (K), Sodium (NA), Protein Total, BUN, and ALT.
- Uric Acid
- LDH
- Coagulation Assessments: PT/INR, PTT, Fibrinogen, and D-Dimer.
- Ferritin/CRP/ESR
- Research Studies as per schedule of events (Table 1)
    - T-Cell Persistent Studies—Will not be repeated once there are two consecutive negative results.
    - Cytokine Studies
    - Neurological assessments
    - T-cell repertoire diversity testing from peripheral blood

5.4.7 Follow-Up Visits (Day+10 to 2 years)

ONGONG RESPONSE

Patients with an ongoing response will be followed for up to 2 years after CAR-20/19-T cells infusion as per this protocol. After Day 28, testing can be performed at the local oncologist office and results sent to MCW/FH other than dates mentioned in the study calendar. The following procedures will be performed at specified intervals, **see Table 1 per schedule of events.**

- Physical Examination, including weight, Performance Status Assessment (Karnofsky score), concomitant medications, and review of adverse events.
- Vital signs: Blood Pressure, Temperature, Respiratory rate, Pulse & Pulse Ox
- CBC with differential
- Comprehensive Metabolic Panel: Albumin, Alkaline Phosphatase, AST, Bicarb, Bili T, Calcium (total), Chloride, Creatinine, Glucose, Potassium (K), Sodium (NA), Protein Total, BUN, and ALT.

- Uric Acid
- LDH
- Coagulation Assessments: PT/INR, PTT, Fibrinogen, and D-Dimer
- Lipid Panel
- Thyroid Function Tests: TSH, Free T4
- Quantitative Immunoglobulins (IgM, IgG, IgA)
- Urinalysis
- Bone Marrow Transplant markers
- Beta-2-Microglobulin
- Ferritin/ESR/CRP
- Radiologic imaging:
    - CT neck/chest/abd/pelvis or PET/CT skull base to mid-thigh
    - At 1 month, 6 months, and 1 year. From 1 year to 2 years, scans will be done yearly.
        a) Scans would only be completed in follow-up only for patients who remain in remission since entering the study. Patients with progressive disease will not require any further imaging.
- Bone Marrow Biopsy and aspirate as per Table 1
- Electrocardiogram (ECG)
- Research Studies:
    - T-Cell Persistent Studies—Will not be repeated once there are two consecutive negative results.
    - Q-PCR for Integrated Vector DNA—Will not be repeated once there are two consecutive negative results.
    - Detectable RCL by Q-PCR for VSV-G—Will not be repeated once Q-PCR for integrated vector DNA is negative at two consecutive timepoints.
    - Cytokine Studies
    - Neurological assessments
    - T-cell repertoire diversity testing from peripheral blood
    - Quality of Life Studies
    - Tryptophan Metabolites
    - PaxGene RNA gene expression

VISITS REQUIRED AT MCW/FH: Beyond Day +28, patients will have required visits at MCW/FH on Day +90 (+/- 7 days), Day+180 (+/- 14 days), Day 365 (+/- 1 month), +1.5 years (+/- 1 month) and 2 years (+/- 1 month). For timepoints not falling on those dates, local oncology evaluation will suffice and records will be obtained accordingly.

PROGRESSIVE DISEASE

For patients who develop **progressive disease** will not follow the above study calendar and will placed in a follow-up plan as detailed below with visit with their local oncologist every 6 months starting from CAR-T infusion date for up to 2 years and if alive will subsequently roll into our long-term follow-up protocol. At these visits the following should be performed

- Physical Examination, including vitals
- Survival/Progression status
- Subsequent Treatments Post-CAR-T infusion
- Targeted concomitant medications
    - Including IVIG, immunosuppressive medications, prophylactic anti-infective medications

- Targeted adverse events recording
    - Including New malignancies, new incidence or exacerbation of pre-existing neurologic disorder, new incidence or exacerbation of prior rheumatologic disorder, or other autoimmune condition, and new incidence of hematologic disorder
- CBC with differential
- Comprehensive Metabolic Panel: Albumin, Alkaline Phosphatase, AST, Bicarb, Bili T, Calcium (total), Chloride, Creatinine, Glucose, Potassium (K), Sodium (NA), Protein Total, BUN, and ALT.
- Quantitative Immunoglobulins (IgM, IgG, IgA)
- Absolute B and T cell lymphocyte counts
- Research Studies:
    - Patients will be required to have blood sent to MCW (See **Appendix K** for instructions on sending samples to MCW) or return to MCW for testing at the following timepoints until testing is negative for persistence of CAR-T cells: +6 months, +1 year, +1.5 years, and +2 years post-CAR-T infusion and will include the following listed below. Once there are TWO negative results, no further visits to MCW or blood samples to MCW will be mandated:
        a) T-Cell Persistent Studies—Will not be repeated once there are two consecutive negative results. Additional samples (blood, CSF, ascites, tissue, etc.) for T-cell persistence can be performed per the investigators discretion as felt to be clinically indicated.
        b) Q-PCR for Integrated Vector DNA—Will not be repeated once there are two consecutive negative results.
        c) Detectable RCL by Q-PCR for VSV-G—Will not be repeated once Q-PCR for integrated vector DNA is negative at two consecutive timepoints.

5.4.8 Long-term/Survival Follow-Up Procedures

Patients will be followed for up to 2 years after CAR-20/19-T cells infusion or until progression. Patients who progress will be followed for survival, progression, persistence of CAR-T cells, and future treatments. Patients who progress post treatment will not have any further required disease assessment on study, and management of their disease will return to their treating physician. They will be required to return for other study laboratory testing and evaluations as mandated per the protocol as outlined above. Patients who are alive beyond 2 years will be placed on a long-term follow-up protocol for a total of up to 15 years for monitoring of post-genetically modified therapies.

5.5 Study Withdrawal Procedures

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

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 that prevents further administration of treatment, a patient's request to end participation, a patient'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 patient's compliance with the prescribed treatment regimen.

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

6 TREATMENT PLAN

6.1 Lymphodepletion (within 1 week prior to CAR-20/19-T cell infusion)

Patients will receive lymphodepleting conditioning regimen prior to CAR-20/19-T cells starting on Day -4. Lymphodepletion will start based on the planned date of infusion of CAR-20/19-T cells. Tumor lysis prevention with allopurinol daily (dose per institutional standards) will be initiated the day of chemotherapy. Levetiracetam (Keppra) prophylaxis dosed per institutional standards will be initiated in all patients to prevent possible neurologic toxicity. Anti-emetics and steroids can be provided pre-treatment as per standard of care protocols for these treatment regimens. Please see Appendix E for guidelines for CAR-T cell infusion. The lymphodepletion regimens is as follows:

Fludarabine 30 mg/m^2 on Day -4, -3, -2 and Cyclophosphamide 500 mg/m^2 on Day -2[41]

This regimen is based on published lymphodepletion protocols for adult patients receiving CD19 CAR-T cells. Fludarabine and Cyclophosphamide combination is the most utilized lymphodepletion regimen prior to CAR-T cell therapy and the above regimen was presented at ASH 2016 and used in a large cohort of >100 patients with published safety and toxicity data. [41, 48] Fludarabine and Cyclophosphamide dosing will be based on actual body weight. For patients who are neutropenic (ANC<1000 k/μL) at the start of lymphodepletion due to underlying hematologic disease or as a result of prior chemotherapy related toxicity, cyclophosphamide will be omitted from the lymphodepletion regimen.

Phase 1 Dose Escalation/Expansion: CAR-20/19-T cells will be administered per actual body weight in the lymphopenic phase on Day 0 & Day 1. Dose will be maxed at a weight of 80 kilograms.

Phase 1b Cohort : CAR-20/19-T cells will be administered as a single dose on Day 0. Dose will be maxed at a weight of 80 kilograms

6.2 Preparation of CAR-20/19-T Cell Infusion

Cell Manufacturing

The MNC, Apheresis product obtained from the patient on Day -15 will be washed free of platelets and plasma and the $CD4^+$ and $CD8^+$ T cells will be purified from the MNC, activated through the CD3 and CD28 antigens, then transduced with the CD20/CD19 CD3ζ/41BB lentiviral vector. The cells will be expanded *in vitro,* harvested from culture, and then administered within 48 hours of production in an isotonic electrolyte solution (Plasma-Lyte® A or Normosol® R) containing 2.5% HSA or cryopreserved for administration in a freezing solution of Plasmalyte A or Normosol R containing 10% DMSO and 4% HSA.

Testing will be performed at two mandatory time points (in process day-6 and at harvest) and will include the following (Please see **Appendix D** for details on testing, including those tests for which results are required for product release):

In-Process Testing

- Transduction efficiency
- Viability
- Gram Stain
- In-house Mycoplasma

Final Product Testing (Harvest)

- Sterility
    - endotoxin
    - mycoplasma
    - bacterial and fungal cultures
- Phenotype
    - CD3, CD4, CD8, central memory and effector memory T cell subsets,
    - NK cells at harvest
    - NK-T cells at harvest
    - B cells at harvest
    - T regs at harvest
- Viability and viable cell numbers
- Effector activity (lysis and cytokine product in response to B cell targets)
- Copy number (qPCR) at harvest
- VSVg (qPCR) at harvest
- % transduced cells (light chain expressing T cells)

Cells will be harvested on Day 14 of production for fresh administration or cryopreserved for later infusion **(Appendix D)**. The day cells are ultimately administered will be deemed Day 0 for study calendar and schedule of activities. All testing will be performed in accordance with FACT and FDA regulations. The entire manufacturing process up until removal from culture will be performed by a computer- controlled program using the CliniMACS Prodigy device.

Cell thawing (if indicated)

For patients who require cryopreservation the following will be performed: The frozen cells will be thawed in the cell processing lab and transported by laboratory staff to the subject's bedside either at the inpatient oncology unit of FH or the outpatient day hospital under the care of a Hematology/Oncology attending. If the CAR-20/19-T cell product appears to have a damaged or leaking bag, or otherwise appears to be compromised, it should not be infused, and should be returned to production laboratory.

Positive Sterility Test (after infusion)

In the scenario that CAR-T cells are administered prior to completion of the 14 days of sterility testing, it is possible that a sterility test returns positive after administration. In that scenario, we will follow our standard operating procedure for positive cultures after infusion of cell therapy products. In general, this involves the following:

1. Cell processing lab will contact the PI on the study at the time of this finding.
2. The PI will discuss these findings with the patient

3. The PI will order appropriate infectious work-up and provide antimicrobial coverage as indicated based on the results of the sterility tests.
4. Reporting—the PI on the clinical trial will report this to the DSMC, IRB, and the FDA

6.3 Administration of CAR-20/19-T cell infusion on Day 0 and Day +1 (Phase 1) or Day 0 (Phase 1b)

Patients will receive the infusion of the CAR-20/19-T cells as an inpatient on the oncology unit or in the outpatient Day Hospital under the clinical care of hematology/oncology attending. Patients will receive institutional standard for infusion reaction prophylaxis (e.g., Acetaminophen and diphenhydramine hydrochloride, or other acceptable alternatives, as pre-treatment). Patients will be on allopurinol for tumor lysis prevention and levetiracetam (Keppra) with initiation of lymphodepleting chemotherapy **(See Appendix E for details)**. Pretreatment with steroids is contraindicated. Patients will receive CAR-20/19-T cells by IV infusion via central line for a total viable, transduced, cell dose ranging from 1.0×10^5 cells/kg to 2.5×10^6 cells/kg based on phase of study. The dose of cells may be limited if calculations performed at the time of administration or cryopreservation indicates that the patient would receive > 1.8×10^8 non-viable cells/kg. Patients who fail to meet the target cell dose will be allowed infusion and will be followed for toxicity and adverse events as all other patients. The infusions will occur approximately 1-7 days following chemotherapy.

Premedication
Potential side effects following T cell infusions include transient fever, chills, and/or nausea. The subject will be pre-medicated with acetaminophen and/or diphenhydramine hydrochloride as per institutional standards prior to the infusion of CAR-20/19-T cells. These medications may be repeated every six hours as needed. Epinephrine will be readily available in the rare scenario of an allergic reaction. A course of non-steroidal anti-inflammatory medication may be prescribed if the patient continues to have fever not relieved by acetaminophen. It is recommended that patients not receive systemic corticosteroids such as hydrocortisone, prednisone, prednisolone (Solu-Medrol) or dexamethasone (Decadron) at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on T cells. If corticosteroids are required for an acute infusional reaction, an initial dose of hydrocortisone 100 mg is recommended.

Prophylaxis Therapies
Patients will receive appropriate prophylaxis with CAR-T cell therapy as recommended in Appendix E. Briefly levetiracetam will be given for seizure prophylaxis, institutional standards will be applied for pneumocystis pneumonia (PCP), viral, and neutropenic prophylaxis.

Infusion
On the day of the first infusion (Day 0), patients will have a CBC with differential, and assessment of T-cell subsets prior to administration of CAR-20/19-T cells since chemotherapy is given in part to induce lymphopenia.

The CAR-20/19-T cell dose will be administered by IV infusion. Dosing will be split during the phase 1 portion of the trial, with 30% given on day 0, and 70% given on day 1. If there is no significant infusional toxicity, in the phase 1b portion of the trial cells will be administered as a single dose on Day 0. As detailed above, the cells will be administered fresh or thawed if indicated in the LPL and placed in a validated transport container at 1-10^{o}C and brought directly to the patient's bedside. If the CAR-20/19-T cell product appears damaged or leaking, or otherwise appears to be compromised, it should not be infused, and should be returned to production laboratory. The infusion will take place in the patient room on the oncology inpatient

units or in the outpatient day hospital BMT rooms, using precautions for immunosuppressed patients.

The transduced T cells will be administered by intravenous infusion. The infusion nurse spikes HPC product bag and administers through blood tubing accordingly. The duration of the infusion will be approximately 5-15 minutes. Each infusion bag will have affixed to it a label containing the following: “FOR AUTOLOGOUS USE ONLY”. In addition, the label will have at least two unique patient identifiers such as the subject’s initials, birth date, and study number. Prior to the infusion, two individuals will independently verify all this information in the presence of the subject and so confirm that the information is correctly matched to the participant. Subjects will be infused and premedicated as described.

Subjects’ vital signs (temperature, respiration rate, pulse, and blood pressure, pulse oximetry) will be done prior to dosing, at the end of the infusion and every 15 minutes thereafter for a minimum of 1 hour. A blood sample for determination of a baseline CAR-20/19-T cell level is obtained any time prior to the infusion and Day+1 (and sent to the LPL Labs) and then will be completed as per the study calendar. Emergency medical equipment (i.e., emergency trolley) will be available during the infusion in case the subject has an allergic response, or severe hypotensive crisis, or any other reaction to the infusion.

Patients experiencing toxicities from their preceding cytoreductive chemotherapy will have their infusion schedule delayed until these toxicities have resolved. The specific toxicities warranting delay of T cell infusions include:

1) Pulmonary: Requirement for supplemental oxygen >2L by nasal cannula to keep saturation greater than 95% or presence of radiographic abnormalities on chest x-ray that are progressive;
2) Cardiac: New cardiac arrhythmia not controlled with medical management
3) Hypotension: requiring pressor support
4) Active Infection: Fever>101 F and/or positive cultures for bacteria, fungus, or virus. Patients who are unable to receive the CAR-20/19-T cells within 7 days of completion of chemotherapy will be removed from the protocol.

Febrile reaction
In the unlikely event that the subject develops sepsis or systemic bacteremia following CAR-T-cell infusion, appropriate cultures and medical management should be initiated.

