Phase I/Ib Clinical Trial of Autologous CD22 Chimeric Antigen Receptor (CAR) T cells in Adults with Recurrent or Refractory B Cell Malignancies

DEPARTMENT OF MEDICINE-BLOOD & MARROW TRANSPLANTATION
STANFORD CANCER INSTITUTE

Principal Investigator:	**Matthew Frank, MD, PhD**
IND-holder:	**Stanford University**
Study Agent:	**Autologous T cells Transduced with lentiviral vector (CD22.BB.Z) chimeric antigen receptor (CD22-CAR); following lymphodepleting chemotherapy**
Protocol Version:	**Amd 3, version 22 July 2022**

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Coordinating Center/Participating Sites:	**Single Site**

DOCUMENT HISTORY	NOTES
Amd 0 Version Date: 13 May 2019	Initial protocol submitted to SRC, FDA, IRB and APB
Amd 0 Version Date: 30 Jun 2019	Modification during FDA review. Changes include: • Additional language clarifying weight-based dosing for subjects with BMI > 30 • Change treatment stagger to 21 days for subjects with aggressive B-cell NHL during dose escalation and 21 days for the first 3 subjects with ALL within a dose level. • Add data collection related to use of bridging anti-neoplastic therapy and subject disease response. • Update infectious disease marker testing in response to AABB Cell Therapy Standards 9th Edition to require HIV, HBV, and HCV testing within 28 days of enrollment. • Revise DLT definition for neurotoxicity. • Clarify additional doses of CD22-CAR will not be offered until preliminary safety data is submitted to FDA showing the dose is safe in the relevant disease cohort. • Update APB safety reporting criteria to report all SAEs assessed as possibly, probably or definitely related to CD22-CAR T cells, regardless of expectedness.
Amd 0 Version Date: 08 Jul 2019	Modification during APB review. Changes include: • New section 2.3.1 discussing similarities and differences in CD22-CAR T cell manufacturing process between Stanford product and NCI POB cell product. • Clarify steps before resuming enrollment after triggering a feasibility stopping rule. • Addition of enrollment table for each objective to Section 12.4.
Amd 0 Version Date 08 Aug 2018	Modification during SRC review. Changes include • Modify maximum enrollment number for subjects with aggressive B-cell NHL to account for a maximum of 18 subjects, rather than 15, during dose escalation • Remove BMI-adjustment for dose calculation. Cap weight at 100kg • Update Correlative Sample Collection Matrix (Section 13.7).
Amd 1 Version Date 20 Sep 2020	Changes include • Addition of LCGM manufacturing facility, update language regarding manufacturing and Action Plan for positive sterility result. • Remove Adaptive collaboration language. • Personnel change

Amd 2 Version Date 20 Oct 2020	Modifications include: • Change PI from Dr. Muffly to Dr. Frank • Remove wash out period specific to inotuzumab • Clarify circulating CAR testing in patients previously receiving CAR therapy. • Added Appendix to allow two stages for enrollment: 1st stage for leukapheresis and 2nd stage for investigational intervention, if required by patient or manufacturing scheduling. • Re-organize procedure schedules to refer to Calendar of Events to reduce discrepancies and inconsistencies. • Updated laboratory testing to reflect data accumulated to date. • Updated to most current disease evaluation criteria for ALL • Updated Appendix B to be consistent with Adult CCT team practice • Minor revisions to eligibility for clarity
Amd 2-1 Version Date 24 Feb 2022	Modifications include: • Administrative changes to personnel • Correction to lymphodepletion chemotherapy eligibility and study calendar • Add collaborations with Adaptive and Syncopation Life Sciences
Amd 3 Version Date 22 July 2022	Modifications include: • Given the shortage of fludarabine, allows for lymphodepletion with clofarabine and cyclophosphamide or bendamustine. Adds potential risks of these agents • Cell dose to be calculated from a weight collected within 14 days of start of cell manufacture • Change IV hydration instructions to follow current institutional practice. • Clarify targeted adverse event collection required for long term gene therapy followup, and update reporting requirements for Stanford APB • Remove patient RCL sample banking after Year 1, if all testing was negative

SYNOPSIS

Protocol Number	IND 19103/ CCT5029 / IRB-50836
Protocol Title	Phase I/Ib Clinical Trial of Autologous CD22 Chimeric Antigen Receptor (CAR) T cells in Adults with Recurrent or Refractory B Cell Malignancies
Sponsor	Stanford University
Principal Investigator	Matthew Frank, M.D., Ph.D.
Name of Investigational Product	Autologous T cells transduced with lentiviral vector (CD22.BB.Z) Chimeric Antigen Receptor (CD22-CAR); following lymphodepleting chemotherapy
Clinical Phase	Phase I/Ib
Background and Rationale	Relapsed/refractory (R/R) B-cell acute lymphoblastic leukemia (ALL) is a predominantly CD19+/CD22+ hematologic malignancy that is associated with dismal long-term survival in both children and adults. Complete response (CR) to conventional salvage chemotherapy occurs in < 30% and long-term survival following salvage therapy with or without allogeneic stem cell transplant (SCT) occurs in < 25% of younger adults and < 10% of adults ≥ 50 years[1],[2], [3]. Newer agents such as blinatumomab, a bi-specific T-cell engaging antibody targeting CD19+, and inotuzumab ozogamicin, a humanized anti-CD22 antibody conjugated to calicheamicin, result in higher response rates but are rarely durable responses without subsequent definitive therapy such as SCT[4],[5],[6]. Similarly, R/R diffuse large B-cell lymphoma (DLBCL) is a CD19+/CD22+ non-Hodgkin lymphoma that represents the most common aggressive B-cell non-Hodgkin lymphoma (NHL) in adults. Subjects who are not cured with up-front rituximab-containing therapy regimens are typically offered salvage therapy followed by autologous SCT. Subjects with chemorefractory disease, or those who relapse within one year of autologous SCT have very poor outcomes with long-term event-free survival (EFS) of < 20%[7]. CARs are non-native receptors that link an antigen-binding domain to cell signaling domain(s). When expressed in T cells, CARs endow MHC-unrestricted antigen specificity. Most commonly, CARs utilize a single chain variable fragment (scFv) coupled to TCR zeta and CD28 or 4-1BB endodomains. CAR T cells targeting CD19 (CD19-CAR) in subjects with R/R B-ALL demonstrate dramatic activity in both children and adults with complete responses (CR) in ≥ 70% [8],[9],[10],[11],[12],[13],[14],[15]. CD19-CAR in R/R DLBCL similarly is associated with response rates of >70% and CR rates of approximately 50%[16, 17]. Further, in some patients, responses appear to be durable beyond one year suggesting for the first time the possibility of cure of R/R B-cell ALL and R/R DLBCL without SCT. Despite the enthusiasm and potential for CD19-CAR therapy for B-cell malignancies, CD19-CAR resistance and disease relapse are ongoing challenges. As the experience with CD19-CAR in B-cell ALL and DLBCL has expanded, CD19 immune escape has emerged as a primary etiology of CAR resistance. [18],[10],[19]. For example, amongst 16 relapses following tisagenlecleucel (Kymriah ®) reported in a Phase II trial of children and young adults, 15 patients had CD19-negative disease at the time of relapse[15]. Similar antigen negative relapse patterns have been reported in ALL following CD19/CD3 bispecific