Table 2: Regimen Description					
Study Drug	**Premedication[3]**	**Dose**	**Route**	**Schedule**	**Cycle Length**
CAR-20/19-T cells[1] (Phase 1)	Tylenol PO Benadryl IV/PO	1.0 x 10^5-2.5 x 10^6cells/kg[2]	IV	30% on Day 0 70% on Day 1	28 days
CAR-20/19-T cells (Phase 1b)	As above	2.5 x 10^6 cells/kg[2]	IV	100% on Day 0	28 days

[1]Patients with ≥ grade 2 non-hematologic CTCAE toxicity will have the 2nd dose held up to 7 days until symptoms resolve or will not be administered at the discretion of the PI

[2]Cell number include only viable T cells expressing the CAR as determined by flow cytometry. Cell dose maxed at 80 kg or if the infusion contains >1.8 x 10^8/kg non-viable cells.

[3]Recommended premedication, dosed per institutional standards

6.4 Dose Escalation Schedule

For this phase 1/1b study we plan to include multiple histologies including CLL/SLL and B-cell NHL. Based on previous CAR-T studies (as outlined in Section 1.6) our goal treatment dose is 2.5 x 10^6 cells/kg. Due to concern that a dual CAR-T cell may increase risk of CRS our starting dose for this study will be 2.5 x 10^5 cells/kg (1 log reduction) and we will escalate based on DLTs to our goal dose. We will utilize a 3+3 escalation design for this study **(Figure 9)**. *At each dose level, the first two patients will be monitored for a full 28 days after CAR-T infusion prior to subsequent patient's treatment with CAR-T cells to allow monitoring of acute and sub-acute toxicities. After that patients will be infused no sooner than at 14-day intervals among the specified dose level or in-between two dose levels.* The first three patients with NHL or CLL/SLL will be treated with 2.5 x 10^5 cells/kg. If 0/3 patients experience a DLT, the dose will increase to 7.5 x 10^5 cells/kg. If 1/3 patients at the 2.5 x 10^5 cells/kg experiences a toxicity, then an additional three patients will be treated at this dose level. If ≤1/6 patients have a DLT at this level, the dose will increase to 7.5 x 10^5 cells/kg. If ≥2/6 patients have a DLT at 2.5 x 10^5 cells/kg, the dose will de-escalate to 1.0 x 10^5 cells/kg. If 2/3 patients in our first cohort have DLTs, the dose will also de-escalate to 1.0 x 10^5 cells/kg. If at the 1.0 x 10^5 cells/kg there are ≥2 DLTs out of a maximum of 6 patients, the study will close due to excessive toxicity. If there is 0-1 DLTs in 3-6 patients, the 1.0 x 10^5 cells/kg dose will be selected for expansion cohort (**Figure 10)**.

We will utilize a similar dosing schema should we escalate to the 7.5 x 10^5 cells/kg dose. If there are 0-1 toxicities out of 3-6 patients, the dose will escalate to our goal dose of 2.5 x 10^6 cells/kg. If there are ≥2 toxicities in 3-6 patients at 7.5 x 10^5 dose, then the 2.5 x 10^5 cells/kg dose will be selected for our expansion cohort. At our goal dose level (2.5 x 10^6 cells/kg) if there is 0-1 DLTs out of 3-6 patients, this dose will be selected for expansion. If there are ≥2 toxicities in 3-6 patients at the 2.5 x 10^6 cells/kg dose, then the 7.5 x 10^5 cells/kg dose will be selected.

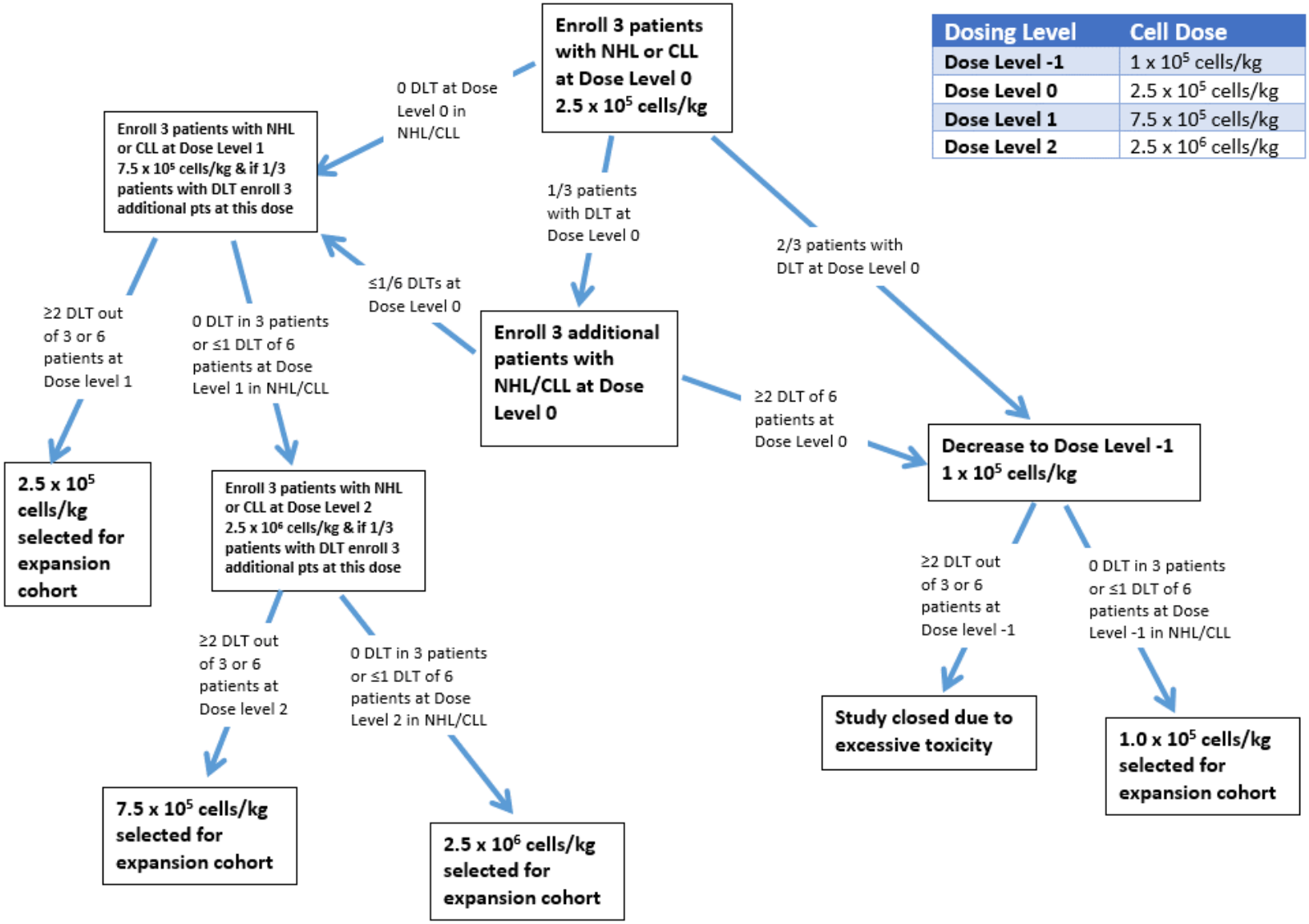
Once a dose has been selected within the NHL and CLL/SLL cohort, this dose will be utilized for the Dose Expansion phase of the study (Section 6.5). The dose expansion cohort will proceed only after approval from the local IRB/DSMC and the FDA.

Table 3: Dose Escalation CAR-T cells		
Dose Level	**Dose of Study Drug***	**Minimum # of Patients/Dose Level**
-1	1.0 x 10^5 cells/kg	2
0	2.5 x 10^5 cells/kg	2
1	7.5 x 10^5 cells/kg	2
2	**2.5 x 10^6 cells/kg (goal cell dose)**	2

Patients who Fail to Produce Target Cell Dose

It is possible that some patients fail to make the targeted cell dose as required per our dose escalation/expansion schema (**Figures 9 & 10**). For those patients, we will allow administration of CAR-20/19-T cells but these patients must be replaced on the study for the purposes of our Phase 1/1b study. Despite not achieving the desired dose level there are still potential toxicities associated with CAR-20/19-T cell administration and so these patients will undergo identical study procedures and will be followed for DLTs, AEs, and survival as all other patients. For this cohort of patients who receive a non-specified dose, if there is ≥33% rate of DLT as defined in section 3.4, then future patients who fail to make the desired dose level will no longer be allowed to be treated in this manner. Standard operating procedures for patients treated at a non-specified cell dose are listed in **Appendix H**.

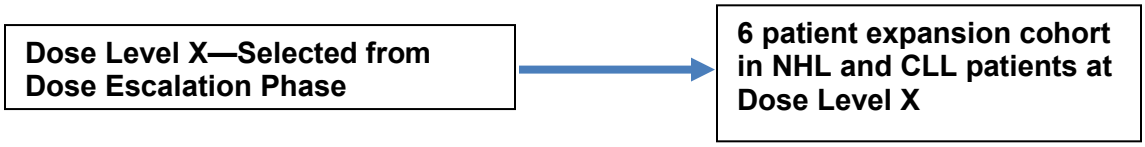
Figure 9: Dose Escalation Phase



Dosing Level	Cell Dose
Dose Level -1	1 x 10^5 cells/kg
Dose Level 0	2.5 x 10^5 cells/kg
Dose Level 1	7.5 x 10^5 cells/kg
Dose Level 2	2.5 x 10^6 cells/kg

6.5 Dose Expansion Phase

Figure 10: Dose Expansion Phase



Once a dose has been identified in our Dose Escalation phase we will perform a Dose Expansion at that dose level (termed Dose Level X). We will evaluate an additional 6 patients in NHL/CLL at this Dose Level X **(Figure 10).** In this dose expansion phase is there are ≥2 patients with DLTs, no further patients will be allowed enrollment.

The dose expansion phase will commence after identification of a dose level in the dose escalation cohort and after approval from both internal and external monitoring agencies (DSMB/IRB/FDA).

6.6 Phase 1b (Single Dose of CAR-T cell)

Once the final patient on the Phase 1 protocol has enrolled on the trial, we will initiate enrollment on the Phase 1b portion to test the safety of single dose administration compared to fractionated dosing. This phase will include a 9 patient expansion at 2.5 x 10^6 cells/kg CAR-T cells administered in a single dose (rather than split dose). The first two patients at this dose level will be admitted to the hospital and monitored for 14-days prior to subsequent infusion to ensure there are not unexpected toxicities associated with single-dosing. If there are no DLTs in these first two patients, then additional patients can receive cells either outpatient or inpatient and be treated as indicated. This cohort will close if there is ≥3 DLTs among the nine treated patients.

6.7 Treatment/Dose Limiting Toxicity

Management and dose modifications associated with the AEs are outlined in Section 7: Dosing Delays/Dose Modifications.

Dose-limiting toxicity (DLT) will be defined as a grade 3 or 4 non-hematologic toxicity possibly, probably, or definitely related to the infusion of CAR-20/19-T cells. **See section 3.4 for a detailed definition.** The dose limiting toxicity will be based on the tolerability observed during the first 28 days of treatment/observation. If multiple toxicities are seen, the presence of dose limiting toxicity should be based on the most severe toxicity experienced.

Grade 3 or 4 CRS will be treated initially with IL-6 inhibition with tocilizumab and/or corticosteroids (**Appendix F**). Additional agents that can be used for refractory CRS include siltuximab or other immunosuppression at the discretion of the PI. A detailed algorithm for the management of CRS and neurotoxicity related to CAR-T cells for this study is provided in **Appendix F**. Supportive care measures to consider for patients receiving CAR-T cells are provided in **Appendix G**.

As cytokine release syndrome is a new entity observed with novel cellular therapies, a modified grading system has been proposed for more appropriate assessment of dose limiting toxicities.

[1, 60] We will employ this grading system to accurately grade toxicity of our CAR-20/19-T cells and a copy of the grading system can be found in **Appendix F.**

6.8 Usage of Concurrent/Concomitant Medications

The investigational agent in this protocol is autologous CAR-20/19-T cells which as a living product will not undergo metabolism as would a pharmaceutical therapy. As such, concurrent/concomitant medications can be prescribed at the discretion of the treating physician for treatment of symptomatic conditions such as fever, nausea/vomiting, diarrhea, anxiety, etc. Antimicrobial prophylaxis can be started as per treating physician. This can include ciprofloxacin or cefepime (or other appropriate antibiotic if allergic) for antibacterial prophylaxis, acyclovir or alternative agent for antiviral prophylaxis, fluconazole (or alternative agent as indicated) for fungal prophylaxis, bactrim (or other PCP prophylaxis if sulfa allergic). Prohibited and limited therapies are detailed in the following section 6.9.

6.9 Dietary Restrictions

On the inpatient service, patients will follow the FDA's Food Safety for Cancer Patient Guidelines. Otherwise no specific dietary recommendations are indicated for this study.

6.10 Prohibited Medications

Patients should be off all systemic steroids, oral or IV, (not including inhaled or intranasal steroids) at the time of apheresis (for at least 7 days prior) and again starting Day 0 after administration of the CAR-20/19-T cells. Exception to this will be steroids for replacement dosing for adrenally insufficient patients or steroids for the purposes of the treatment of CRS at the discretion of the investigators listed on this protocol.

With regards to growth factors, long-acting products such as Neulasta (pegfilgrastrim) will not be permitted starting Day -7 to Day +28. Given the potential risk of exacerbation of CRS or triggering of CRS, short-acting growth factors such as filgrastrim (neupogen) or sargramostim (leukine) will be limited to patients with persistent neutropenia starting Day+21 provided that there are no active signs or symptoms consistent with CAR-T cell associated CRS or neurotoxicity.

6.11 Monitoring Subject Compliance

CAR-20/19-T cells will be administered only to eligible patients under the supervision of the investigator or identified sub-investigator(s). The appropriate study personnel will maintain records of study drug receipt and dispensing. The study agent will be given on the inpatient oncology units. There will be no self-administered agents as part of the investigational portion of this protocol.

6.12 Re-treatment

Patients who have received treatment on the CAR-20/19-T cell protocol may be retreated if they meet the same inclusion/exclusion criteria as listed in the clinical protocol and meet the following requirements:

1. Patient must be ≥100 days from first dose of CAR-20/19-T cells but ≤24 months from initial treatment.

2. Have <1% detectable CAR-T cells by flow cytometry in the peripheral blood
3. Had demonstrated clinical benefit from the first dose (either PR or CR at Day 28 evaluation).
4. Have a repeat biopsy that confirms either CD19 or CD20 positive disease post-CAR-T cell relapse.
5. Have not received another genetically modified CAR-T cell product either commercially or as part of a separate clinical trial.

Patients who are re-treated will not count towards our primary safety endpoint but AEs and testing will be monitored/performed in an identical fashion as their first infusion (see schedule of events). Research studies including t-cell repertoire studies, Paxgene RNA analysis, tryptophan metabolites, and QOL studies will not be repeated. Cells will be given as a single infusion on Day 0 and the lymphodepletion regimen outlined in this protocol will be utilized. Patients will not undergo repeat apheresis and CAR-T cell production, rather, they will receive remainder CAR-T cells that are cryopreserved from their initial production (*see section 9.1.5 on stability data of CAR-T cells post cryopreservation*). Patients will receive no more than the goal dose of 2.5 x 10^6 cells/kg (max 80 kg). If the patient does not have that dose, they will receive the remainder of the CAR-T cells left.

To ensure safety of re-treatment, DLTs through Day 28 will be monitored specific to this cohort. At first, a maximum of 3 patients will be allowed to be treated in this manner with concurrent enrollment allowed. If there is ≤1 DLT within the first three patients, further patients will be allowed re-treatment with CAR-20/19-T cells as clinical indicated and if they have met above criteria. After enrollment of the 4th patient or beyond in this cohort, if the DLT rate exceeds ≥33%, no further patients will be allowed retreatment.

All patients will be followed for AEs, survival, response rates as per Table 1.

7 DOSING DELAYS/DOSE MODIFICATIONS

7.1 Dosing Administration/Delays

Patients will receive the modified CAR-20/19-T cells in one fixed dose on Day 0 or two doses given 24 hours apart on Day 0 and Day +1 of this protocol. Patients will be either admitted to the oncology unit for this infusion or seen daily in the outpatient BMT Day hospital suite and will be under the care of a hematology/oncology attending. Criteria for infusion of cellular therapy include the following:

1) No new medical complication that would unduly increase risk of infusion
2) No requirement of supplemental oxygen >2L by nasal cannula to keep saturation >95%
3) No active cardiac arrhythmias not controlled with medical management
4) No hypotension requiring pressor support
5) Study treatment administration will not be delayed as a result of neutropenia, thrombocytopenia, or anemia, which are all potential risks of lymphodepleting chemotherapy
6) No uncontrolled active infectious complications
7) No fever>101° F in last 24 hours prior to infusion

Patients who do not meet these criteria will have the first dose of their cells delayed for up to a maximum of 7 days beyond their goal treatment date (Day 0). If there is not resolution of those issues, the patients will be taken off protocol.

For patients who received split dose cells, the second dose of cells given on Day 1, patients must meet the above same criteria. In addition, patients who develop clinically significant toxicities within 24 hours after their first infusion will not have their subsequent infusion given. These include grade 2 or higher infusion reactions, allergic responses, cytokine release syndrome, cardiac or pulmonary toxicities, or others deemed clinically significant by the study investigators. Transient grade 1 chills, nausea, vomiting and other mild expected infusion-related toxicities that respond to usual supportive care measures will not require holding subsequent doses. Patients with fever≥101° F after the first cell dose felt to be related to CRS will have the second dose held for up to 7 days until there is resolution. Any questions about subsequent dosing should be discussed with the Principal Investigator. The second dose can be delayed up to maximum of 7 days after the initial dose as per the discretion of the PI.

Dose Modifications and Dosing Delay Table for Specific Adverse Events

Table 4: Potential Dose Modifications for Dose #2 in setting of Cytokine Release Syndrome	
Grade of Event	**Management**
≤ Grade 1	No change in planned 2nd infusion
Grade 2*	Hold 2nd infusion until ≤ Grade 1 Resume at same dose level
Grade 3	Hold 2nd infusion
Grade 4	Hold 2nd infusion
Management: CRS management (Appendix F)	
***Patients requiring a delay of > 7 days will not receive the 2nd dose of modified CAR-20/19-T cells**	

7.2 Monitoring and Toxicity Management

Each patient receiving CAR-20/19-T cells will be evaluable for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical findings, and spontaneous reports of adverse events reported to the investigator by patients. Each patient will be assessed periodically for any toxicity development as per the schedule of procedures (Table 1). Toxicity will be assessed per the NCI CTCAE v5.0 except for CRS which will be graded as previously defined.

For expected cytopenias patients will be transfused platelets and red blood cells as per **Appendix G**.

We will monitor specifically for the development of CRS, a known complication that can occur with the treatment of CAR modified T cells. Signs/symptoms related to CRS were listed in section 1.7 (**Figure 8**). Grading of CRS will be by the revised recommended grading system for CRS found in **Appendix F**. Any patient who develops fever as an outpatient will be admitted for work-up and evaluation of CRS. Detailed algorithm for CAR-T cell associated CRS or neurotoxicity based on current recommendations can be found in **Appendix F.** The current recommendations for CRS management include Tocilizumab and/or steroids as first-line therapies. Tocilizumab is a humanized, IgG1κ antihuman IL-6R monoclonal antibody approved

for the treatment of several rheumatological conditions. Tocilizumab prevents IL-6 binding to both cell associated and soluble IL-6Rs inhibiting signaling and has led to clinical improvement in patients with life-threatening CRS within 24 hours of administration.[1, 53] For patients failing to respond after two doses of tocilizumab, steroids will be second line for CRS. Further management beyond second line therapy will depend upon the judgment of the clinician and may include additional immunosuppression such as Siltuximab or other agents that can inhibit T cell proliferation/function.

7.3 Other Toxicities

Other toxicities include those associated with lymphodepleting chemotherapy regimen which includes fludarabine with cyclophosphamide.

Hematologic Toxicities	Grade 3 or 4 anemia, neutropenia, and thrombocytopenia are potential toxicities associated with lymphodepleting chemotherapy.
Infection	Neutropenic fever is a potential toxicity associated cytotoxic chemotherapy.
Tumor Lysis Syndrome	Can occur as a result of tumor cell death with treatment with cytotoxic chemotherapy.
Organ toxicity	Can be pulmonary, renal, liver, or cardiac toxicity related to chemotherapy administration

8 ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS

8.1 Definitions

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

The investigator and his or her team will follow the Medical College of Wisconsin policies related to adverse event reporting. This information may be found on the Human Research Protection Program website. (http://www.mcw.edu/hrpp/InvestigatorsandStudyStaff.htm)

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

- **Death.** Results in death.
- **Life threatening**. Is life threatening (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- **Hospitalization**. Requires inpatient hospitalization or prolongation of an existing hospitalization (see clarification in the paragraph below on planned hospitalizations).
- **Disability/incapacity**. Results in persistent or significant disability or incapacity. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- **Medically important event**. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent 1 of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization; any organism, virus, or infectious particle (e.g., prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term severe is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as serious, which is based on patient/event outcome or action criteria described above, and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations. Planned hospitalizations (e.g. admission for CAR-T infusion) will not be considered an adverse event.

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

The investigator and his or her team will follow the Medical College of Wisconsin policies related to unanticipated problems involving risks to subjects or others. This information may be found on the Human Research Protection Program website (http://www.mcw.edu/hrpp/InvestigatorsandStudyStaff.htm)

8.3 AE Attribution and Grading

Adverse Event Grading

Will be performed using the NCI CTCAE Version 5.0 except for CRS which will be graded as described in Appendix F per clinical guidelines.

Adverse Event Attribution

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

Relationship Assessment: In-Depth Definitions

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

Definitely Related: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to CAR-20/19-T cell administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Probably Related: There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time sequence to CAR-20/19-T cell administration, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

Possibly Related: There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse event from CAR-20/19-T cell infusion 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 CAR-20/19-T cell 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 CAR-20/19-T cell 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.

Known AEs List

Potential risks associated with this treatment include symptoms/complications associated with CRS and neurotoxicity. Section 1.7 lists the known symptoms/complications associated with CRS and other known risks of CAR-T cell administration.

Time Period for AE Capture

AE collection schedule detailed in Table 1: Schedule of Events

8.4 Monitoring and Recording an Adverse Event

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

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

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

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

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

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

Follow-up of Adverse Events

All adverse events will be followed with appropriate medical management 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.

8.5 Procedure for Reporting Drug Exposure during Pregnancy and Birth Events

If a woman becomes pregnant, or suspects that she is pregnant, while participating in this study, she must inform the investigator immediately. The sponsor-investigator must notify the DSMC and IRB. The pregnancy must be followed for the final pregnancy outcome. As this study utilizes an investigational agent under IND with the FDA, this will be reported to the FDA.

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

8.6 Subject Complaints

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

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

A product complaint is a verbal, written or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality or stability of a drug product. 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.

8.7 Routine Reporting Procedures for AEs

Expedited Reporting Procedures for SAEs, UPIRSOs and DLTs.

Since this is an investigator-initiated study, the principal investigator, also referred to as the sponsor-investigator, is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the sponsor- investigator's IRB. Regardless of expectedness or causality, all SAEs (including serious pretreatment events) must also be reported to the DSMC as soon as possible, but no later than five calendar days of the sponsor-investigator's observation or awareness of the event.

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

The investigator will assess all adverse events and determine reporting requirements to the MCWCC Data and Safety Monitoring Committee (DSMC) and MCW's Institutional Review Board, and, when the study is conducted under an Investigational New Drug Application (IND), to the Food and Drug Administration (FDA), if it meets the FDA reporting criteria. The investigator will report SAEs to any regulatory agency and to the sponsor- investigator's IRB.

All adverse events, whether or not unexpected, and whether or not considered to be associated with the use of the study drug, will be entered into OnCore®.

Reporting to the Data and Safety Monitoring Committee

Regardless of expectedness or causality, all SAEs (including serious pretreatment events) must also be reported to the DSMC as soon as possible, but no later than **five calendar days** of the sponsor-investigator's observation or awareness of the event.

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 as a guideline whenever possible.

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

Reporting to MCW Committee Institutional Review Board

The principal investigator must report unanticipated problems involving risks to subjects or others (UPIRSOs) to the MCW IRB within five business days of his/her awareness of the event.

[Guidance on Adverse Event Reporting to the IRB is available online at MCW IRB Policies and Procedures.] (http://www.mcw.edu/hrpp/policiesprocedures.htm)

Expedited Reporting to the Food and Drug Administration

As this study is being conducted under an IND, the sponsor-investigator is responsible for determining whether the suspected adverse reaction meets the criteria for expedited reporting in accordance with Federal Regulations (21 CFR §312.32).

The investigator will report in an IND safety report any suspected adverse reaction that is both serious and unexpected. In addition, per FDA recommendations, the sponsor-investigator will report all CRS ≥Grade 3 and Neurotoxicity ≥Grade 3 that occurs as a result of CAR-20/19-T cell infusion. For other events, the sponsor-investigator needs to ensure that the event meets all three definitions: *suspected adverse reaction, 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. The timeline for submitting an IND safety report to FDA is no later than **15 calendar days** after the investigator determines that the suspected adverse reaction qualifies for reporting (21 CFR 312.32(c)(1)).

Any unexpected fatal or life-threatening suspected adverse reaction will be reported to FDA no later than **seven calendar days** after the Investigator's initial receipt of the information (21 CFR 312.32(c)(2)).

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

Any other form deemed appropriate by the sponsor-investigators

9 PHARMACEUTICAL INFORMATION

9.1.1 CAR-20/19-T cells

Product Description
Each CAR-20/19-T cell line is a unique product prepared from patient peripheral blood CD4+ and CD8+ T cells. The cells have been engineered to express two different extracellular single chain antibodies (scFv) recognizing CD20 and CD19. The antibodies are linked to tandem signaling domains comprised of the CD3ζ signaling module linked to the 4-1BB costimulatory domain (**Figure 3**). The CAR-20/19-T cells if given fresh without cryopreservation will be in an infusion medium of an isotonic electrolyte solution (Plasma-Lyte® A or Normosol® R) containing 2.5% HSA. If needed, the CAR-T cells will be cryopreserved in infusible grade cryomedium at two different cell numbers. For cryopreserved cells the infusion medium will consist of 10% di-methylsulfoxide, 4% human serum albumin, in 85% Normosol R.

Mechanism of Action:

When the antibody chains on the CAR-20/19-T cells bind to their ligands on the surface of B cells the T cells are triggered to kill the cells to which they bind. A given T cell can bind and kill multiple target cells.

Metabolism:

The CAR-20/19-T cells will be activated by ligand engagement and are expected to expand in vivo. Transduced cells are expected to persist for a period of months to years.

Contraindications:

Please see section 7.1 for contraindications to infusion

Side Effects:

Side effects are thoroughly detailed in section 1.7. Briefly major toxicities include:

- B cell ablation- Since CD19 and CD20 are also expressed on healthy B cells, the CAR-20/19-T cells are expected to eliminate these cells from the circulation as long as the cells persist. The loss of B cells could result in an increased risk of infection.

- Allergic Reaction- The scFv on the T cell surface may invoke an immune reaction that might eliminate the CAR-20/19-T cells and secondarily cause an autoimmune response, though such responses have not been reported in clinical trials to date.

- Transformation- The genetic modification of the CAR-20/19-T cells could result in transformation. To date transformations of CAR-T cells have not been reported

- Tumor lysis syndrome (TLS) - Patients with a high tumor burden may experience a reaction due to lysis of the tumor cells either from the lymphodepleting preparative regimen or from the CAR-20/19-T cells. Such reactions have been reported and can be severe. With careful monitoring, appropriate therapies are available that can mitigate this reaction.

- Cytokine Release Syndrome (CRS)- In vivo expansion of the infused CAR-20/19-T cells may be associated with a release of inflammatory cytokines. Such responses have been reported, some have been severe. Close monitoring and appropriate interventions are available that can mitigate this reaction.

- Neurotoxicity- CAR-T therapy has led to some patients having unpredictable neurotoxicity in the form of seizures, aphasias, and in rare cases life threatening cerebral edema. Clinical vigilance and early intervention can help prevent and/or treat this complication.

- Unknown- Given this first-in-human use of CAR-20/19-T cells unknown side effects may occur either associated with the infusion or long term. Monitoring for such effects is described in this protocol

9.1.2 Solution Preparation

The CAR-20/19-T cells will be administered either fresh following harvest or within 6 weeks of cryopreservation in the appropriate doses for infusion. Cryopreserved cells will be thawed within the LPL prior to administration. The CAR-T cells will be transported at 1-10^{o}C to the infusion site. If the dose level changes from the time of product cryopreservation to time of infusion, the volume of product infused will be adjusted accordingly.

9.1.3 Investigational Agent Administration

The cells will be administered from 1-7 days after completion of chemotherapy to deplete lymphocytes. The patient will be premedicated. Vital signs will be monitored 15 minutes prior to infusion and every 15 minutes after infusion for the first hour. Monitoring will be continued until all signs are stable. The cells will be administered over a period of 5-15 minutes by IV injection. See Section 6 for further details.

9.1.4 Storage Requirements

For patients requiring cryopreservation, the cells will be stored cryopreserved in the LPL until the time of infusion. Cryopreservation storage is in vapor phase liquid nitrogen at temperatures <-150^{o}C in devices that are continuously monitored for temperature according to standard laboratory practices.

Fresh cells will be stored at 1-10^{o}C in a monitored refrigerator and will be given within 48 hours of harvest or cryopreserved accordingly.

9.1.5 Stability

Fresh cells have been demonstrated to be stable in infusion medium up to 48 hours after harvest when stored at a concentration <20 x 10^{6} per mL at 1-10^{o}C. Longer term phenotypic and functional stability data up to 1 year is shown in the below table and Figure 11. Based on the 1-year data, the product is likely stable indefinitely while in the cryopreserved state. Once thawed, the cell infusion must start within 45 minutes.