	therapy[20]. There is therefore a need to develop CARs targeting alternative antigens in B-cell ALL. In DLBCL, approximately 25% of relapses following CD19-CAR are associated with loss of CD19 expression[21]. CD22 is a sialic acid binding adhesion molecule, which belongs to the Siglec superfamily. Signaling via CD22 mediates inhibitory signals on B cell receptor signaling via immunoreceptor tyrosine-based inhibitory motifs, which are essential for maintenance of B cell tolerance[22]; [23]; [24]. CD22 expression is restricted to cells of the B-lineage, and is not found on pluripotent hematopoietic stem cells[25],[26]. The vast majority of B-lineage malignancies express CD22 including B-ALL and B cell NHL[27],[28],[29],[30],[31],[32, 33]. Several therapeutics targeting CD22, including inotuzumab ozogamicin, have been studied in B-cell ALL and DLBCL with some success and no evidence of on-target, off-tissue toxicity has been observed[4]. A phase III study evaluating inotuzumab ozogamicin in relapsed/refractory adults with ALL achieved an 80% complete remission or complete remission without hematologic recovery (CRi) rate leading to FDA approval of this targeted agent for adults with relapsed/refractory ALL.[4] Objective responses have also been seen following inotuzumab ozogamicin in DLBCL[34, 35] The Pediatric Oncology Branch of the National Cancer Institute has developed a CAR T cell construct targeting CD22 (CD22-CAR). In a Phase I safety and dose escalation study conducted in 21 children and young adults ages 1-30 years with relapsed/refractory B-cell ALL, CR was achieved in 73% (11 of 15) of patients treated at the recommended dose level of $\geq 1x10^6$ CD22-CAR T cells/kg[36],[37]. Many of those treated had relapsed with CD19-negative disease following CD19-CAR, and responses were seen in all CD19 dim/absent patients treated at this dose level. Cytokine release syndrome (CRS) occurred in 16 of 21 patients and was limited to Grade 1/2. Therefore, we believe that CD22-CAR is a potentially promising agent in adults with B-cell malignancies and in the current protocol we aim to evaluate this therapy in adults with B-cell ALL and aggressive B-cell non-Hodgkin lymphoma (NHL), including DLBCL, primary mediastinal large B-cell lymphoma (PMBCL) and transformed DLBCL (tDLBCL) arising from Follicular Lymphoma and Marginal Zone Lymphoma. The aims of this clinical trial are three-fold: 1. To evaluate the feasibility of manufacturing CD22-CAR T cellular products in the Miltenyi CliniMACS Prodigy® system across both diseases, ALL and aggressive B-cell non-Hodgkin lymphoma. The CliniMACS Prodigy® represents the next generation in automated cell processing, combining and streamlining cell processing workflows into one closed system. 2. To establish safety in two adult disease populations. CD22-CAR T cells have not been previously administered to subjects with aggressive B-cell non-Hodgkin lymphoma, and we will establish the maximum tolerated dose (MTD) or recommended Phase 2 dose (RP2D) of CD22-CAR T cells in this patient population. Simultaneously, we will seek to expand the prior pediatric and young adult experience with this CD22-CAR T cell agent in adults with CD22+ expressing B-cell ALL at the established dose of $1x10^6$ transduced T cells/kg. Once MTD/RP2D is determined in subjects with aggressive B-cell non-Hodgkin lymphoma, this disease population will undergo the same safety evaluation. 3. To evaluate in a preliminary fashion efficacy of CD22-CAR T cells in two disease populations, subjects with relapsed/refractory ALL and subjects with relapsed/refractory aggressive B-cell non-Hodgkin lymphoma. The precedent for conducting clinical trials of this scope was established by the

	ongoing clinical trials CCT5001/IRB-41382 and CCT5007/IRB-41383 conducted by Stanford Center for Cancer Cell Therapy.
Objectives	Primary Objectives: ✓ Determine the feasibility of manufacturing CD22-CAR T cells using the Miltenyi CliniMACS Prodigy® system for administration to adults with relapsed/refractory CD22 expressing B-cell ALL or relapsed/refractory aggressive B-cell NHL. ✓ Establish the maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D) of CD22-CAR T cells in adults with relapsed/refractory aggressive B-cell NHL. ✓ Determine the safety of an established dose of CD22-CAR T cells in adults with relapsed/refractory CD22 expressing B-cell ALL and the safety of the MTD/RP2D of CD22-CAR T cells in adults with relapsed/refractory aggressive B-cell NHL. Secondary Objective ✓ Assess the clinical activity of CD22-CAR T cells in adults with R/R CD22 expressing B-cell ALL and R/R aggressive B-cell NHL, including overall survival (OS) and progressive free survival (PFS). Exploratory Objectives ✓ Analyze alterations in early B cell development induced by immune pressure exerted via CD22-CAR T cells. ✓ Evaluate whether subjects receiving CD22-CAR T cells relapse with loss or diminished expression of CD22, when feasible. ✓ Measure persistence of CD22-CAR T cells in the blood, bone marrow and CSF, and explore correlations between CD22-CAR T cell properties and CAR T cell efficacy and persistence. ✓ Establish the utility of chromatin structure and epigenomic technology to characterize CAR T cell therapies. ✓ Analyze CD22 expression in aggressive B-cell NHL and correlate with disease response.
Primary Endpoint(s)	The primary endpoints for this study are: ✓ Feasibility defined by the rate of successful manufacture of the CD22-CAR T cells produced with the Miltenyi CliniMACS Prodigy® system to satisfy the targeted dose level and meet the required release specifications. ✓ MTD/RP2D in adults with aggressive B-cell NHL as evidence by the number of dose limiting toxicities (DLTs). MTD is defined as the feasible dose achieving 0 DLTs out of 3 or 1 (or fewer) DLT(s) out of 6 patients. ✓ Safety of CD22-CAR T cells as evidenced by the incidence and severity of dose limiting toxicities (DLT), adverse events, serious adverse events, laboratory abnormalities, changes in vital signs, and changes in physical examination following infusion of CD22-CAR T cells graded according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 (see link) in two disease groups: adults with relapsed/refractory CD22 expressing B-cell ALL and adults with relapsed/refractory aggressive B-cell NHL.
Secondary Endpoints	The secondary endpoints for this study are: ✓ Overall response rate (ORR) as measured by Guidelines for efficacy evaluation in Acute Lymphoblastic Leukemia (ALL) (based on NCCN Guidelines (2013, Appelbaum, ASH and IWG guideline, Cheson 2003.) - Complete Response (CR) - CRi