Phenotypic Stability of CAR-20/19-T Cell Products

CAR-T Cell Phenotype Pre and Post Cryopreservation						
Subjects	**Time from Cryo to Thaw**	**CD3**	**CD4**	**CD8**	**NK**	**NKT**
SHAH-002	Fresh product	99.18%	57.60%	48.34%	0.50%	5.60%
SHAH-002	1 month	97.03%	52.94%	46.80%	0.20%	0.60%
SHAH-002	3 month	99.18%	48.27%	51.41%	0.30%	2.30%
SHAH-002	1 Year	99.63%	53.90%	48.00%	0.10%	2.50%
SHAH 003	Fresh product	99.30%	62.11%	39.18%	0.00%	0.00%
SHAH 003	2 weeks	99.38%	60.14%	40.15%	0.00%	0.50%
SHAH 003	1 month	98.93%	59.97%	41.25%	0.10%	0.60%
SHAH 003	6 month	99.75%	53.79%	47.62%	0.00%	0.70%
SHAH 003	1 Year	99.93%	61.22%	40.75%	0.00%	0.40%
SHAH 004	Fresh product	99.83%	64.34%	42.26%	0.00%	0.50%
SHAH 004	6 month	99.85%	58.64%	45.13%	0.00%	0.20%
SHAH 004	1 Year	99.85%	53.32%	50.72%	0.00%	0.60%

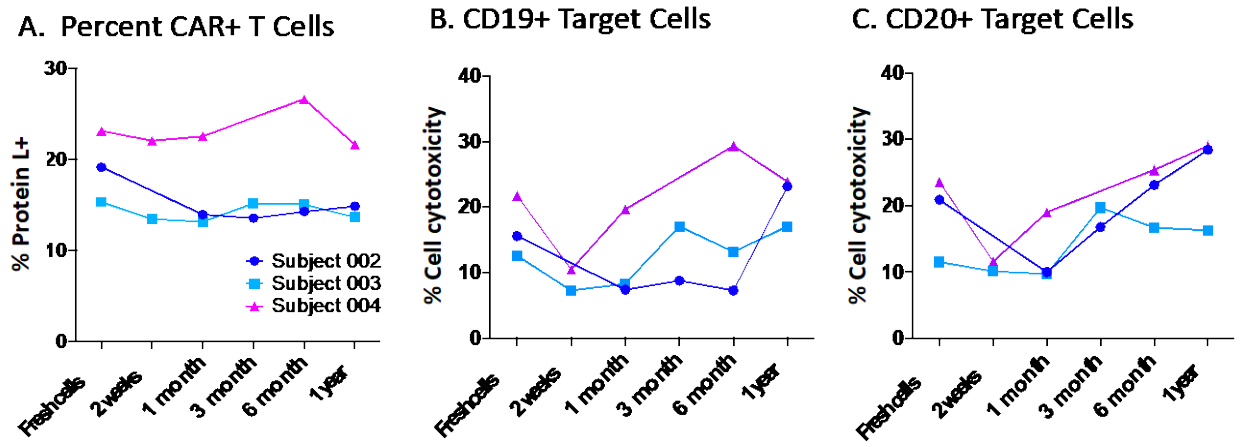


Figure 11: Phenotypic and Functional Stability of CAR-20/19-T Cell Products. CAR-20/19-T cell products generated from Subjects 002-004 in the Phase I trial were cryopreserved, thawed at the indicated time points to assess **(A)** Protein L positivity to assess surface CAR expression, **(B)** cytotoxicity versus CD19+ target cells, and **(C)** cytotoxicity versus CD20+ target cells. Cell cytotoxicity was conducted at an effector:target (E:T) cell ratio of 50:1 (***note***: the E:T ratio was not adjusted based on the percentage of CAR-T cell present in the product; it was based on total T cells).

9.1.6 Route of Administration

The CAR-20/19-T cells will be administered intravenously.

9.1.7 Nursing Implications

Premedication and patient monitoring before, during, and after each split dose infusion is required. See Section 6 for details

9.1.8 Handling

The required dose of CAR-20/19-T cells are transported at 1-10^{o}C. The cells are infused at room temperature over a 5-15-minute period by IV injection. For cryopreserved cells, infusion should start no longer than 45 minutes after thawing.

9.1.9 Availability

Each lot of CAR-20/19-T cells is patient-specific. The cell lines are produced on site by the MCW cell processing laboratories.

9.1.10 Agent Ordering

CAR-20/19-T cells are produced at MCW and are a patient-specific material. A physician order for infusion specifying the dose of transduced cells per kg to be infused is required.

9.1.11 Agent Accountability

CAR-20/19-T cells are identified by a donor identification number (DIN) that is used to track the product at collection to the product at disposition (infused or discarded) and to trace the product at infusion to the donation. CAR-20/19-T cells are for autologous use only and are labeled accordingly.

9.1.12 Agent Destruction and Return

Once issued from the LPL, the CAR-20/19-T cell line cannot be returned for reissue. Aliquots of the lines must be maintained for potential further analysis; however, the cells are for autologous use only and may not be released to another patient.

9.2.1 Tocilizumab

Product Description

Tocilizumab is a monoclonal antibody and immunosuppressant; specifically, tocilizumab is an interleukin-6 (IL-6) receptor antagonist. IL-6 is a pleiotropic pro-inflammatory cytokine and is produced by various cells including T- and B cells, lymphocytes, monocytes and fibroblasts. This agent will be part of the first line treatment for patients with CRS requiring more than supportive measures for medical management (**Appendix F for CRS management guidelines**).

Mechanism of Action

Tocilizumab binds IL-6 receptors (sIL-6R and mIL-6R) and inhibits IL-6-mediated signaling through these receptors. IL-6 has been shown to be involved in T cell activation, induction of immunoglobulin secretion, initiation of hepatic acute phase protein synthesis, and stimulation of hematopoietic precursor cell proliferation and differentiation.

Contraindications

Administration of live vaccines is contraindicated during treatment with tocilizumab. Use is contraindicated in patients with known hypersensitivity to tocilizumab.

Drug Interactions

Use of tocilizumab may cause increased metabolism of medications that are CYP450 substrates including CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Caution should be used in medications that are CYP450 substrates including simvastatin, dextromethorphan and omeprazole, and medications with a narrow therapeutic index.

Side Effects

- Myelosuppression: Tocilizumab may lead to neutropenia and thrombocytopenia.
- Hepatic enzyme and lipid parameter alterations: Tocilizumab may lead to an elevation in liver enzymes and an increase in lipid parameters including: total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol.
- Immunosuppression: Tocilizumab may lower patient's resistance to infections.
- Gastrointestinal perforation: Events have occurred in clinical trials, caution in patients at increased risk of developing gastrointestinal perforation

9.2.2 Preparation

Tocilizumab should be diluted in 100 mL of 0.9% sodium chloride. Prior to addition of tocilizumab, withdraw a volume of 0.9% sodium chloride from the infusion bag equal to the volume of tocilizumab required (total volume of final preparation should be 100 mL). Gently mix the infusion to avoid foaming

9.2.3 Tocilizumab Administration

Tocilizumab should be administered over 60 minutes as an intravenous infusion. The infusion should have a dedicated line and not be infused concomitantly with other drugs.

9.2.4 Storage

Vials must be refrigerated at 2ºC to 8ºC (36°F to 46°F). Do not freeze. Vials should be protected from light and stored in the original package until time of use. Prepared parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Do not use the product if visibly opaque particles, discoloration, or other foreign particles are observed in the solution.

9.2.5 Stability

Prepared solutions may be stored at 2ºC to 8ºC (36°F to 46°F) or room temperature for up to 24 hours.

9.2.6 Handling

Tocilizumab will be handled following the institutional hazardous medication policy.

9.2.7 Availability

Tocilizumab is available in single dose vials (20 mg per mL) of 80 mg per 4 mL, 200 mg per 10 mL, and 400 mg per 20 mL.

9.2.8 Agent Ordering

Tocilizumab is commercially available

9.2.9 Agent Destruction and Return

Tocilizumab will be destroyed based on institutional standards

10 REPORTING AND DOCUMENTING RESPONSE (MEASUREMENT OF EFFECT)

10.1 Evaluation of Efficacy (or Activity)

Definitions
Evaluable for toxicity
- All patients will be evaluable for toxicity from the time of their first study drug treatment.

Evaluable for objective response
- Only those patients who have received CAR-20/19-T cells will be re-evaluated and considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the first disease assessment at Day +28 will also be considered evaluable.)

10.2 Response Criteria

Response assessment will vary by disease subtype and specific criteria are listed below for CLL/SLL and NHL.

10.3 Chronic Lymphocytic Leukemia Response Criteria

Response criteria as per 2008 guidelines for diagnosis and treatment of CLL/SLL [61]

Complete Response (CR)
1. Peripheral blood lymphocytes (evaluated by blood and differential count) below 4.0 x 10^9/L (4000/µL)
2. Absence of significant lymphadenopathy (e.g., lymph nodes >1.5 cm in diameter) by physical examination and CT scan
3. No hepatomegaly or splenomegaly by physical examination or CT imaging
4. Absence of constitutional symptoms
5. ANC >1500/µL, Hemoglobin >11.0 g/dL, Platelets >100,000/ µl
6. Bone Marrow Biopsy confirmation

Complete response with incomplete marrow recovery (CRi)

1. Fulfill all criteria for a CR but have persistent anemia, thrombocytopenia, or neutropenia felt to be unrelated to CLL but related to drug toxicity.

Partial Remission (PR)

1. A decrease in the number of blood lymphocytes by 50% or more from value before therapy
2. Reduction in lymphadenopathy by CT scans as defined by the following
    a. A decrease in lymph node (LN) size by 50% or more either in the sum products of up to 6 lymph nodes, or in the largest diameter of the enlarged node(s) detected prior to therapy
    b. No increase in any LN and no new enlarged LN. In small LN (<2 cm), an increase of less than 25% is not considered to be significant.
3. A reduction in the pretreatment enlargement of the spleen or liver by 50% or more as detected by CT scan
4. Blood count should show one of the following results
    a. Neutrophils >1500/µl without exogenous growth factors
    b. Platelet count >100,000/µl or 50% improvement over baseline without need for exogenous growth factors
    c. Hemoglobin >11.0g/dL or 50% improvement from baseline without requiring RBC transfusions or exogenous erythropoietin.

Progressive Disease

1. Lymphadenopathy
    a. Appearance of any new lesion, such as enlarged lymph nodes (>1.5 cm), splenomegaly, hepatomegaly, or other organ infiltrates.
    b. An increase by 50% or more in greatest determined diameter of any previous site.
2. An increase in the previously noted enlargement of the liver or spleen by 50% or more or the de novo appearance of hepatomegaly or splenomegaly.
3. An increase in the number of blood lymphocytes by 50%or more with at least 5000 B lymphocytes per microliter.
4. Transformation to a more aggressive histology (e.g., Richter syndrome). Whenever possible, this diagnosis should be established by lymph node biopsy.
5. Occurrence of cytopenia (neutropenia, anemia, or thrombocytopenia) attributable to CLL

Stable Disease

1. Patients who have not achieved a CR or a PR, and who have not exhibited progressive disease, will be considered to have stable disease (which is equivalent to a nonresponse).

10.4 Non-Hodgkin's Lymphoma Response Criteria

2014 Lugano Classification will be utilized for response assessment in patients with NHL. [62]

Figure 12: 5 point Deauville Scale

Score 1: no uptake

Score 2: uptake ≤ mediastinum

Score 3: uptake > mediastinum but ≤ liver

Score 4: moderately increased uptake > liver

Score 5: markedly increased uptake > liver and/or new lesions related to lymphoma

Score X:
New areas of uptake unlikely to be related to lymphoma

Complete Response (CR)
PET/CT Based Response

- Complete metabolic response: Score 1, 2, 3 with or without a residual mass on the 5 point Deauville Scale (**Figure 12**)
- No evidence of FDG-avid disease in marrow

CT-Based Response

- Target nodes/nodal masses must regress to ≤1.5 cm in longest transverse diameter (LDi)
- No extralymphatic sites of disease
- Negative BM biopsy if positive at baseline
- Regression of organ enlargement to normal

Partial Response (PR)
PET/CT Based Response

- Score 4 or 5 on Deauville scale with reduced uptake compared with baseline and residual masses of any size

CT-Based Response

- ≥50% decrease sum of the product of the perpendicular diameters (SPD) of up to 6 dominant measurable nodes and extranodal sites; no criteria for progressive disease are met
    - When a lesion is too small to measure on CT, assign 5 mm x 5 mm as the default value. When no longer visible, 0 x 0 mm
    - For a node >5 mm x 5 mm, but smaller than normal, use actual measurement for calculation
- If enlarged, spleen must have regressed by >50% in length

Stable Disease (SD)
PET/CT Based Response

- Score 4 or 5 on Deauville scale with no significant change in FDG uptake from the baseline scan

CT-Based Response

- <50% decrease from baseline SPD of up to 6 dominant measurable nodes and extranodal sites, no criteria for progressive disease are met.
- No increase in non-measured lesions consistent with progression
- No increased in organ enlargement consistent with progression.

Progressive Disease (PD)
PET/CT Based Response

- Score 4 or 5 on Deauville scale with increase in intensity of uptake from baseline
- New FDG-avid foci consistent with lymphoma
- New or recurrent FDG-avid foci in bone marrow

CT-Based Response

- An individual node/lesion must be abnormal with longest transverse diameter >1.5 cm and have an increase by ≥50% from cross product of the LDi and perpendicular diameter and have an increase in the LDi or shortest axis perpendicular (SDi) from nadir as follows:
    - 0.5 cm for lesions ≤2 cm
    - 1.0 cm for Lesions >2 cm
- In the setting of splenomegaly, the splenic length must increase by >50% of the extent of its prior increase beyond baseline (eg: a 15-cm spleen must increase to >16 cm). If no prior splenomegaly, must increase by at least 2 cm from baseline
- New or recurrent splenomegaly
- New or clear progression of pre-existing non-measured lesions
- Re-growth of previously resolved lesions
- A new node >1.5 cm in any axis
- A new extra nodal site > 1.0 cm in any axis; if <1.0 cm in any axis is presence must be unequivocal and must be attributable to lymphoma
- Assessable disease of any size unequivocally attributable to lymphoma

10.5 Response Definitions

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria. The first disease assessment will occur at 4 weeks' post treatment (Day+28). Response assessment will continue up to 2 years or until progression/death.

Duration of Response

Duration of overall response

The overall response duration is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The overall CR duration is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease

Stable disease is measured from the start of the treatment until the criteria progression are met, taking as reference the smallest measurements recorded since the treatment started.

Progression-Free Survival

Progression-free survival (PFS) is defined as the time duration from treatment start to progression time.

10.6 Evaluation of Safety

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the CTCAE v5.0 for reporting of non-hematologic adverse events and modified criteria for hematologic adverse events.

11 CORRELATIVE STUDIES/SPECIAL STUDIES

11.1 T Cell Diversity Analysis During CAR-T Cell Therapy

Diversity of the T cell repertoire (as measured by T cell receptor sequencing) has been associated with effective T cell immunity. Conversely, low levels of T cell diversity have been correlated with aging, infection, and the development of cancer. The relation between the diversity of the T cells used for CAR-T therapy and outcomes are not understood. Neither is the effect of generating CAR-T on repertoire diversity understood. Both could be important parameters in therapy outcomes.

We will measure the diversity of the circulating T cell repertoire at three time points during the study to examine: 1) the effect of generating the CAR-T cells and 2) any possible effect of infusion of the CAR-T product on the overall circulating repertoire of subject. The initial sample of 10^7 mononuclear cells will be obtained at time of apheresis and the T cell repertoire diversity will act as the benchmark for the two comparative analyses. The second sample will consist of the same number of cells taken from the final CAR-T product prior to infusion into the subject. The final sample will consist of 10ml of blood and will be obtained as part of the 28-day disease assessment.