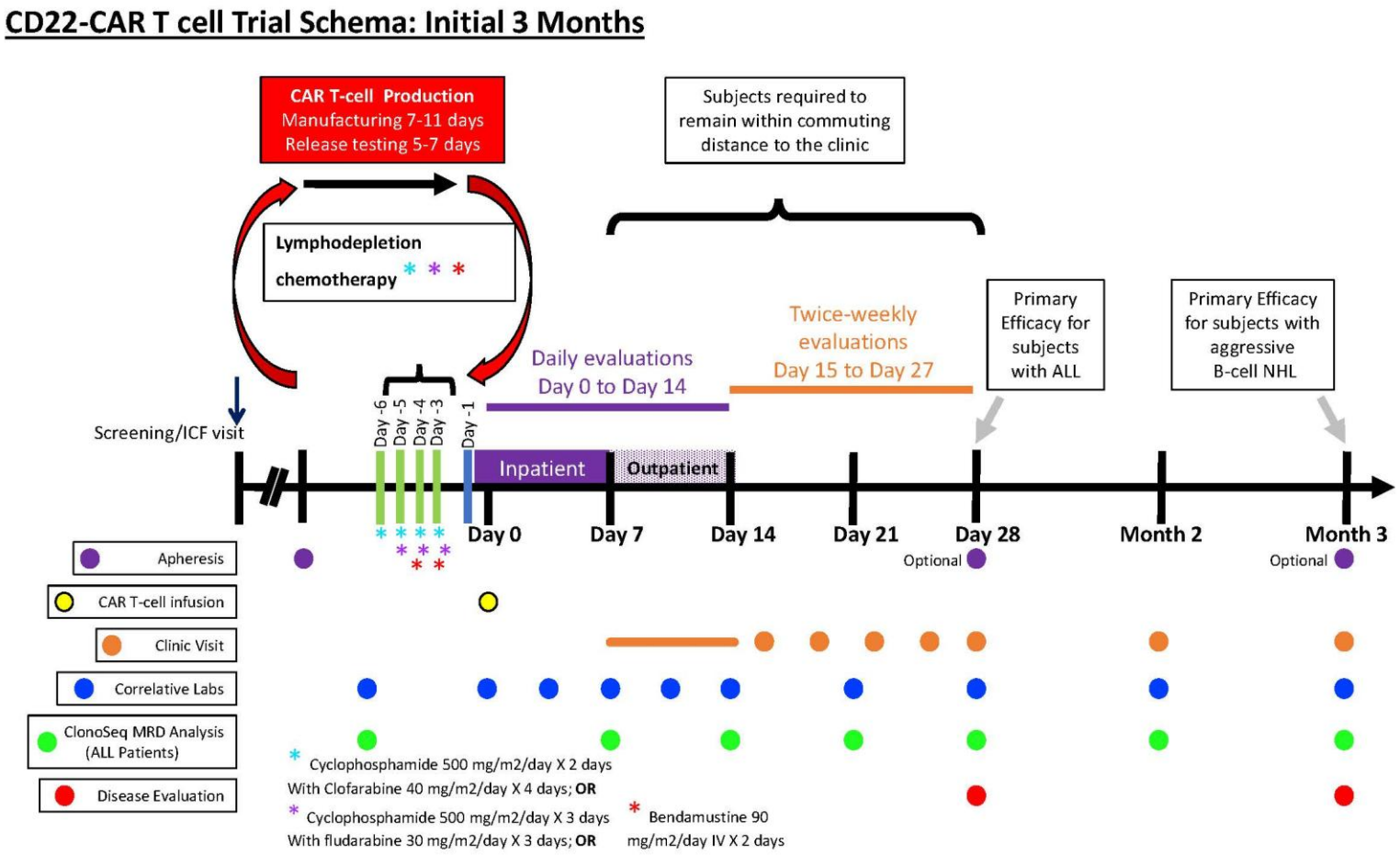
	- No Response - Relapsed Disease - Unknown ✓ Overall response rate (ORR as measured by Response Criteria for Lymphoma (From: Cheson BD, et al 2014[38]): - Complete Response (CR) - Partial Response (PR) - No response or Stable Disease (SD) - Progressive Disease (PD)
Study Centers	This is a Stanford University, investigator initiated protocol conducted at Stanford Health Care (SHC).
Sample Size	This study has 3 part analysis to establish sample size: 1. Feasibility of manufacture; 2. Safety in subjects with relapsed/refractory aggressive B-cell non-Hodgkin lymphoma after establishing MTD/RP2D dose, and safety in subjects with relapsed/refractory ALL; and 3. Efficacy of administration of CD22-CAR T cells in two disease groups: adults with relapsed/refractory CD22 expressing B-cell ALL or relapsed/refractory aggressive B-cell non-Hodgkin lymphoma. For relapsed/refractory aggressive B-cell non-Hodgkin lymphoma patients, a maximum of 18+10+33 = 61 subjects may be required to establish safety and feasibility, and indicate sufficient efficacy. For relapsed/refractory ALL patients, a maximum of 28+6= 34 subjects may be required to establish safety and feasibility, and indicate sufficient efficacy.
Overall Duration of the Study	The recruitment period for this study is expected to be 2 – 3.5 years. Subjects will be followed for a total of 15 years post treatment. The total duration of this study is expected to be 7-8.5 years of active treatment and short term follow up, and a total of 15 years of long term follow up after the last subject completes study therapy.
Duration of Study per Subject	Subject's active participation in this study is expected to be 5 years, with a total of 15 years of long term follow up from the time of cell infusion.
Subject Population	Subjects ≥ 18 years of age, with relapsed/refractory CD22 expressing B-ALL or relapsed/refractory aggressive B-cell non-Hodgkin lymphoma, including DLBCL, PMBCL, and tDLBCL, after standard therapies, with no alternative curative options or have refused alternative options, who meet the eligibility criteria.
Eligibility criteria	✓ <u>For ALL</u>, Must have chemotherapy refractory disease (defined as progression or stable disease after a standard line of therapy), or relapsed disease after achieving CR. o Subjects with persistent or relapsed MRD require verification of MRD on 2 occasions at least 2 weeks apart. o Subjects with Ph+ALL are eligible if have progressed, had stable disease, or relapsed after receiving a tyrosine kinase inhibitor o Subjects with recurrence of isolated CNS relapse after CR are eligible ✓ <u>For aggressive B-cell NHL</u>, Must have histologically confirmed disease as defined by WHO 2008:

	○ DLBCL not otherwise specified; T cell/histiocyte rich large B cell lymphoma; DLBCL associated with chronic inflammation; Epstein Barr virus (EBV)+ DLBCL of the elderly; OR ○ Primary mediastinal (thymic) large B cell lymphoma, OR ○ transformation of follicular lymphoma, marginal zone lymphoma or chronic lymphocytic leukemia/small lymphocytic lymphoma to DLBCL ○ Follicular Lymphoma Grade 3B ○ Subjects with DLBCL, Follicular Lymphoma Grade 3B –or- subjects with transformed FL, MZL, or CLL/SLL who have not received chemotherapy prior to transformation: must have received an anthracycline regimen and an anti-CD20 monoclonal antibody (unless documented CD20-neg) and be relapsed/refractory after second line of DLBCL treatment. Subjects with PR to second line therapy must be ineligible for autologous transplant. ○ Subjects with transformed FL, MZL, or CLL/SLL who have received anthracycline-containing chemotherapy prior to transformation: must have progressed, had SD or recurred with transformed disease after initial treatment for DLBCL ✓ Measurable Disease: Subjects with ALL must have evaluable or measurable disease (MRD positive by flow cytometry, NGS, or PCR is acceptable). Subjects with aggressive B-cell NHL must have evaluable or measurable disease according to the revised IWG Response Criteria for Malignant Lymphoma[38]. Lesions that have been previously irradiated will be considered measurable only if progression has been documented following completion of radiation therapy. ✓ CD22 expression: For ALL, must have confirmed positive CD22 expression of the malignant cells by immunohistochemistry or flow cytometry. For aggressive B-cell NHL, CD22 expression at any level, including undetectable, will be acceptable and subjects must have archival tissue available for analysis of CD22 expression, or must be willing to undergo biopsy of easily accessible disease. ✓ Subjects who have progressed or relapsed after prior autologous SCT must be at least 100 days post-transplant, have no evidence of GVHD, and have been without immunosuppressive drugs at least 30 days. ✓ Meet required prior therapy washout windows (see inclusion criteria for leukapheresis for details). ✓ Subjects with prior CAR therapy must be at least 30 days post CAR infusion and have < 5% CD3+ cells express the previous CAR, if a validated assay is available. ✓ Toxicities from prior therapy stable or resolved (except for clinically non-significant toxicity and cytopenias covered in * footnote) ✓ Age: ≥ 18 years of age. ✓ Adequate performance status (ECOG 0, 1, or 2; or Karnofsky ≥ 60% ✓ Adequate organ and marrow function as defined by: ○ ANC ≥ 750/uL *, platelet count ≥ 50,000/uL *, ALC ≥ 150/uL * ○ Creatinine ≤ 2 mg/dL OR Creatinine Clearance ≥ 60 mL/min ○ Serum ALT/AST ≤ 10 times the ULN (institutional normal) [Elevations related to leukemia involvement of the liver will not disqualify a subject] ○ Total bilirubin ≤ 1.5 mg/dL (except in subjects with Gilbert's disease) ○ Cardiac ejection fraction (LVEF) ≥ 45% and no evidence of pericardial effusion ○ No clinically significant ECG findings ○ No clinically significant pleural effusion ○ SaO_2 > 92% on room air ✓ Subjects with CNS involvement or a history of CNS involvement are eligible only in the absence of neurologic symptoms that may mask or interfere with neurological assessment of toxicity. ✓ Females of child-bearing potential must have negative pregnancy test.

	✓ Females of child-bearing potential and males of child-fathering potential must be willing to practice birth control from time of enrollment and for 4 months post preparative lymphodepletion regimen. ✓ Must be able to provide informed consent (LAR is permitted if subject able to provide verbal assent). ✓ May NOT have ALL limited to isolated testicular disease. ✓ May NOT have hyperleukocytosis (≥ 50,000 blasts/μL) or rapidly progressive disease that in the estimation of the investigator and sponsor would compromise ability to complete study therapy. ✓ May NOT have history of other malignancies, apart from non-melanoma skin cancer or carcinoma in situ, unless disease free for at least 3 years, or in remission 1-2 years and Principal Investigator assesses other malignancy as unlikely to return within 1 year or interfere with CAR T cell safety. ✓ May NOT have active fungal, bacterial, viral or other infection requiring intravenous antimicrobials Simple UTI or uncomplicated bacterial pharyngitis is permitted if responding to active treatment. ✓ May NOT have ongoing HIV, HBV or HCV infection. History of HBV or HCV is permitted if viral load is undetectable by qPCR and/or nucleic acid testing. ✓ May NOT have active cerebrovascular ischemia/hemorrhage, dementia, cerebellar disease, or autoimmune disease with CNS involvement that in investigator's judgement impair ability to evaluate neurotoxicity. ✓ May NOT have history of MI, cardiac angioplasty or stenting, unstable angina or other clinically significant cardiac disease with 12 months of enrollment. ✓ May NOT have severe, immediate hypersensitivity reaction attributed to compounds of similar chemical or biologic composition to any agents used in study. ✓ May NOT be pregnant or breastfeeding. ✓ May NOT have primary immunodeficiency or history of autoimmune disease (e.g. Crohns, rheumatoid arthritis, systemic lupus) requiring systemic immunosuppression/systemic disease modifying agents within the last 2 years. ✓ May NOT, in investigator's judgment, have any medical condition likely to interfere with assessment of safety or efficacy, or be unlikely to complete all protocol-required visits and procedures. * A subject will not be excluded because of pancytopenia ≥ Grade 3 if it is felt by the investigator to be due to underlying leukemia/lymphoma. Subjects may be enrolled using two stages (1) Leukapheresis and 2) Lymphodepletion Chemotherapy and Cell Infusion) using these same criteria (see **Section 13.8** Appendix H) if patient or manufacturing scheduling require.
Investigational Product, Dose, and Mode of Administration	Autologous peripheral blood mononuclear cells (PBMC) will be obtained by leukapheresis and cryopreserved. Cryopreserved PBMC stored from participation in other institutional cell therapy or cell collection studies or standard of care may be used to generate the cellular product on this study as long as they meet the criteria established in this IND. PBMC will be transported to the manufacturing facility (MBI or LCGM), where they will undergo selection, activation, transduction with the lentiviral vector, expansion, and formulation for the manufacture of CD22-CAR T cells. The product will be cryopreserved and returned to Stanford's Cell Therapy Facility (CTF), from which the product will be distributed to the patient care unit for infusion. Autologous CD22-CAR T cells will be administered intravenously at Dose Level 1 in subjects with ALL. Autologous CD22-CAR T cells will be administered in 3 escalating doses (Dose Level 1, 2, and 3) in subjects with aggressive B-cell NHL to determine MTD/RP2D. A conditioning lymphodepletion chemotherapy regimen will be administered prior to cell infusion. If the rate of DLT ever exceeds the maximum acceptable rate in a disease group, the next lower dose level will be

	explored in that disease group. ▪ Dose Level -1: 3 x 10^5 transduced T cells/kg (± 20%) ▪ Dose Level 1: 1 x 10^6 transduced T cells/kg (± 20%) ▪ Dose Level 2: 3 x 10^6 transduced T cells/kg (± 20%) ▪ Dose Level 3: 1 x 10^7 transduced T cells/kg (± 20%) Lymphodepletion chemotherapy regimenprior to CD22-CAR T cell infusion (Day 0) will occur according to one of the following regimens, at the discretion of the investigator: • Fludarabine 30 mg/m^2 per day IV for days -5, -4, -3 • Cyclophosphamide 500 mg/m^2 per day IV for days -5, -4, -3 OR • Bendamustine (Bendeka®) 90 mg/m2 per day IV over 10 minutes on Days -3, -2 OR • Clofarabine 40 mg/m2 per day IV over 2 hours on Days -6, -5, -4, -3 Cyclophosphamide 500 mg/m2 per day IV over 60 minutes on days -6, -5
Study Design and Methodology	This is a Phase 1 study in adult subjects with B-ALL and aggressive B-cell NHL who have relapsed or refractory disease after standard therapies. Subjects will receive a conditioning lymphodepletion chemotherapy regimen followed by infusion of CD22-CAR T cells. Subjects in each disease cohort will be evaluated after treatment for toxicity, antitumor effects and for persistence of CAR in blood samples and functionality of transduced T cells. Feasibility will be assessed initially in each disease population. Subjects with ALL will receive CD22-CAR T cells at the established dose of 1 x 10^6 transduced T cells/kg (± 20%) based on previous experience, whereas the dose has yet to be established in subjects with aggressive B-cell NHL. Hence, a standard 3 + 3 dose escalation design will be used in subjects with aggressive B-cell NHL to establish MTD/RP2D. The safety of CD22-CAR T cells will be evaluated in subjects with ALL at the established dose and subjects with aggressive B-cell NHL at the MTD/RP2D separately. In addition a futility analysis will be conducted in each disease group (ALL at the established dose and aggressive B-cell NHL at the MTD/RP2D) to evaluate whether the treatment regimen is efficacious enough to warrant a Phase 2 study.
Statistical Considerations	This is a Phase I trial simultaneously evaluating feasibility and safety with a possible dose-expansion cohort to indicate efficacy in patients with relapsed/refractory ALL and aggressive B-cell NHL. Initially, 50% feasibility is required in the first 6 and 10 patients with R/R aggressive B-cell NHL and R/R ALL, respectively; after which, 80% feasibility is desired and will allow the study to replace a total of 10 or 6 patients with R/R aggressive B-cell NHL and R/R ALL, respectively. The expected DLT rate is 20% for each disease population, however, the maximum acceptable DLT rate is 30%. The traditional '3+3' design will be used for possible dose-escalation in patients with R/R aggressive B-cell NHL across three dose levels. If a feasible dose is established as MTD/RP2D for this disease population, the trial will expand for these patients to investigate an overall response rate (CR or PR at 3 months) greater than 25% (using a Minimax Simon two-stage design to motivate sample sizes and decision rules). At the previously established dose level for R/R ALL patients, the trial will continuously monitor safety for a maximum 30% DLT rate and investigate an overall response rate (CR at 28 days) greater than 50% (using a Minimax Simon two-stage design to motivate sample sizes and decision rules).