- Sample 1: Will be obtained from the apheresis product collected at Day -15
- Sample 2: From final CAR-T cell product prior to patient infusion
- Sample 3: Peripheral blood sample (10 ml) on Day +7
- Sample 4: Peripheral blood sample (10 ml) on Day +28 (+/- 3 days)

The samples will be labeled with a unique patient identifier and sent to BloodCenter of Wisconsin (BCW) for T cell repertoire analysis. At BCW the de-identified samples will be subject to cell separation into CD4 and CD8 T cells, cDNA tagged with unique barcodes will be prepared and subject to BV gene-specific amplification and single cell RNA sequencing (scRNA-Seq). The amplicons will be pooled and subject to NGS sequencing on the Illumina platform. The sequence data will be clustered on the basis of unique CDR3 sequence (clonotype), whole transcriptome and the number of unique cDNA-defining barcodes counted to quantify the clonotype. Diversity and complexity measures will be derived, the latter involving not just the number of unique species but their frequency distribution. A similar analysis will be performed on the basis of the amino acids encoded by the CDR3 sequence (motif analysis). As needed the depth of sequencing can be modulated to bring up the resolution level.

The repertoire diversity analyses and scRNA-Seq will describe the initial diversity of the repertoire and show whether the CliniMACS Prodigy-derived CAR-T cells have undergone major shifts in the T cell population being infused into the patient. Both results will be most informative when compared to other clinical parameters and outcomes. Comparison of the initial circulating repertoire diversity with that of the circulating repertoire after infusion of the CAR-T product will show if there have been significant changes in the repertoire and differentiation

states due to the functioning of the CAR-T cells, and comparison of the data with clinical outcomes should be informative. The risk associated with these analyses is minimal. The lab at BCW will be working with de-identified samples. The investigators at BCW are experts in this area of analysis. The information gained will be useful for the overall interpretation of outcomes.

11.2: Circulating Tryptophan Metabolites and Gene Expression Patterns and risk for CNS toxicity during CAR-T cell Therapy

Cytokine release syndrome (CRS) is a systemic inflammatory reaction that is observed in a high proportion of patients receiving CAR-T-based therapies. CRS is driven by an increase in the release of proinflammatory cytokines that are released after the activation of monocytes, macrophages and some lymphocyte populations. In addition to CRS, and perhaps as a consequence, patients receiving CAR-T therapies also experience CNS toxicity, including tremor, seizures and life-threatening cerebral edema. Although the pathophysiology of these neurological effects is not clear, inflammatory cytokines are thought to be involved.

Human studies, primarily carried out in patients with major depressive illness, indicate that the kynurenine pathway is a critical link between inflammation and damage to the brain. The kynurenine pathway is activated by inflammatory cytokines (predominantly interferon gamma) released in the brain by activated T cells and leads to the production of kynurenine from tryptophan as a result of up-regulation of the enzyme indoleamine 2,3-dioxygenase (IDO) by microglia or brain-resident macrophages. Kynurenine is further metabolized to neurotoxic metabolites 3-hydroxykynurenine (3-HK) and quinolinic acid (QA) and to the neuroprotective metabolite, kynurenic acid (KA). The ratios of serum KA/3-HK and/or KA/QA have been used previously as indices of the role of this inflammatory pathway on brain function. Low ratios are seen in patients with mood disorders, for example, compared to controls. Further, we have identified a gene expression profile of 53-genes termed the “conserved transcriptional response to adversity” (CTRA), a gene expression pattern associated with exposure to chronic stressors and whose largest gene subset is comprised of proinflammatory genes. This CTRA gene expression has been associated with worse outcomes following HCT.

We hypothesize that neuroinflammation is responsible for the adverse effect of CAR-T cell therapy on the brain and that the serum KA/3-HK and KA/QA ratios as well as CTRA profiles will serve as biomarkers for neuroinflammation, and thus, as biomarkers for the risk of CNS toxicity.

We will measure the serum concentrations of tryptophan, kynurenine, 3-HK, QA, KA, and gene expression patterns (CTRA) at 4 time points during the study to examine: (1) the trajectory in the concentration of each metabolite and gene expression in the month following CAR-T cell therapy; and (2) the correlation between the KA/3-HK and KA/QA ratios as well as gene expression and the occurrence of CNS toxicity. A baseline sample will be assessed on the day of apheresis and will serve as a benchmark for the trajectory study. Two additional blood samples will be analyzed during the first month after treatment (14 and 28 days) and one sample will be analyzed after three months (day 90) to assess recovery. The samples will be collected along with other blood samples at these time points in the study.

The samples will be collected via standard venipuncture in serum separating tubes (metabolites) and PaxGene RNA tubes (gene expression) and will be collected using standard protocols and stored at -80 degrees in cryotubes labeled with unique patient identifiers until analysis. The samples will be sent to the laboratory of Dr. Cecilia Hillard in the Neuroscience Research Center. The serum metabolite tubes will be processed for the concentrations of 5 analytes: tryptophan, kynurenine, 3-HK, QA and KA using high performance liquid

chromatography with tandem mass spectrometry. The extraction and derivatization of the samples will be carried out in the Hillard laboratory and the mass spectroscopy will be performed in the Department of Pharmacology at the Medical College of Wisconsin. The PaxGene RNA tubes will be stored until they can be shipped in batch to the UCLA Social Genomics Core (Directed by Dr. Steve Cole, Professor, Hematology/Oncology, UCLA), who will conduct all gene expression profiling on obtained blood samples by using Illumina HT-12 human gene expression bead arrays. Total RNA will be extracted from the whole blood samples stored at MCW, subjected to quality assurance assays to test suitable mass (by spectroscopy) and integrity (by Agilent Bioanalyzer RNA Integrity Score) for analysis, and subjected to microarray target synthesis and hybridization in collaboration with the UCLA Neuroscience Genomics Core Laboratory using standard Illumina assay equipment and protocols. The output of these analyses is quantification of whole genome RNA production. The laboratory staff will work with de-identified samples and the data will be provided to the study PI for correlation with CNS toxicity in the same patients.

The information gathered from this study will provide important mechanistic information regarding the relationships among the tryptophan-kynurenine pathway, proinflammatory gene expression, and the occurrence of CNS toxicity, and could elucidate these metabolites and gene expression clusters as potential biomarkers for the occurrence of CNS toxicity in patients undergoing CAR-T therapy.

11.3: Patient-reported outcome (PRO) measures following CAR-T cell therapy

Related to or as a consequence of CNS and its neurotoxicities, including neuroinflammation, patients receiving CAR-T therapies may be at risk for diminished quality of life and patient-reported outcomes (PROs), including the development of depression, anxiety, fatigue, sleep disturbances, and pain. There are several mechanistic modalities by which engineered autologous CAR-T cells may influence and/or be affected by PRO measures.

First, immune activation, inflammation, and cytokine activation - as occurs with CRS - are known to affect cognitive and/or emotional functioning,[63] including among cancer patients.[64] Second, while patient-reported symptomatology may be less salient during the initial acute period of potentially serious illness, as can occur with CRS [1, 53, 54], the acute and likely subsequent inflammation may play a key role in the development of depression and other psychopathologic symptoms; it is unknown what longer-term QOL sequelae may be present after an initial severe (and potentially ongoing) inflammatory insult. Prior research indicates that inflammation itself is involved in both the genesis and foreshadowing of future depressive or psychotic episodes.[65]

Third, altered central nervous system (CNS) functioning and subsequent emotional/cognitive responses influence immunity and T cell function among cancer patients.[66]Further, these adverse responses are predictive of compromised immune reconstitution[67] and adverse outcomes following HCT.[68-70]Therefore, it is critical to assess whether or how CNS and psychological functioning will impact the efficacy of this new and likely increasingly used cancer treatment modality. As infection remains a significant risk of this therapy, it will be important to understand and optimize any factors that could contribute to increased infectious susceptibility, including neuroimmunologic influence.

Finally, it is unknown what longer-term (1-2 years and beyond) effects such enhanced immune activation will have. Given the aforementioned immune/CNS effects and increasing emphasis

on QOL and PROs as mainstay parameters in assessing cancer treatment efficacy,[71, 72] it is important to ascertain late effect PROs.

We will measure the following PROs (see **Appendix I** for questionnaires) during the ancillary study at the same time points as the biomarkers described in section 11.2 (at apheresis, D+14, D+28, D+90), as well as at the additional time points of 6 months, 1 year, and 2 years:

Depression: Depression will be assessed through the General Depression subscale of the Inventory of Depression and Anxiety Symptoms (IDAS) and includes 20 questions with a scoring range of 20-100 (mean in community dwelling adult of 44.99 and standard deviation of 14.75).[73]

Anxiety: Anxiety will be assessed using two subscale items of the IDAS including panic (health population mean = 12.58, SD = 5.26) and traumatic intrusions (healthy population mean = 7.60, SD = 4.20)[73]

Fatigue: The Fatigue Severity Index (FSI) will be utilized to assess fatigue; a score of 3 or greater on items assessing fatigue in the past week (average of items 1-3; FSI Composite) indicates clinically meaningful fatigue.[74] The FSI can also be evaluated using the average rating of the degree to which fatigue interfered with some general activities (0-10; FSI Interference); participants' ratings of the number of days in the past week they felt fatigued (0-7; FSI Days); and participants' rating of what percent of each day in the past week, on average, they felt fatigued (0-100; FSI Percent).[75] Individuals scoring at or above the cutoff also report significantly greater scores on these other subscales.

Sleep: Sleep will be assessed using the Pittsburgh Sleep Quality Index (PSQI), with a score of >5 considered disturbed sleep as adjusted for cancer populations.[76, 77]

Pain: The Brief Pain Inventory (BPI) assesses pain intensity as well as pain-related interference in function.[78] BPI Pain Severity score ranges from 0-40 (first four items), and the BPI Pain Interference score is a mean of the last 7 items (5a-5g) with a range of 0-10.

The information gathered from this study will provide important data regarding the impact of CAR-T therapy on patient QOL outcomes.

Guidelines for reporting of Depression and/or Suicidal Ideation:

Should the patient endorse any thoughts of suicidality or self harm per the IDAS, the study co-investigator for this portion of the ancillary testing (Dr. Jennifer Knight) will contact them by phone and standard of care referrals will be made. Should completion of the study surveys prompt participants to want treatment for any of the other symptoms, participants will be offered a referral for appropriate care through the Quality of Life Center at the Froedtert Cancer Center.

12 STATISTICAL CONSIDERATIONS

12.1 Study Endpoints

There are two primary endpoints in this study—feasibility and safety.

Feasibility of CAR-20/19-T cell production by the CliniMACS Prodigy processing device (Miltenyi Biotec) will be measured. At minimum, we expect to reach our target CAR-20/19-T cell dose in at least 75% of patients with an adequate T cell starting dose. Feasibility will be measured at the end of the study after completion of accrual. If >25% of patients with an adequate T cell starting dose were unable to achieve goal CAR-20/19-T cell dose, we would deem that this technique is not a feasible method for production.

Safety of administration of CAR-20/19-T cells in patients will be assessed as outlined in Section 6 Treatment plan.

12.2 Accrual Estimates

This is a Phase 1/1b study evaluating the safety and efficacy CAR-20/19-T cells as part of a first-in-human study. We will utilize a 3+3 escalation design to identify the target dose level followed by a dose expansion at the selected dose level. The minimum number of patients enrolled will be 4 patients (2 at dose level 0 and 2 at dose level -1). The maximum number of patients enrolled would be 24, which is dependent on the number of DLTs experienced on trial.

Should this treatment be safe and feasible, we would use the identified dose to open a larger phase II study to demonstrate the efficacy of our treatments.

12.3 Interim Analyses and Stopping Rules

There are no planned interim analyses as part of this Phase 1/1b Study.

Toxicity will be considered unacceptable and lead to clinical hold of the study if any of the following occur in the first 5 patients: one treatment related mortality (TRM) or three grade 4 non-hematological toxicity events as assessed by PI or DSMB. This ensures that there is only a 5% probability of stopping if the actual TRM is $\leq$1%. For Grade 4 non-hematological events not resulting in death, this rule ensures a 5% probability of stopping if the true incidence is <20% and an 68% probability of stopping if the incidence is 60% or more. Additionally, if there are ≥ 2 DLTs at any dose level, no further patients will be allowed administration at that dose level.

In terms of production, if within the first 8 patients with an adequate starting T-cell dose ≥3 patients fail to meet the targeted CAR-20/19-T cell dose, the study will be placed on a clinical hold to re-evaluate production methods and optimize manufacturing.

12.4 Statistical Analyses Plans

Dosing Level	Cell Dose
Dose Level -1	1×10^5 cells/kg
Dose Level 0	2.5×10^5 cells/kg
Dose Level 1	7.5×10^5 cells/kg
Dose Level 2	2.5×10^6 cells/kg

Phase 1

The first 3 patients with NHL or CLL/SLL will be treated consecutively at Dose Level 0 and will be monitored for DLTs for a full 28 days. At each dose level, the first two patients treated

will be treated no sooner than 28 days apart to allow monitoring of acute and sub-acute toxicities. After that patients will be infused no sooner than at 14 day intervals among the specified dose level or in-between two dose levels.

Dose escalation will proceed within each cohort per the following scheme: A cohort of three patients with NHL or CLL/SLL will receive 2.5 x 10^5 cells/kg (Dose Level 0). If there are no DLTs in the first three patients, the dose will be escalated to 7.5 x 10^5 cells/kg (Dose Level 1). If 1/3 patients have a DLT at Dose Level 0, an additional three patients will be enrolled at this dose level. If there is ≤1/6 patients with a DLT, the dose will increase to Dose Level 1. A similar dose escalation schema will be used to escalate from Dose Level 1 to Dose Level 2 (2.5 x 10^6 cells/kg) which is our goal dose. Dose escalations will NOT occur until all patients at the current level are monitored for 28 days after CAR-20/19-T cell infusion.

If two of the first three or six patients with NHL or CLL/SLL develop DLT at Dose Level 0, the dose will de-escalate to 1.0 x 10^5 cells/kg and accrual will proceed per the same schema as above. If two of the first three or six patients with NHL or CLL have a DLT at the lower dose, the study will be stopped for failure to achieve the desired safety endpoint. The minimum number of patients enrolled will be 4 patients (2 at dose level 0 and 2 at dose level -1) if the first four patients all experience a DLT. The maximum number of patients enrolled in our Dose Escalation phase would be 18.

Once a safe dose has been identified, the Dose Expansion phase of the study will be open to accrual once approval is obtained from local and federal regulatory bodies. In this Phase, there will be a 6-12 patient expansion cohort in NHL and CLL/SLL. DLT monitoring will continue in the Dose Expansion phase. If ≥2 patients experience a DLT within this expansion cohort, the expansion phase will be placed on hold and will be re-opened only after discussion with the local DSMC and IRB.

Phase 1b

The Phase 1b cohort will be open to accrual once the final subject has enrolled on the Phase 1 portion of the study. This will test the safety of single day CAR-T infusion.

Expansion Single Infusion

9 patients will be enrolled at the goal dose of 2.5 x 10^6 cells/kg. The first two patients at this dose level will be observed for 14 days prior to additional enrollment. Among this cohort if there are ≥3 DLTs, this arm will be closed early.

Dose limiting Toxicity

The dose limiting toxicity will be based on the tolerability observed during the first 28 days of treatment/observation. If multiple toxicities are seen, the presence of dose limiting toxicity will be based on the most severe toxicity experienced. If a patient dies from a cause felt to be *unrelated* to CAR-T cells (e.g., progressive disease) before completion of the 28 day DLT monitoring period, that patient will be replaced. Patients who experience toxicity from lymphodepletion chemotherapy that prevents administration of CAR-20/19-T cells will also be replaced. All patients who fail to produce the desired dose level will be followed for DLTs, AEs, and survival but will be replaced for this dose-finding and dose-expansion study.

The definition of DLT for this study is detailed in **Section 3.4**

The statistical analysis will be primarily descriptive in keeping with the exploratory nature of the study. We will present summary tables of baseline demographic and clinical characteristics of the patients. Adverse events will be tabulated for both overall and within major categories and by grade. Analysis of other secondary endpoints such as anti-tumor activity will be presented as response rates by disease specific sub-type.