SCHEMA


CD22-CAR T cell Trial Schema: Initial 3 Months
CAR T-cell Production
Manufacturing 7-11 days
Release testing 5-7 days
Subjects required to remain within commuting distance to the clinic
Lymphodepletion chemotherapy * * *
Daily evaluations Day 0 to Day 14
Twice-weekly evaluations Day 15 to Day 27
Primary Efficacy for subjects with ALL
Primary Efficacy for subjects with aggressive B-cell NHL
Screening/ICF visit
Day -6
Day -5
Day -4
Day -3
Day -1
Inpatient
Outpatient
Day 0
Day 7
Day 14
Day 21
Day 28
Month 2
Month 3
Optional
Optional
Apheresis
CAR T-cell infusion
Clinic Visit
Correlative Labs
ClonoSeq MRD Analysis (ALL Patients)
Disease Evaluation
* Cyclophosphamide 500 mg/m2/day X 2 days With Clofarabine 40 mg/m2/day X 4 days; OR
* Cyclophosphamide 500 mg/m2/day X 3 days With fludarabine 30 mg/m2/day X 3 days; OR
* Bendamustine 90 mg/m2/day IV X 2 days

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADL	Activities of daily living
AE	Adverse event
ALC	Absolute lymphocyte count
ALL	Acute lymphoblastic leukemia
ALT/ AST	aspartate transaminase/ alanine transaminase
ANC	Absolute neutrophil count
APB	Administrative Panel on Biosafety
BID	Twice daily
BITE	Bi-specific T-cell Engager
BLA	Biologic license application
BSA	Body surface area
CAR	Chimeric Antigen Receptor
CBC	Complete blood count
CI	Confidence interval
CLS	Capillary Leak Syndrome
CMAX	Maximum concentration of drug
CMS	Centers for Medicare & Medicaid Services
CMV	Cytomegalovirus
CNS	Central nervous system
COA	Certificate of Analysis
CR	Complete response
CRF	Case report/Record form
CRi	complete response with incomplete blood count recovery
CRP	C reactive peptide
CRS	Cytokine release syndrome
CSF	cerebral spinal fluid
CTCAE	Common Terminology Criteria for Adverse Events
CTF	Cellular Therapeutics Facility
DIC	Disseminated intravascular coagulation
DLBCL	Diffuse large B-cell lymphoma
DLCO	Diffusing capacity of the lungs for carbon monoxide
DLT	Dose Limiting Toxicity
DMSO	Dimethyl Sulfoxide
DOR	Duration of Recovery
DSMC	Data Safety Monitoring Committee
EBV	Epstein-Barr virus
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EFS	Event free survival
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FIH	First in Human
FL	Follicular lymphoma

GI	Gastrointestinal
GMP	Good Manufacturing Practices
GvHD	Graft versus Host Disease
HbsAG	Hepatitis B surface antigen
HDT	high dose platinum-based therapy
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
HLH	Hemophagocytic lymphohistiocytosis
HPF	High-power field
HSCT	Hematopoietic Stem Cell Transplant
HSV	herpes simplex virus
HTN	Hypertensions
HUS	Hemolytic Uremic Syndrome
IDB	ideal body weight
IL-2	interleukin-2
IRB	Institutional Review Board
IV	Intravenous
LAR	Legally Authorized Representative
LCGM	Stanford's Laboratory for Cell and Gene Medicine
LLN	Lower limit of normal
LP	Lumbar puncture
LV	Lentivirus
LVEF	left ventricular ejection fraction
MAS	macrophage activation syndrome
MHC	Major Histocompatibility Complex
MI	myocardial infarction
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
MUGA scan	multigated acquisition scan
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NGS	Next generation sequencing
NHL	Non-Hodgkin lymphoma
OKT3	anti-CD3 antibody
OR	Overall response
OS	Overall survival
PBMC	Peripheral blood mononuclear cells
PCR	polymerase chain reaction
PD	Progressive disease
PET	Positron Emission Tomography
PFS	Progression free survival
PFT	Pulmonary function test
PLT	Platelet

PMBCL	Primary mediastinal large B-cell lymphoma
POB	Pediatric Oncology Branch
PR	Partial response
QD	Once daily
R-CHOP	rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone
R/R	Relapsed/refractory
RCL	Replication competent lentivirus
RECIST	Response evaluation criteria in solid tumors
RP2D	Recommended phase 2 dose
RR	Response rate
RV	Retrovirus
SAE	Serious adverse event
SaO_2	oxygen saturation level
scFv	single chain variable fragment
SCT	stem cell transplant
SD	Stable disease
SMC	Safety Monitoring Committee
SRC	Scientific Review Committee
TCR	T-cell Receptor
TCS	T-cell selection
tDLBCL	transformed DLBCL
TLS	Tumor lysis syndrome
TTP	Time to progression
ULN	Upper limit of normal
UNK	Unknown
VZV	varicella zoster virus
WBC	White blood cell
WHO	World Health Organization

1 OBJECTIVES

1.1 PRIMARY OBJECTIVE

- Determine the feasibility of manufacturing CD22-CAR T cells using the Miltenyi CliniMACS Prodigy® system for administration to adults with relapsed/refractory CD22 expressing B-cell ALL or relapsed/refractory aggressive B-cell NHL.
- Establish the maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D) of CD22-CAR T cells in adults with relapsed/refractory aggressive B-cell NHL.
- Determine the safety of an established dose of CD22-CAR T cells in adults with relapsed/refractory CD22 expressing B-cell ALL and the safety of the MTD/RP2D of CD22-CAR T cells in adults with relapsed/refractory aggressive B-cell NHL.

1.2 SECONDARY OBJECTIVE

- Assess the clinical activity of CD22-CAR T cells in adults with R/R CD22 expressing B-cell ALL and R/R aggressive B-cell NHL, including overall survival (OS) and progressive free survival (PFS).

1.3 EXPLORATORY OBJECTIVES

As part of participation

- Analyze alterations in early B cell development induced by immune pressure exerted via CD22-CAR T cells.
- Evaluate whether subjects receiving CD22-CAR T cells relapse with loss or diminished expression of CD22, when feasible.
- Measure persistence of CD22-CAR T cells in the blood, bone marrow and CSF, and explore correlations between CD22-CAR T cell properties and CAR T cell efficacy and persistence.
- Establish the utility of chromatin structure and epigenomic technology to characterize CAR T cell therapies.
- Analyze CD22 expression in aggressive B-cell NHL and correlate with disease response.

2 BACKGROUND AND RATIONALE

2.1 RELAPSED/REFRACTORY B-CELL MALIGNANCIES IN ADULTS

2.1.1 Acute Lymphoblastic leukemia (ALL)

Relapsed/refractory (R/R) B-cell acute lymphoblastic leukemia (ALL) is a predominantly CD19+/CD22+ hematologic malignancy that is associated with dismal long-term survival in both children and adults. The incidence of ALL follows a bimodal distribution with the first peak occurring in early childhood, and a second occurring in older adults. Although survival rates for pediatric ALL have consistently improved over the last 20 years with most recent estimates of long-term disease free survival of 90% in children with standard-risk B-cell ALL,[39] outcomes for children and adults with relapsed/refractory B-cell have remained particularly poor.