Statistical support will be provided by Mei-Jie Zhang, PhD the senior statistician on this study. Dr. Zhang will participate in the study design, data analyses, and the scientific manuscript writing associated with this project.

12.5 Analysis Population

All patients enrolled and consented for this protocol who underwent CAR-20/19-T cell production will be evaluable for our feasibility endpoint. Only patients who receive CAR-20/19-T cells will be evaluable for our safety endpoint.

12.6 Lost to Follow-up

Should a subject fail to return to the clinic for a scheduled protocol specific visit, personnel at the investigational site will need to make 2 attempts by a combination of telephone/e-mail and mail to contact the subject. Sites must document both attempts to contact the subject. If a subject does not respond within 1 month after the second contact the subject will be considered lost to follow-up and no additional contact will be required.

13 DATA AND SAFETY MONITORING PLAN (DSMP)

Data and Safety Management Overview

The Medical College of Wisconsin (MCW) Data and Safety Monitoring Committee (DSMC) and the MCW 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 monthly (or as each new patient is dosed if no patients enrolled in the month) safety and progress reports submitted to the DSMC. For the purpose of this study, a medical monitor will be appointed to evaluate serious AEs (Grade 3-5) on a real-time basis. This medical monitor is responsible for evaluating serious AEs and can convene a conference call of DSMC chair, DSMC reviewers, or external reviewers when necessary.

13.1 Study Team

The study team consists of the investigators, clinical research coordinators/assistants, regulatory specialist, pharmacists, laboratory team, and the study biostatistician.

13.2 Quality Assurance

The MCWCC Clinical Trials Office provides ongoing quality assurance audits

13.3 Clinical Trials Office

The MCWCC Clinical Trials Office [CTO] provides administrative assistance and support to the DSMC.

13.4 DSMC

The Medical College of Wisconsin Cancer Center places the highest priority on ensuring the safety of patients participating in clinical trials. Every cancer interventional trial conducted at MCW includes a plan for safety and data monitoring.

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

This study will be reviewed by the Medical College of Wisconsin Cancer Center Data Safety Monitoring Committee (MCW-CC DSMC). External reviewers with CAR-T experience will serve on this committee to provide their expertise. A summary of the MCW CC DSMC activities are as follows:

- Review the clinical trial for data integrity and safety
- Review all unexpected grade 3, and all grade 4, and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol. (Grades 4 & 5 events must be reported to the DSMC within 5 calendar days of study staff's knowledge.) Review all DSM reports
- Submit a summary of any recommendations related to study conduct
- Terminate the study if deemed unsafe for patients

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

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

14 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 Institutional Review Board

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

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.

Informed consent from the patient will be obtained using forms approved by the local institution's IRB. Informed consent will be obtained by the Principal Investigator or Co-Investigators. Consent forms will be IRB-approved and the subject 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 or representative legal guardian will sign and date the informed consent document prior to any procedures being done specifically for the study. 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 MCWCC CTO will follow the MCW/FH IRB's policy for subjects who demonstrate limited English proficiency or limited literacy. The original consent is kept with the subject's study file, and a copy of the consent is sent to the OCRICC office, which will then submit to HIM a copy of the signed consent to be scanned into EPIC, the legal medical record.

14.4 Subject Confidentiality and Access to Source Documents/Data

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

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

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

While data are being collected and after all data have been collected but are still in the process of being analyzed, the subject's data/PHI are stored in the locked Clinical Research office in the

Clinical Trials Office. Databases in which the study subject information is stored and accessed are password protected, allowing for limited access by authorized personnel only. Data/PHI kept in the Case Report Forms in Oncore contain the study identifiers, subject initials, date of birth and date of service.

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

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

Onsite Audits

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

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

14.5 Quality Assurance

This protocol will be reviewed internally every six months by the Cancer Center Clinical Trials Office Quality Assurance Staff. Approximately 30% of subject files will be selected randomly for review (max 10 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 be reviewed at the time of each review.

14.6 Changes in the Protocol

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

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

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

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

15 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. MCW Cancer Center database will be utilized for data collection and case report forms through OnCore.

15.2 Study Record Retention

The principal investigator will maintain adequate records of the disposition of the CAR-20/19-T cells, including dates, quantity and use by subjects and the disposition of cells manufactured but not infused.

The principal investigator, along with assistance from the research coordinators, 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 CAR-20/19-T cells. 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.

15.3 Publishing Data

Data from this study will be managed by the clinical trials office at MCW and the investigators listed on this study. Upon completion of the study, the data will be submitted to national meeting followed by a plan to publish in peer-reviewed journal.

16 REFERENCES

1. Lee, D.W., et al., *Current concepts in the diagnosis and management of cytokine release syndrome.* Blood, 2014. **124**(2): p. 188-95.

2. *Facts and Statistics*. Leukemia and Lymphoma Society 2015 [cited 2015 12/21]; Available from: http://www.lls.org/http%3A/llsorg.prod.acquia-sites.com/facts-and-statistics/facts-and-statistics-overview/facts-and-statistics.

3. Scheuermann, R.H. and E. Racila, *CD19 antigen in leukemia and lymphoma diagnosis and immunotherapy.* Leuk Lymphoma, 1995. **18**(5-6): p. 385-97.

4. Fearon, D.T. and M.C. Carroll, *Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex.* Annu Rev Immunol, 2000. **18**: p. 393-422.

5. Stamenkovic, I. and B. Seed, *CD19, the earliest differentiation antigen of the B cell lineage, bears three extracellular immunoglobulin-like domains and an Epstein-Barr virus-related cytoplasmic tail.* J Exp Med, 1988. **168**(3): p. 1205-10.

6. Maude, S.L., et al., *Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia.* New England Journal of Medicine, 2014. **371**(16): p. 1507-1517.

7. Maude, S.L., et al., *CD19-targeted chimeric antigen receptor T cell therapy for acute lymphoblastic leukemia*. 2015.

8. Garfall, A.L., et al., *Chimeric Antigen Receptor T Cells against CD19 for Multiple Myeloma.* New England Journal of Medicine, 2015. **373**(11): p. 1040-1047.

9. Kochenderfer, J.N., et al., *Chemotherapy-Refractory Diffuse Large B-Cell Lymphoma and Indolent B-Cell Malignancies Can Be Effectively Treated With Autologous T Cells Expressing an Anti-CD19 Chimeric Antigen Receptor.* Journal of Clinical Oncology, 2014.

10. Schuster, S.J., et al., *Sustained Remissions Following Chimeric Antigen Receptor Modified T Cells Directed Against CD19 (CTL019) in Patients with Relapsed or Refractory CD19+ Lymphomas.* Blood, 2015. **126**(23): p. 183-183.

11. Tedder, T.F. and P. Engel, *CD20: a regulator of cell-cycle progression of B lymphocytes.* Immunol Today, 1994. **15**(9): p. 450-4.

12. Shan, D., J.A. Ledbetter, and O.W. Press, *Apoptosis of Malignant Human B Cells by Ligation of CD20 With Monoclonal Antibodies.* Blood, 1998. **91**(5): p. 1644-1652.

13. Till, B.G., et al., *CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results.* Blood, 2012. **119**(17): p. 3940-50.

14. Wang, Y., et al., *Effective response and delayed toxicities of refractory advanced diffuse large B-cell lymphoma treated by CD20-directed chimeric antigen receptor-modified T cells.* Clin Immunol, 2014. **155**(2): p. 160-75.

15. Curran, K.J. and R.J. Brentjens, *Chimeric Antigen Receptor T Cells for Cancer Immunotherapy.* Journal of Clinical Oncology, 2015.

16. Hiraga, J., et al., *Down-regulation of CD20 expression in B-cell lymphoma cells after treatment with rituximab-containing combination chemotherapies: its prevalence and clinical significance.* Blood, 2009. **113**(20): p. 4885-4893.

17. Kaiser, A.D., et al., *Towards a commercial process for the manufacture of genetically modified T cells for therapy.* Cancer Gene Ther, 2015. **22**(2): p. 72-8.

18. Apel, M., et al., *Integrated Clinical Scale Manufacturing System for Cellular Products Derived by Magnetic Cell Separation, Centrifugation and Cell Culture.* Chemie Ingenieur Technik, 2013. **85**(1-2): p. 103-110.

19. Spohn, G., et al., *Automated CD34+ cell isolation of peripheral blood stem cell apheresis product.* Cytotherapy, 2015. **17**(10): p. 1465-71.

20. Bunos, M., et al., *Automated isolation of primary antigen-specific T cells from donor lymphocyte concentrates: results of a feasibility exercise.* Vox Sang, 2015. **109**(4): p. 387-93.

21. Kochenderfer, J.N., et al., *Construction and preclinical evaluation of an anti-CD19 chimeric antigen receptor.* J Immunother, 2009. **32**(7): p. 689-702.

22. Imai, C., et al., *Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia.* Leukemia, 2004. **18**(4): p. 676-84.

23. Picanco-Castro, V., E.M. de Sousa Russo-Carbolante, and D. Tadeu Covas, *Advances in lentiviral vectors: a patent review.* Recent Pat DNA Gene Seq, 2012. **6**(2): p. 82-90.

24. Dropulic, B., *Lentiviral vectors: their molecular design, safety, and use in laboratory and preclinical research.* Hum Gene Ther, 2011. **22**(6): p. 649-57.

25. Kenderian, S.S., et al., *Chimeric Antigen Receptor T-cell Therapy to Target Hematologic Malignancies.* Cancer Research, 2014. **74**(22): p. 6383-6389.

26. Milone, M.C., et al., *Chimeric Receptors Containing CD137 Signal Transduction Domains Mediate Enhanced Survival of T Cells and Increased Antileukemic Efficacy In Vivo.* Mol Ther, 2009. **17**(8): p. 1453-1464.

27. Carpenito, C., et al., *Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains.* Proc Natl Acad Sci U S A, 2009. **106**(9): p. 3360-5.

28. Chen, F., et al., *Construction of Anti-CD20 Single-Chain Antibody-CD28-CD137-TCRzeta Recombinant Genetic Modified T Cells and its Treatment Effect on B Cell Lymphoma.* Med Sci Monit, 2015. **21**: p. 2110-5.

29. Schneider, D., et al., *A Tandem CD19/CD20 CAR Lentiviral Vector Drives On-Target and Off-Target Antigen Modulation in Leukemia Cell Lines.* Journal for Immunotherapy of Cancer, 2017.

30. Schneider, D., et al., *Minimizing leukemia escape: implementing a dual anti-CD20- and CD19-scFv-based chimeric antigen receptor (CAR).* Journal for Immunotherapy of Cancer, 2015. **3**(Suppl 2): p. P122-P122.

31. Keever-Taylor, C.A., et al., *Characteristics of CliniMACS(R) System CD34-enriched T cell-depleted grafts in a multicenter trial for acute myeloid leukemia-Blood and Marrow Transplant Clinical Trials Network (BMT CTN) protocol 0303.* Biol Blood Marrow Transplant, 2012. **18**(5): p. 690-7.

32. Stroncek, D.F., et al., *Myeloid cells in peripheral blood mononuclear cell concentrates inhibit the expansion of chimeric antigen receptor T cells.* Cytotherapy, 2016. **18**(7): p. 893-901.

33. Wang, X. and I. Rivière, *Clinical manufacturing of CAR T cells: foundation of a promising therapy.* Molecular Therapy — Oncolytics, 2016. **3**: p. 16015.

34. Zheng, Z., N. Chinnasamy, and R.A. Morgan, *Protein L: a novel reagent for the detection of Chimeric Antigen Receptor (CAR) expression by flow cytometry.* Journal of Translational Medicine, 2012. **10**(1): p. 1-6.

35. Curran, E. and W. Stock, *How I treat acute lymphoblastic leukemia in older adolescents and young adults.* Blood, 2015. **125**(24): p. 3702-3710.

36. Frey, N.V. and S.M. Luger, *How I treat adults with relapsed or refractory Philadelphia chromosome negative acute lymphoblastic leukemia.* Blood, 2015.

37. Plosker, G.L. and D.P. Figgitt, *Rituximab: a review of its use in non-Hodgkin's lymphoma and chronic lymphocytic leukaemia.* Drugs, 2003. **63**(8): p. 803-43.

38. Armitage, J.O., *How I treat patients with diffuse large B-cell lymphoma.* Blood, 2007. **110**(1): p. 29-36.

39. Wang, K., G. Wei, and D. Liu, *CD19: a biomarker for B cell development, lymphoma diagnosis and therapy.* Experimental Hematology & Oncology, 2012. **1**(1): p. 1-7.

40. Porter, D.L., et al., *Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia.* N Engl J Med, 2011. **365**(8): p. 725-33.

41. Neelapu, S.S., et al., *Kte-C19 (anti-CD19 CAR T Cells) Induces Complete Remissions in Patients with Refractory Diffuse Large B-Cell Lymphoma (DLBCL): Results from the Pivotal Phase 2 Zuma-1.* Blood, 2016. **128**(22): p. LBA-6-LBA-6.

42. Turtle, C.J., et al., *CD19 CAR-T Cells Are Highly Effective in Ibrutinib-Refractory Chronic Lymphocytic Leukemia.* Blood, 2016. **128**(22): p. 56-56.

43. Gross, G., T. Waks, and Z. Eshhar, *Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity.* Proceedings of the National Academy of Sciences of the United States of America, 1989. **86**(24): p. 10024-10028.

44. Mullaney, B.P. and M.G. Pallavicini, *Protein-protein interactions in hematology and phage display.* Exp Hematol, 2001. **29**(10): p. 1136-46.

45. June, C.H., B.R. Blazar, and J.L. Riley, *Engineering lymphocyte subsets: tools, trials and tribulations.* Nat Rev Immunol, 2009. **9**(10): p. 704-16.

46. Gattinoni, L., et al., *Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells.* J Exp Med, 2005. **202**(7): p. 907-12.

47. Dummer, W., et al., *T cell homeostatic proliferation elicits effective antitumor autoimmunity.* J Clin Invest, 2002. **110**(2): p. 185-92.

48. Park, J.H., M.B. Geyer, and R.J. Brentjens, *CD19-targeted CAR T-cell therapeutics for hematologic malignancies: interpreting clinical outcomes to date.* Blood, 2016. **127**(26): p. 3312-20.

49. Batlevi, C.L., et al., *Novel immunotherapies in lymphoid malignancies.* Nat Rev Clin Oncol, 2016. **13**(1): p. 25-40.

50. Frey, N.V., et al., *Optimizing chimeric antigen receptor (CAR) T cell therapy for adult patients with relapsed or refractory (r/r) acute lymphoblastic leukemia (ALL).* ASCO Meeting Abstracts, 2016. **34**(15_suppl): p. 7002.

51. Porter, D.L., et al., *Randomized, phase II dose optimization study of chimeric antigen receptor (CAR) modified T cells directed against CD19 in patients (pts) with relapsed, refractory (R/R) CLL.* ASCO Meeting Abstracts, 2016. **34**(15_suppl): p. 3009.

52. Brudno, J.N., et al., *Allogeneic T Cells That Express an Anti-CD19 Chimeric Antigen Receptor Induce Remissions of B-Cell Malignancies That Progress After Allogeneic Hematopoietic Stem-Cell Transplantation Without Causing Graft-Versus-Host Disease.* Journal of Clinical Oncology, 2016.

53. Maude, S.L., et al., *Managing cytokine release syndrome associated with novel T cell-engaging therapies.* Cancer J, 2014. **20**(2): p. 119-22.

54. Frey, N.V., et al., *Refractory Cytokine Release Syndrome in Recipients of Chimeric Antigen Receptor (CAR) T Cells.* Blood, 2014. **124**(21): p. 2296-2296.

55. Henter, J.I., et al., *HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis.* Pediatr Blood Cancer, 2007. **48**(2): p. 124-31.

56. Brudno, J.N. and J.N. Kochenderfer, *Toxicities of chimeric antigen receptor T cells: recognition and management.* Blood, 2016. **127**(26): p. 3321-30.

57. Lee, D.W., et al., *T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial.* Lancet, 2015. **385**(9967): p. 517-28.

58. Davila, M.L., et al., *Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia.* Sci Transl Med, 2014. **6**(224): p. 224ra25.

59. Rosca, J. *Juno Therapeutics Cancer Trial Stops After 3 Deaths, But Other Gene Therapy Trials Continue*. 2016 [cited 2016 August 15]; Available from: http://www.natureworldnews.com/articles/25003/20160709/juno-therapeutics-cancer-trial-stops-3-deaths-gene-therapy-trials.htm.

60. Porter, D.L., et al., *Cytokine Release Syndrome (CRS) after Chimeric Antigen Receptor (CAR) T Cell Therapy for Relapsed/Refractory (R/R) CLL.* Blood, 2014. **124**(21): p. 1983-1983.

61. Hallek, M., et al., *Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute–Working Group 1996 guidelines.* Blood, 2008. **111**(12): p. 5446-5456.

62. Cheson, B.D., et al., *Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification.* J Clin Oncol, 2014. **32**(27): p. 3059-68.

63. Dowlati, Y., et al., *A meta-analysis of cytokines in major depression.* Biol Psychiatry, 2010. **67**(5): p. 446-57.

64. Alesci, S., et al., *Major depression is associated with significant diurnal elevations in plasma interleukin-6 levels, a shift of its circadian rhythm, and loss of physiological complexity in its secretion: clinical implications.* J Clin Endocrinol Metab, 2005. **90**(5): p. 2522-30.

65. Khandaker, G.M., et al., *Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life: a population-based longitudinal study.* JAMA Psychiatry, 2014. **71**(10): p. 1121-8.

66. Sommershof, A., et al., *Chronic stress suppresses anti-tumor TCD8+ responses and tumor regression following cancer immunotherapy in a mouse model of melanoma.* Brain Behav Immun, 2017. **65**: p. 140-149.

67. Knight, J.M., et al., *Peri-transplant psychosocial factors and neutrophil recovery following hematopoietic stem cell transplantation.* PLoS One, 2014. **9**(6): p. e99778.

68. Satin, J.R., W. Linden, and M.J. Phillips, *Depression as a predictor of disease progression and mortality in cancer patients: a meta-analysis.* Cancer, 2009. **115**(22): p. 5349-61.

69. El-Jawahri, A., et al., *Impact of pre-transplant depression on outcomes of allogeneic and autologous hematopoietic stem cell transplantation.* Cancer, 2017. **123**(10): p. 1828-1838.

70. Knight, J.M., et al., *Psychosocial factors and hematopoietic stem cell transplantation: potential biobehavioral pathways.* Psychoneuroendocrinology, 2013. **38**(11): p. 2383-93.

71. Shaw, B.E., et al., *Can we agree on patient-reported outcome measures for assessing hematopoietic cell transplantation patients? A study from the CIBMTR and BMT CTN.* Bone Marrow Transplant, 2016. **51**(9): p. 1173-9.

72. Barata, A., et al., *Unmet Needs for Psychosocial Care in Hematologic Malignancies and Hematopoietic Cell Transplant.* Curr Hematol Malig Rep, 2016. **11**(4): p. 280-7.

73. Watson, D., et al., *Development and validation of the Inventory of Depression and Anxiety Symptoms (IDAS).* Psychol Assess, 2007. **19**(3): p. 253-68.

74. Hann, D.M., et al., *Measurement of fatigue in cancer patients: development and validation of the Fatigue Symptom Inventory.* Qual Life Res, 1998. **7**(4): p. 301-10.

75. Donovan, K.A., et al., *Identifying clinically meaningful fatigue with the Fatigue Symptom Inventory.* J Pain Symptom Manage, 2008. **36**(5): p. 480-7.

76. Vargas, S., et al., *Sleep dysfunction and psychosocial adaptation among women undergoing treatment for non-metastatic breast cancer.* Psychooncology, 2010. **19**(6): p. 669-73.

77. Carpenter, J.S. and M.A. Andrykowski, *Psychometric evaluation of the Pittsburgh Sleep Quality Index.* J Psychosom Res, 1998. **45**(1): p. 5-13.

78. Cleeland, C.S. and K.M. Ryan, *Pain assessment: global use of the Brief Pain Inventory.* Ann Acad Med Singapore, 1994. **23**(2): p. 129-38.

APPENDIX A: *PERFORMANCE STATUS CRITERIA*

	Karnofsky Performance Scale
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

APPENDIX B: NEUROLOGICAL ASSESSMENT

The Mini-Mental State Examination will be performed as per Table 1: Schedule of Events.

MINI MENTAL STATE EXAMINATION (MMSE)

Name:

DOB:

Hospital Number:

One point for each answer	DATE:		
ORIENTATION Year Season Month Date Time	...../ 5	...../ 5	...../ 5
Country Town District Hospital Ward/Floor	...../ 5	...../ 5	...../ 5
REGISTRATION Examiner names three objects (e.g. apple, table, penny) and asks the patient to repeat (1 point for each correct. THEN the patient learns the 3 names repeating until correct).	...../ 3	...../ 3	...../ 3
ATTENTION AND CALCULATION Subtract 7 from 100, then repeat from result. Continue five times: 100, 93, 86, 79, 65. (Alternative: spell "WORLD" backwards: DLROW).	...../ 5	...../ 5	...../ 5
RECALL Ask for the names of the three objects learned earlier.	...../ 3	...../ 3	...../ 3
LANGUAGE Name two objects (e.g. pen, watch).	...../ 2	...../ 2	...../ 2
Repeat "No ifs, ands, or buts".	...../ 1	...../ 1	...../ 1
Give a three-stage command. Score 1 for each stage. (e.g. "Place index finger of right hand on your nose and then on your left ear").	...../ 3	...../ 3	...../ 3
Ask the patient to read and obey a written command on a piece of paper. The written instruction is: "Close your eyes".	...../ 1	...../ 1	...../ 1
Ask the patient to write a sentence. Score 1 if it is sensible and has a subject and a verb.	...../ 1	...../ 1	...../ 1
COPYING: Ask the patient to copy a pair of intersecting pentagons 	...../ 1	...../ 1	...../ 1
TOTAL:	...../ 30	...../ 30	...../ 30

MMSE scoring
24-30: no cognitive impairment
18-23: mild cognitive impairment
0-17: severe cognitive impairment

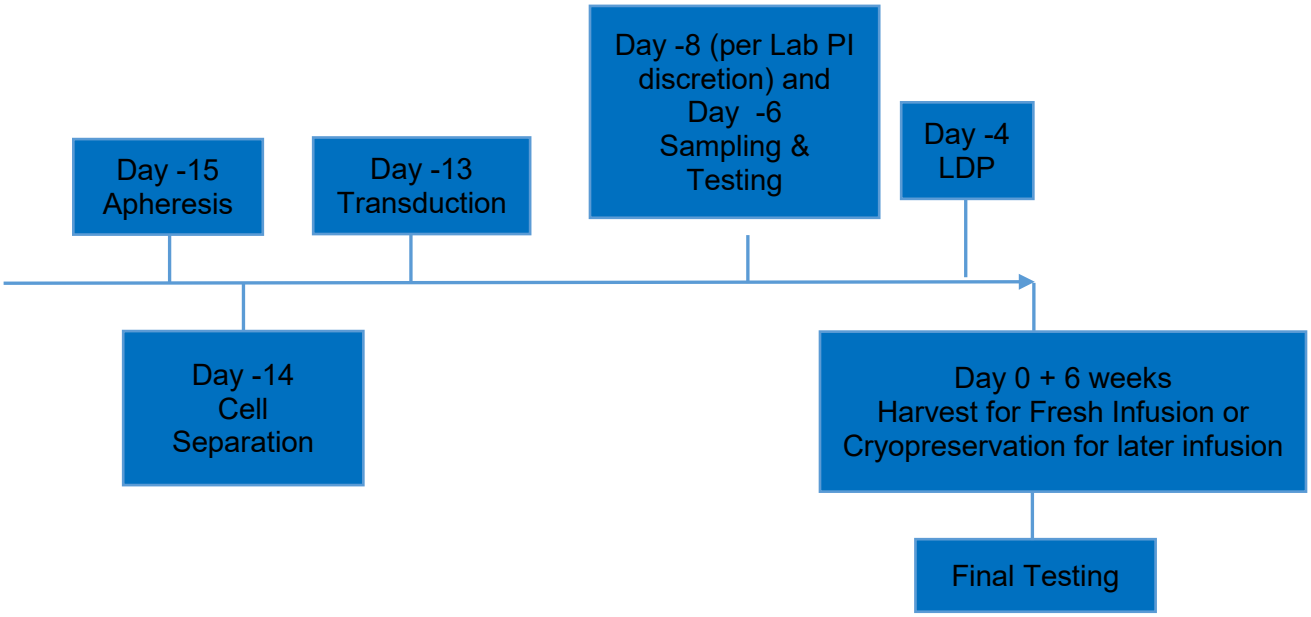
OME Oxford Medical Education

APPENDIX C: 2004 MAS/HLH DIAGNOSTIC CRITIERA

Diagnosis of MAS/HLH requires meeting 5/8 following criteria

- Fever
- Splenomegaly
- Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood)
    - Hemoglobin <9 g/dL
    - Platelets <100,000/µL
    - Absolute neutrophil count<1000/µL
- Hypertriglyceridemia and/or hypofibrinogenemia:
    - Fasting triglycerides >265 mg/dl
    - Fibrinogen <1.5 g/L
- Hemophagocytosis in bone marrow or spleen or lymph nodes
- Low or absent NK-cell activity (according to local laboratory reference)
- Ferritin >500 ng/mL
- Soluble CD25 (i.e., soluble IL-2 receptor) >2,400 U/ml

APPENDIX D: SCHEMA FOR TESTING OF CART CELLS FOR FRESH INFUSION OR CRYOPRESERVATION (FOR LATER INFUSION) IN PHASE 1 DOSE ESCALATION/EXPANSION



Day -6 In-Process Testing (minimal testing requirements)

(***Rationale***: For those receiving CAR-T cells as a fresh infusion, lymphodepleting chemotherapy would be initiated on Day -4 This allows 2 days to verify CAR-T potency before lymphodepletion is initiated.)

1. Sterility cultures
2. Transduction efficiency (Protein L staining for CD20_19 expression)
3. Basic phenotyping (CD3, CD4, CD8 from Protein L panel)
4. Viability (from Protein L panel) and viable cell numbers
5. Chromium release assays versus $CD19^+$/ $CD20^+$ Raji target cells (potency)
6. Mycoplasma testing (in-house)
7. Endotoxin testing (sendout)

Day 0 Testing of Final Product

(***Important Note***: For those receiving CAR-T cells as a fresh infusion, results of gram stains, flow cytometry (to assess transduction efficiency) and 7-AAD viability will be required for product release.)

1. Sterility cultures
2. Gram stain
3. Transduction efficiency (by Protein L staining)
4. Comprehensive phenotyping
5. Chromium release assays versus $CD19^+$/ $CD20^+$ Raji target cell and transfected $CD19^+$ and $CD20^+$ target cells (potency)
6. Intracellular cytokine staining assays (potency)
7. VCN & VSVg testing (sendout)
8. Sendout for final mycoplasma and endotoxin testing

APPENDIX E: CAR-T CELL TREATMENT GUIDELINES

- ☐ **Tumor Lysis Prevention**
  - Allopurinol 300 mg daily for tumor lysis prevention starting Day -4 until Day+14[1]
- ☐ **Seizure prophylaxis**
  - Keppra (Levetiracetam) 500 mg twice daily from Day -4 until Day +28
- ☐ **Prophylactic Antibiotics**
  - PCP prophylaxis to be started Day -4 through Day+28 (agent and dosing per institutional standards)
  - Anti-viral prophylaxis: Acyclovir or valacyclovir starting Day -4 through Day+28. Valganciclovir or IV ganciclovir can be given as a substitute as indicated by treating provider.
  - Neutropenic prophylaxis: As per institutional standards, not mandatory (e.g. ciprofloxacin or cefepime)
  - Fungal prophylaxis as per treating physician, not mandatory
- ☐ **Pre-medications for CAR-20/19-T cells (1 hour prior to CAR-T cell infusion)**
  - Acetaminophen 650 mg x 1 orally
  - Diphenhydramine hydrochloride 50 mg x 1 oral or IV[2]

Transfusion Parameters

- Maintain Platelets>20 K/μL and Hemoglobin>8.0 mg/dL
- Filgrastrim *can* be given at the discretion of the treating provider starting Day +21, provided the patient does not have signs/symptoms consistent with CAR-T cell neurotoxicity or cytokine release syndrome.

1- Can be stopped at discretion of treating physician
2- Alternatives anti-histamines in place of diphenhydramine hydrochloride can be given per discretion of treating physician (e.g. cetirizine, ranitidine) Dosed per institutional standards.

APPENDIX F: ASSESSMENT/MANAGEMENT OF CYTOKINE RELEASE SYNDROME AND NEUROTOXICITY ASSOCIATED WITH CAR-T CELLS [1, 56]

Lee et al. CRS Grading System[1]

Grade	Toxicity
Grade 1	Symptoms are not life threatening and require symptomatic treatment only, eg, fever, nausea, fatigue, headache, myalgias, malaise
Grade 2	Symptoms require and respond to moderate intervention Oxygen requirement <40% or Hypotension responsive to fluids or low dose[2] 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* 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)
Grade 5	Death

High-dose vasopressors (all doses are required for ≥3 hours)

Pressor	Dose
Norepinephrine monotherapy	≥20 μg/min
Dopamine monotherapy	≥10 μg/kg/min
Phenylephrine monotherapy	≥200 μg/min
Epinephrine monotherapy	≥10 μg/min
If on vasopressin	Vasopressin + norepinephrine equivalent of ≥10 μg/min*
If on combination vasopressors (not vasopressin)	Norepinephrine equivalent of ≥20 μg/min*

1. Lee, D.W., et al., *Current concepts in the diagnosis and management of cytokine release syndrome.* Blood, 2014. **124**(2): p. 188-95.