In 2017, FDA approved Kymriah™ (CTL019, tisagenlecleucel) the first chimeric antigen receptor (CAR) T cell therapy for children and young adults (up to 25 years of age) with R/R B-cell

ALL[40]. The approval was based on an overall remission rate of 83% (52/63). Two other studies conducted using CTL019 reported overall remission rates of 69%-95%, while all three reported similar complete remission rates (62-69%)[12],[18, 41]. Importantly, OS at 12 months was improved relative to OS reported with other therapeutic agents such as clofarabine, blinatumomab or combination therapy with clofarabine/etoposide/cyclophosphamide.

Despite improvements in pediatric ALL survival rates, the survival rate in adult ALL has remained flat at 30-40%[42]. Outcomes for adults with relapsed/refractory B-cell ALL have been particularly poor. For these subjects, complete response (CR) to conventional salvage chemotherapy occurs in < 30% and disease-free survival following salvage therapy with or without allogeneic SCT has been reported to be < 25% in younger adults and < 10% in adults ≥ 50 years[1],[2],[43],[3]. Toxicity associated with chemotherapy and SCT are substantial, and adult subjects who relapse following SCT rarely experience long-term survival[44].

Therapeutic advances in adult B-cell ALL have been achieved in recent years. Notably, the development and FDA approval of the anti-CD19/CD3 bi-specific T-cell engaging (BITE) agent blinatumomab has resulted in complete response rates in adult subjects with R/R B-cell ALL of 50-80%[45],[46] and 30-40% in children[47]; however, the responses are often not durable and many subjects relapse without consolidative allogeneic stem cell transplant. Further, approximately 20% of subjects treated with CD19 targeting BITE therapy will experience CD19 escape, relapsing with CD19 negative leukemia[20].

Inotuzumab ozogamicin, a humanized anti-CD22 antibody conjugated to the cytotoxic molecule calicheamicin, was FDA-approved in August 2017 for the treatment of R/R B-cell ALL in adults. In the pivotal Phase III trial conducted in adults with R/R B-cell ALL expressing CD22, CR was achieved in approximately 80%, but remission duration was only a median of 4.6 months (95% CI, 3.9 to 5.4 months[6]). Additional agents are thus needed in order to improve the outcomes of adults with B-cell ALL.

2.1.2 Lymphoma

Diffuse Large B-Cell Lymphoma (DLBCL) is a CD19+/CD22+ non-Hodgkin lymphoma that represents the most common aggressive lymphoid malignancy in the United States and Europe, accounting for approximately 30-40% of all non-Hodgkin lymphoma (NHL)[48],[49]. There are approximately 25,000 new cases of DLBCL in the US each year[50].

Therapeutic advances have been achieved in standard first-line treatment of DLBCL with the addition of the anti-CD20 monoclonal antibody rituximab to cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP)[51],[52]. Despite this, about one-third of subjects with DLBCL do not achieve durable remission and/or relapse following intial response. [53],[54],[55].

The current approach to R/R DLBCL is salvage chemotherapy followed by high dose platinum-based therapy (HDT) and autologous stem cell transplantation (SCT)[56]. Unfortunately, half of subjects with primary relapsed/refractory disease are not eligible for HDT and autologous SCT right because of age (typically older than 70-75 years), comorbidities, or lack of adequate social support to help with the care needed after transplantation [57]. Of the remaining 50% initially eligible, an additional 50% do not receive HDT-SCT mainly because their disease shows no sensitivity to salvage chemotherapy[7]. Thus, only a fraction of patients with relapsed DLBCL will be cured with HDT followed by autologous SCT[54].

The large SCHOLAR-1 retrospective study highlights the poor outcomes across all subgroups of chemorefractory DLBCL. For these subjects, expected median OS is only 6-7 months, with the lowest OS rates observed in subjects refractory to second or later line therapy and those who relapse less than 12 months after SCT. In aggregate, response to therapy in refractory DLBCL was 26%, with a CR rate of only 7%[58].

Similarly, the recently reported REFINE collaborative retrospective study analyzed the outcomes of subjects with primary refractory DLBCL. They reported an overall response rate [(OR) = complete response (CR) + partial response (PR)] of approximately 40% to salvage therapy. For the subgroup undergoing autologous SCT, the 2-year OS rate was only 54.9%, with the subgroup with germinal center B type DLBCL with positive c-myc expression (by FISH) having a far worse OS rate of 23.7%[59]. Thus, there is a pressing need to develop and incorporate novel therapeutic agents into the treatment of subjects with R/R DLBCL.

In October 2017, CAR T cell therapy directed at CD19+ R/R B-cell lymphomas entered the clinical landscape as a very promising new approach. Objective response rates of approximately 70% and CR rates of approximately 50% in heavily pre-treated R/R adult DLBCL patients led to the recent FDA approval of axicabtagene ciloleucel (Yescarta™) and tisagenlecleucel (Kymriah™) in DLBCL refractory to at least two lines of previous therapy[60];[15].

2.2 CHIMERIC ANTIGEN RECEPTOR (CAR) THERAPY

Chimeric Antigen Receptor (CAR) expressing T-cells is a new therapy wherein a subject's own T-cells are harvested and subsequently genetically modified in order to target cell surface antigens on specific cancer cells. CARs are non-native receptors that link an antigen-binding domain to cell signaling domain(s). When expressed in T cells, they endow major histocompatibility complex (MHC)-unrestricted antigen specificity. Most commonly, CARs utilize a single chain variable fragment (scFv) coupled to TCR zeta and CD28 or 4-1BB endodomains. In addition to their specificity, these CAR T-cells can be modified to be highly proliferative and possess the ability to negate immunosuppressive mechanisms making them ideal agents against highly aggressive cancers. Beginning in 2010, several groups published clinical results using CAR T cells targeting CD19 for B cell malignancies.

2.2.1 CD19 CAR T Cell Therapy

CD19 is a 95 kD transmembrane protein with expression restricted to the B cell lineage. Expression of CD19 is present from the time of immunoglobulin rearrangement through B cell development and maturation until it is lost with terminal plasma cell differentiation[61]. It is not expressed on primitive hematopoietic stem cells. CAR T cells targeting CD19 (CD19-CAR)first demonstrated impressive clinical efficacy in small case series in adults with B cell lymphomas and chronic lymphocytic leukemia [62-64]. The first pediatric report followed shortly after and demonstrated striking responses in two children with refractory B-ALL[18]. The demonstration of exquisite sensitivity of acute B cell leukemias inspired multiple centers to advance translation of CD19 targeting CARs. Despite utilizing varying strategies, clinical trials from multiple institutions have consistently shown dramatic activity of CD19-CAR T cells in pediatric and adult subjects with relapsed and refractory B-ALL with complete responses rates of ≥ 70% across trials [8],[9],[10],[6, 11, 15, 47].

Independent of the infused T cell dose, anti-leukemic effects generally do not occur in the absence

of expansion of the CD19-CAR T cells *in vivo*. Dramatic CD19-CAR T cell expansion can be observed, with > 1000-fold expansion occurring within 1-2 weeks in subjects with high tumor burden. CAR T cell expansion can be quantified using polymerase chain reaction (PCR) to measure the number of cells with integrated virus or by flow cytometry, where is it not uncommon to observe during expansion that > 70% of all circulating T cells express the engineered CD19-CAR receptor. Expansion of CAR T cells is antigen driven, but is greatly enhanced following a lymphodepleting preparative chemotherapy regimen. Extensive preclinical work has demonstrated that lymphopenia induces elevations in the availability of cytokines that drive T cell expansion, notably IL-7 and IL-15, and that increased availability of such factors leads to enhanced expansion and improved efficacy[65]. Based upon this paradigm, the vast majority of clinical trials using CAR T cells incorporate a pre-infusion preparative regimen comprising cyclophosphamide and fludarabine, agents known to induce profound lymphocyte depletion.

Another major insight gleaned from the substantial clinical experience with CD19-CAR T cells for B cell malignancies is the differential impact of distinct costimulatory domains on T cell function. It has become evident that CD28 costimulation facilitates more rapid and higher peak T cell expansion[9, 10]; it also predisposes T cells to early exhaustion, which leads to poor long-term T cell persistence as a result of activation induced cell death[36]. In contrast, 4-1BB costimulation is associated with a slower expansion rate, lower CAR T cell peak level, a diminished risk of T cell exhaustion and more prolonged persistence following adoptive transfer[36]. Although remission induction rates do not appear to differ between CD28 and 4-1BB costimulatory domain containing CD19-CARs, sustained remission likely requires T cell persistence and thus 4-1BB CARs may confer improved long-term outcomes.

2.2.2 CD19 Immune Escape Following CD19 Directed Immunotherapy

CD19 is universally expressed at high levels on B-ALL and DLBCL at diagnosis, and levels of expression do not change appreciably following cytotoxic therapy. Indeed, CD19-based flow cytometry provides powerful prognostications by measuring minimal residual disease (MRD) following standard cytotoxic therapy in B-ALL[66],[67],[68]. However, with the introduction of CD19-based immunotherapies, relapse with diminished or absent surface CD19 has been increasingly observed. CD19 immune escape was first reported following blinatumomab therapy[69], and has been observed by several groups following CD19-CAR therapy for B-ALL and DLBCL [18],[10, 19],[16]. In a recent report from Children's Hospital of Philadelphia of 50 subjects rendered into remission with CD19-CAR therapy, 40% of subjects had relapsed with a median follow-up of 10.5 months; and loss of the CD19 target accounted for 65% of the total relapses[70]. Similarly, in an expanded cohort of 75 subjects, loss of CD19 was seen in 15 of 16 subjects studied at the time of relapse[15]. Finally, in a report of CD19-CAR therapy for B-cell NHL, low expression of CD19 was reported in over 25% of patients with relapsed disease following CAR therapy[13]. While the true incidence of relapse due to CD19 immune escape has not yet been established, our CD19-CAR experience to date has implicated CD19 escape as the most common cause of post CD19-CAR relapse in B-ALL and B-cell NHL.

Investigation into the biology of CD19 immune escape has identified a complex biology guiding the loss or downregulation of CD19 expression. The two distinct patterns of tumor remodeling that have been recognized are categorized as the "isoform switch" and "lineage switch". The majority of cases of CD19 loss in ALL fall into the "isoform switch" category, wherein the cells retain all other characteristics of B-ALL and there is no clear evidence for alteration in fitness. In

the majority of "isoform switch" cases, mRNA specific for CD19 is retained, but is enriched for CD19 isoforms that preferentially remain intracellular, lack a transmembrane domain, and/or lack the epitopes targeted by all CD19-CARs currently under study as well as blinatumomab[71]. Such B-ALL cells resultantly express reduced total surface levels of wild-type CD19 or express aberrant CD19 that lack the epitope targeted by CD19-CARs and blinatumomab. In "lineage switch" cases, tumor cells undergo switching from the lymphoid to the myeloid lineage with a resultant population with myeloid markers and features that clonally relates to the parental B cell leukemia[19].

2.2.3 CD22 Expression in ALL and B-cell NHL

CD22 is a 140-kD phosphoglycoprotein member of the Ig superfamily that functions as a B-cell receptor signal transduction modifier and a cellular adhesion molecule[72],[73],[74],[75]. CD22 is restricted to cells of B-lineage and is expressed on the surface of mature B cells[76]. A variety of B-lineage malignancies are CD22+, including 96 to 100% of cases of childhood B-precursor ALL and 66 to 100% of Burkitt's lymphoma, with the majority of malignant cells expressing this antigen[77],[78],[79],[80],[81],[82]. In addition, CD22 expression is restricted to the B lymphocyte lineage beginning at the stage at which IgD expression is initiated[73],[83],[84],[85]. Targeting CD22 is especially important in the growing era of CD19 targeted therapy for two reasons: 1) Not all patients respond to CD19 targeted therapies and 2) CD19-negative escape has been observed following CD19-CAR therapy, as well as following anti-CD19/CD3 bispecific antibody. Thus, additional targeted strategies are needed.

A recent study performed at the National Cancer Institute (NCI) evaluating CD22 expression in children, adolescents and young adults with relapsed/refractory ALL (***Table 1***), demonstrated that CD22 was expressed on 100% of all samples (n=132)[86]. Median antigen site density of surface CD22 was 3,470 sites/cell (range 417-19,653, n=128). In those with known 11q23 *MLL* rearrangement, site density was lower (median 1,734 sites/cell (range 417-3,624, n=18)) (p=<0.0001) and 5 of 19 cases had sub-populations of blasts lacking CD22 expression (22%, 29%, 73%, 75% and 82% CD22+). CD22 expression was maintained in serial studies of 58 subjects, including those treated with anti-CD22 immunotoxins. The levels of soluble CD22 measured in the blood and marrow by ELISA were low and would not affect the pharmacology of anti-CD22 directed agents. CD22 expression was recapitulated in 9 of 9 human ALL-murine xenografts established with limiting dilution studies, suggesting the presence of CD22 expression on leukemia initiating cells. These characteristics make CD22 an excellent target in ALL, though cases with MLL rearrangement may warrant further study[87].

CD22 has been reported to be nearly universally expressed in B-cell NHL, including in samples obtained from patients with newly diagnosed and/or R/R disease and evaluated for CD22 expression by flow cytometry[32],[88] (***Table 1***). However, a recent study evaluated CD22 expression in 32 patient samples of patients with R/R DLBCL and found variable CD22 expression by immunohistochemistry ranging from 0-100% expression on lymphoma cells[89]. Interestingly, several studies evaluating anti-CD22 agents including antibody-drug conjugates have demonstrated no association between CD22 expression level and response to these agents in CD22+ DLBCL[89],[32],[90].

Table 1: Surface Expression of CD22 in Cases of B-lineage ALL and DLBCL

Series	Cases CD22 +[a]	CD22 + % Blasts Median (range, if available)	CD22 Site Density Sites/cell Median (range)
Bostrom[91]	31/31 (100%)	N/A	N/A
Lones[92]	55/55 (100%)	96% (74-99%)	N/A
Yusuf[93]	48/50 (96%)	N/A	N/A
Chevallier[31]	42/45 (95.5%)	(95.5%) with greater than 90%	N/A
Raponi[28]	397/427 (93%)	(79.9%) with CD22+ on 100% blasts (13.1%) with partial expression	1450 (548-2487) pro-B ALL 2356 (412-8294) B-common ALL 2810 (793-9143) Pre-B ALL
Iwamoto[94]	70.3% (of 1250 early pre-B cases) 87.6% (of 248 pre-B cases) 60.5% (of 45 mature B cases)	N/A	N/A
Shah, et al.	132/132 (100%)	100% (22-100%)	3,470 (417-19,653)
Polson (2010)[32]	59/59 (100%) in DLBCL	N/A	N/A
Boyd (2013)[88]	34/35 (97%) in DLBCL	N/A	N/A
Pfeifer (2015)[89]	26/32 (81%) in DLBCL	N/A	N/A

Importantly, CD22 is not expressed on pluripotent hematopoietic stem cells. While destruction of normal B cells is a drawback to targeting CD22, clinical experience suggests that destruction of normal B cells is a tolerable toxicity of leukemia-directed therapy. When patients receive the anti-CD20 monoclonal antibody rituximab, the number of normal B cells is severely depressed for several months[95], yet patients that receive chemotherapy plus rituximab do not have an increased rate of infections when compared to patients that receive chemotherapy alone[96]. Similarly, patients with congenital absence of B cells have prolonged survival and acceptable quality of life as a result of intravenous infusions of IgG (IVIG).