Table 5: CRS Symptoms, Evaluation, Management, Response

CRS assessed via Lee et al. Revised CRS grading system.	**Grade 1 toxicities**	**Grade 2 toxicities**[1]	**Grade 3 or Grade 4 toxicities**
Symptoms	Fevers, myalgias, nausea/fatigue, malaise, headache	Hypotension that responds to fluid or one low dose pressor, oxygen requirement<40%, Grade 2 organ toxicity per CTCAE 5.0	Hypotension requiring high dose or multiple pressors, Grade 3 or 4 organ toxicity, life threatening symptoms, mechanical ventilation
Evaluation & Management	Infectious work-up, initiation/escalation of antibiotics per institutional standards. IV fluids, anti-pyretic therapy as indicated with cooling blankets and acetaminophen (avoid NSAIDs). Supportive care measure[2]	Admission to hospital (if not admitted). IV fluids, infectious work-up, supportive care measures	Admission to hospital, intensive care unit transfer as indicated. IV fluids, infectious work-up, respiratory support, and renal replacement therapy as indicated
Response & Escalation	Persistent Grade 1 symptoms>24-48 hours or escalation to Grade 3-4 toxicities initiate CRS directed treatment[3]	Persistent symptoms >12-24 hours despite interventions or escalation to Grade 3-4 toxicity initiate CRS directed treatment[3]	Immediate initiation of CRS directed treatments[3]

1-At direction of treating physician, CRS treatment can be initiated in any patient with Grade≥2 toxicity
2-Recommended Supportive care measure listed in Appendix G
3-CRS Directed Treatment Algorithm is on Page 91

Table 6: Neurotoxicity Evaluation and Management

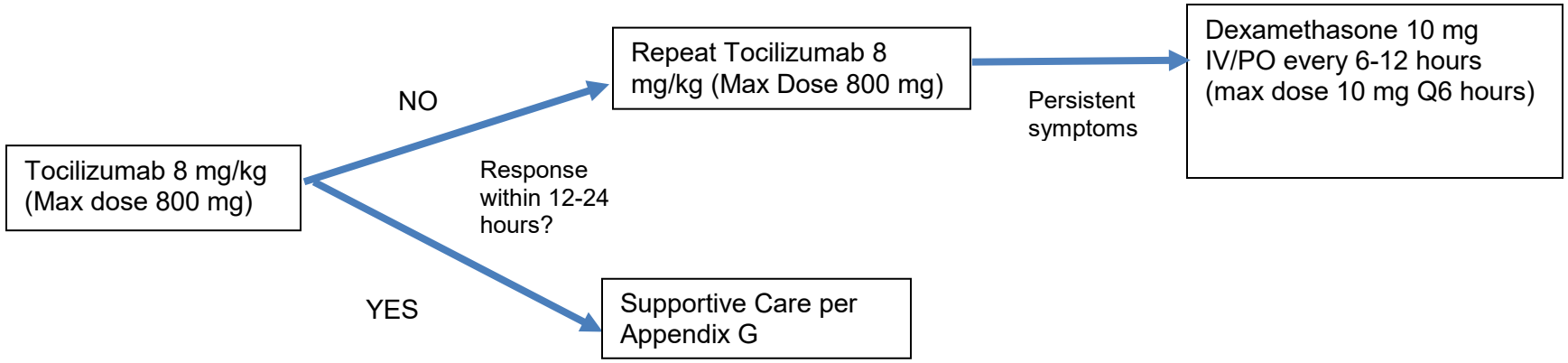
Neurotoxicity assessed via CTCAE 5.0	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Examples	Mild memory impairment, reduced awareness or alertness, mild paresthesia, etc.	Moderate memory impairment or moderate lethargy limiting ADL. Moderate weakness or sensory loss moderate neuropathy limiting instrumental ADL, etc.	Severe memory impairment limiting self-care, severe weakness or sensory loss, multiple seizures, severe neurological deficit, etc.	Life threatening consequence, cerebral edema[1] urgent intervention indicated
Evaluation	Neurological Exam, additional work-up as indicated	Neurological Examination, MRI Brain +/- lumbar puncture and EEG as indicated. Neurological consultation.		
Management	Clinical vigilance, monitoring, supportive care	Admission to hospital (if not admitted), neuro-checks Q4 hours, supportive care, work-up as above. Dexamethasone can be given at discretion of treating physician	Admission to Intensive Care Unit. Dexamethasone 10 mg IV/PO every 6-12 hours (max dose 10 mg Q6 hours). If not improving consider tocilizumab 8 mg/kg IV x 1 (max dose 800 mg). Can give up to 2 doses of Tocilizumab in 24 hours.	

1-For patients with evidence of cerebral edema (Grade 4 toxicity) the following specific interventions will be performed

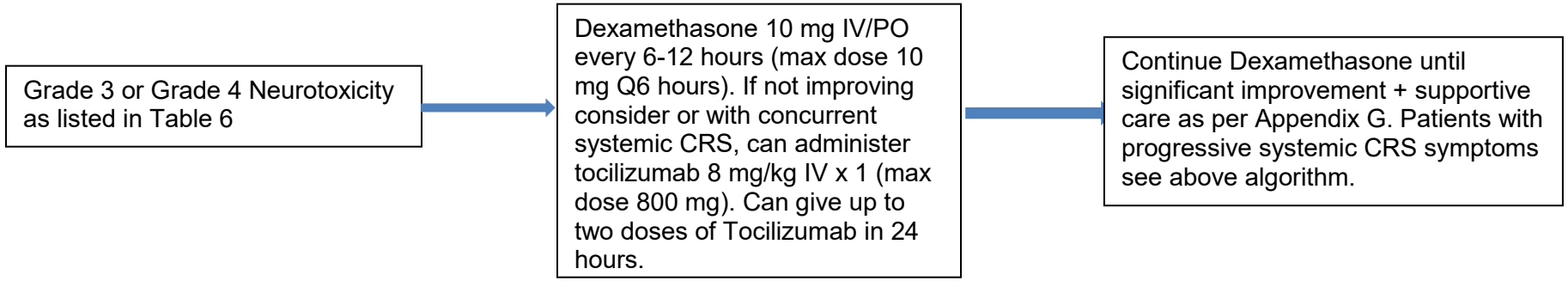
- Intensive Care Unit care
- Neurosurgical consultation
- Mechanical ventilation
- Pharmacological support per intensivist and neurological and neurosurgical recommendations

Cytokine Release Syndrome Directed Treatment[56]

Without Neurological Toxicities



With Neurological Toxicities[1]



1-Patients with neurological toxicities (Grade≥3 by CTCAE 5.0) will be treated with dexamethasone with or without the presence of systemic CRS.

APPENDIX G: SUPPORTIVE CARE MEASURES FOR CAR-T CELL TREATMENT

Guidelines developed and adapted from *Brudno, J. N. and J. N. Kochenderfer (2016). "Toxicities of chimeric antigen receptor T cells: recognition and management." Blood **127**(26): 3321-3330.*

Table 7: Supportive Care Guidelines for Patients Receiving CAR-T cells

Constitutional	• Administer acetaminophen for symptomatic management of fevers in patients with normal hepatic function. • Provide cooling blankets for fevers >40ºC • Avoid corticosteroids and NSAIDs • Avoid meperidine
Cardiovascular	• Minimize anti-hypertensives prior to cell infusion • Monitor vital signs at least every 4 hours for up to 48 hours' post CAR-20/19-T cell infusion • Monitor vital signs Q2H in patients with fevers and tachycardia • Initiate replacement of IV fluids for patients with poor oral intake or high insensible losses to maintain net even fluid balance • IV fluid boluses for patients with SBP less than their pre-infusion baseline or those with hypotension SBP<90 • Persistent hypotension not responsive to fluids should be initiated on vasopressors and patients transferred to intensive care unit • For patient on vasopressors evaluate cardiac function with troponin and ECG. Echocardiogram can be performed as indicated.
Infectious disease	• Prophylactic antibiotics as per **Appendix E** • Neutropenic Fever management as per institutional guidelines with broad spectrum antibiotics. Appropriate fever work-up with chest x-ray, urinalysis, and blood cultures
Hematologic	• Initiate Allopurinol for TLS prevention as per **Appendix E** • Transfuse RBCs for Hgb<8 g/dL • Transfuse platelets for Platelets<20 k/μL • Monitor ANC as per schedule of events (Table 1). Neupogen can be started at Day+21 in patients with persistent neutropenia as per guidelines in **Section 6.9** • Transfuse fresh frozen plasma in patients with PTT prolongation >2.0 fold upper limit of normal • Transfuse cryoprecipitate to maintain fibrinogen ≥100 mg/dL
Neurologic	• Neuro checks Q4H by nursing staff in patients with ≥Grade 2 neurotoxicity • MRI Brain +/- lumbar puncture and EEG in patients with ≥Grade 2 neurotoxicity • Neurological consultation for patients with ≥Grade 2 neurotoxicity • MMSE evaluation as per **Table 1**

APPENDIX H: OPERATING PROCEDURES FOR PATIENTS TREATED WITH CAR-20/19-T CELLS AT A NON-SPECIFIED DOSE

Some patients will have successful production of CAR-20/19-T cells but fail to make the adequate cell dose as required per our protocol. Those patients will be allowed treatment but required to be replaced for the purposes of this Phase 1/1b study. Patients will be followed as per this clinical protocol with monitoring for DLT, safety, adverse events, and long-term survival.

Specific Criteria for Infusion of Non-Specified CAR-20/19-T cell dose

1) Meet all eligibility criteria a listed in Section 4
2) Meet all release criteria for CAR-T cell product
3) Meet same infusion criteria as listed in Section 6.3 and 7.1
4) Patients will be followed per schedule of events listed in Section 5 and Table 1

Analysis of Patients Treated with Non-Specified CAR-20/19-T cell dose

At first, a maximum of 3 patients will be allowed to be treated in this manner. If there is ≤1 DLT within the first 28 days for the first three patients, further patients will be allowed study drug with CAR-20/19-T cells even if they don't make the required treatment dose. After enrollment of the 4^{th} patient or beyond in this cohort, if the DLT rate exceeds ≥33%, no further patients will be allowed treatment outside of a specified cell dose per the clinical protocol.

All patients will be followed for AEs, survival, response rates as per Table 1.

APPENDIX I: QUALITY OF LIFE (QOL) MEASURES

QOL MEASURES

Participant # _____

IDAS

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

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

15. I thought about hurting myself	1	2	3	4	5
16. I did not have much of an appetite	1	2	3	4	5
17. I felt like eating less than usual	1	2	3	4	5
19. I did not feel much like eating	1	2	3	4	5
21. I felt optimistic	1	2	3	4	5
23. I felt that I had accomplished a lot	1	2	3	4	5
24. I looked forward to things with enjoyment	1	2	3	4	5
25. I was furious	1	2	3	4	5
26. I felt hopeful about the future	1	2	3	4	5
27. I felt that I had a lot to look forward to	1	2	3	4	5
28. I felt like breaking things	1	2	3	4	5
29. I had disturbing thoughts of something bad that happened to me	1	2	3	4	5
30. Little things made me mad	1	2	3	4	5
31. I felt enraged	1	2	3	4	5
32. I had nightmares that reminded me of something bad that happened	1	2	3	4	5
33. I lost my temper and yelled at people	1	2	3	4	5
34. I felt like I had a lot of interesting things to do.	1	2	3	4	5
35. I felt like I had a lot of energy	1	2	3	4	5
36. I had memories of something scary that happened	1	2	3	4	5
37. I felt self-conscious knowing that others were watching me	1	2	3	4	5

38. I felt a pain in my chest	1	2	3	4	5
39. I was worried about embarrassing myself socially	1	2	3	4	5
40. I felt dizzy or light headed	1	2	3	4	5
41. I cut or burned myself on purpose	1	2	3	4	5
42. I had little interest in my usual hobbies or activities	1	2	3	4	5
43. I thought that the world would be better off without me	1	2	3	4	5
44. I felt much worse in the morning than later in the day	1	2	3	4	5
45. I felt drowsy, sleepy	1	2	3	4	5
46. I woke up early and could not get back to sleep	1	2	3	4	5
47. I had trouble concentrating	1	2	3	4	5
48. I had trouble making up my mind	1	2	3	4	5
49. I talked more slowly than usual	1	2	3	4	5
50. I had trouble waking up in the morning	1	2	3	4	5
51. I found myself worrying all the time	1	2	3	4	5
52. I woke up frequently during the night	1	2	3	4	5
53. It took a lot of effort for me to get going	1	2	3	4	5
54. I woke up much earlier than usual	1	2	3	4	5
55. I was trembling or shaking	1	2	3	4	5
56. I became anxious in a crowded public setting	1	2	3	4	5
57. I felt faint	1	2	3	4	5

58. I found it difficult to make eye contact with people	1	2	3	4	5
59. My heart was racing or pounding	1	2	3	4	5
60. I got upset thinking about something bad that happened	1	2	3	4	5
61. I found it difficult to talk with people I did not know well	1	2	3	4	5
62. I had a very dry mouth	1	2	3	4	5
63. I was short of breath	1	2	3	4	5
64. I felt like I was choking	1	2	3	4	5

FSI

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

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

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

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

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

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

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

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

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

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

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

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

0	1	2	3	4	5	6	7

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

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

PSQI

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

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

BED TIME _____

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

NUMBER OF MINUTES _____

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

GETTING UP TIME _____

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

HOURS OF SLEEP PER NIGHT _____

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

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

a) Cannot get to sleep within 30 minutes

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

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

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

c) Have to get up to use the bathroom

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

d) Cannot breathe comfortably

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

e) Cough or snore loudly

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

f) Feel too cold

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

g) Feel too hot

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

h) Had bad dreams

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

i) Have pain

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

j) Other reason(s), please describe_____
_____

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

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

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

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

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

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

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

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

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

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

BPI

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

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

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

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

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

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

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

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

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

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

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

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

Please indicate below what best represents your household income:

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

Please indicate which education level best describes you:

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

APPENDIX J: SPECIMEN COLLECTION AND HANDLING

For patients who have progressed, mandatory blood samples for monitoring for CAR-T cell persistence which include T-cell persistence by flow and by quantitative PCR and presence of replication competent virus by Q-PCR for VSV-g can be drawn locally and sent to Medical College of Wisconsin for analysis.

Peripheral blood sample:

- T-cell Persistent Studies: A minimum of 20mL of peripheral blood, separated into 2-10mL EDTA (purple top) tubes.
- Q-PCR Samples: A minimum of 20mL of peripheral blood, separated into 2- 10mL EDTA (purple top) tubes.
- VSV-g Samples: A minimum of 20mL of peripheral blood, separated into 2- 10mL EDTA (purple top) tubes.

Specimen Labeling

All submitted specimens must be labeled with the patient's initials, patient study ID#, patient's date of birth and date/time of specimen collection. (All supplies and airbills will be provided in research sample kit).

Shipping Requirements

All specimens must be accompanied by a Specimen Shipping Requisition Form. Samples must be received by the Medical College of Wisconsin within 24 hours of draw. The Froedtert-Cell Processing Lab is open M-F, 8:00am – 4:30 pm. Please do not draw samples on Fridays for delivery on Saturday.

The sample must be placed in a leak proof primary receptacle (ex: vacutainer tube). Multiple primary receptacles must be individually wrapped or separated to prevent contact.

The primary receptacle must be placed into a leak proof secondary container (ex: resealable biohazard bag) in such a way that under normal conditions of transport, they cannot break or leak. Absorbent material, such as paper towels or absorbent pads, must be placed in the secondary container with sufficient capacity to absorb the entire contents of the primary receptacle(s).

The secondary container must be placed into an outer shipping container with suitable cushioning material. (ie: Gel pack) Ice packs to be added to the bottom of shipping container, and on top of the samples. The shipping container must be labeled with the universal biohazard symbol.

The outer packaging must be marked with the name, address, and phone number of both the sender and recipient. All packages shipped via aircraft must display a 2-inch diamond with "UN3373" inside of the diamond.

Send the container via next day delivery at refrigerated temperature to:

Froedtert- Cell Processing Lab
Attn: Fenlu Zhu
9200 W. Wisconsin Ave
Milwaukee, WI 53226

Please call/email the Cell Processing Lab and Coordinator as soon as the tracking number is available: (414)805-6144, Fenlu Zhu:FZhu@mcw.edu, and (414)805-8378, Sharon Yim: syim@mcw.edu, The Cell

Processing Lab is open M-F, 8:00am – 4:30 pm. Please do not draw samples on Fridays for delivery on Saturday.