Several monoclonal antibodies, including epratuzumab[62] a fully humanized unconjugated monoclonal antibody targeting CD22; inotuzumab ozogamicin[97],[98] an anti-CD22 monoclonal antibody conjugated to the calichaemicin toxin; and moxetumomab pasudotox[99],[100], an anti-CD22 immunotoxin monoclonal antibody conjugated to the pseudomonas exotoxin, have been studied in the treatment of CD22 expressing hematologic malignancies. Experiences to date have demonstrated safety and efficacy of targeting CD22 in patients with relapsed/refractory disease, although the optimal dosing, schedule and incorporation of this approach with combination therapy are under study.

2.2.4 Development of an CD22-CAR for Clinical Adoptive T Cell Transfer

Chimeric antigen receptors (CARs) were developed that could be used to re-target activated T

lymphocytes to recognize and eliminate CD22+ leukemias. The T cells are derived from peripheral blood and activated in tissue culture with either anti-CD3 antibody alone (OKT3) or with beads that have been conjugated to both OKT3 and an anti-CD28 antibody, in the presence of interleukin-2 (IL-2). Activated T cells can be readily transduced with either retrovirus-based (RV) or lentivirus-based (LV) gene vectors. Vectors are produced by transfecting a producer cell line (293-GP for retrovirus) with a plasmid encoding a viral envelope glycoprotein and a second plasmid containing the viral vector backbone construct which includes the gene of interest, bounded by sequences that will insure its packaging into a RV or LV particle and subsequent expression of that backbone gene in the target cell of interest, the activated T cell. The safety of RV or LV is assured by splitting the envelope genes, the backbone containing the gene insert, and the packaging genes, between different plasmids or in the packaging cell itself. The LV system contains even more safety features with regard to segregating the genes required to make a vector particle, and thus making the re-emergence of an infectious particle essentially impossible; although it is tested for replication competency each time a new batch of vector is created. The engineered receptor is called "chimeric" because it is a fusion between an extracellular antibody-derived binding domain (usually called the scFv), and T cell derived transmembrane signaling domains that activate a T cell bearing the CAR once it associates with another cell bearing the target antigen that the scFv can bind to. In short, the CAR gene of interest combines the antigen binding capacity of an antibody with the T cell activation domains that are activated when the T cell recognizes cognate antigen by virtue of the antigen T cell receptor (TCR).

With the assistance of Dr. Ira Pastan, whose group developed the binding domains used in the recombinant CD22 immunotoxin, NCI Pediatric Oncology Branch (POB) investigators incorporated the identical binding domains into CAR constructs. The Pastan lab at NCI had developed both BL22, the original anti-CD22 binding scFv, and HA22, a high-affinity anti-CD22 scFv. HA22 is a specifically mutated version of BL22, where 3 key amino acids in the CDR3 binding domain were changed to increase soluble immunotoxin's potency approximately 10-fold[10]. Through molecular cloning, the BL22 and HA22 CD22 binding sites (the scFv) were ligated to DNA sequences encoding T lymphocyte specific transmembrane and signaling domains[29]. Additional CD22-CAR constructs were generated using another anti-CD22 scFv binding motif, called M971, that had been developed by the laboratory of Dr. Dimiter Dimitrov at NCI Frederick[9] .

We have also collaborated with Dr. Steven Rosenberg and Dr. Richard Morgan in NCI Surgical Oncology Branch. They had developed a series of retroviral vector backbone vector constructs that could be used to rapidly test new binding motifs for CARs. These vectors feature either a second generation signaling package, comprised of CD28 and the CD3 zeta chain, or a third generation signaling package, featuring CD28, 4-1BB (CD137), and CD3-zeta signaling motifs. These vectors also differed in that the second-generation vectors used the native CD28 transmembrane domain, while the third-generation vectors used the transmembrane domain from CD8 (see Zhao, Y., 2009 J Immuno[101], for a full description of these combinations). We also re-engineered the Surgery Branch backbone vectors to create a second generation retroviral backbone that expressed the CD8 transmembrane linked to the 4-1BB (CD137) and CD3-zeta signaling domains. One other major variable we tested for in our vector formats was the inclusion of the CH2CH3 domains from IgG1 in the membrane proximal extracellular domain. To date there is no clear rule with regard to vector format construction, and some vector formats benefit from having the scFv binding domain extended away from the transduced T cell plasma

membrane.

Figure 1: Constructs Created to Evaluate CD22 CAR vector formats

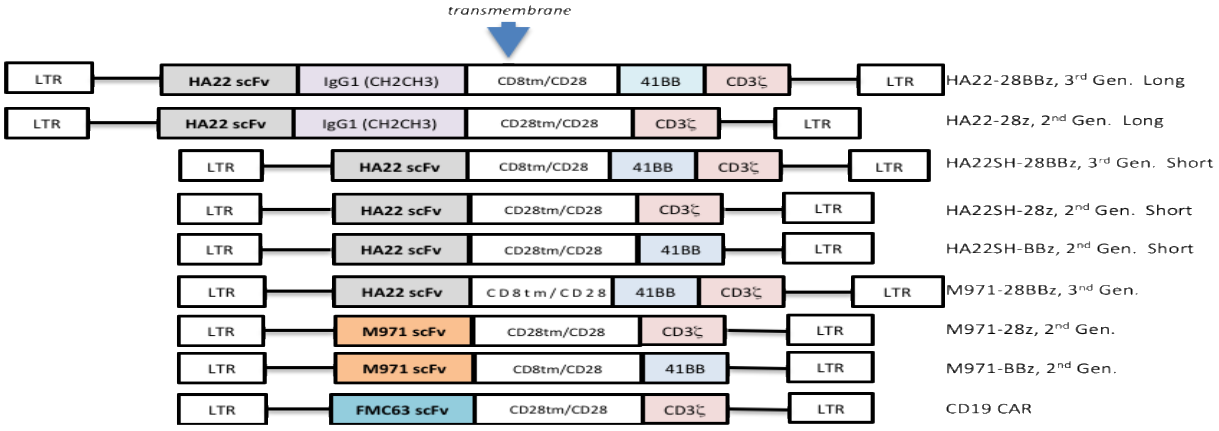


Figure 1. In this figure some of the constructs created to evaluate CD22 CAR vector formats are illustrated. All constructs are bounded by the RV-derived LTR sequences. Not shown but implied are the SA/SD and packaging sites following the 5' LTR that allow RNA from the linear plasmid sequence to be packaged into vector particles as they bud from the plasma membrane of the producer cell line. In addition to transfection with these backbone vectors, an *env* encoding plasmid vector (we use RD114 for RV and VSV-G for LV) must also be transfected into the producer line. Shown above the constructs is a large arrowhead where the transmembrane region of the protein transcribed from this vector would be located. Vectors are oriented around this point to communicate the general orientation of the domains with regard to the T cell surface. Illustrated are three different scFv (HA22, in gray; M971, orange; FMC63 (anti-CD19), blue), and the IgG1 derived CH2CH3 domain for the "Long" vector formats. Third generation vectors encode the CD28 (white), 4-1BB (CD137, light blue), and CD3-zeta chain (pink) signaling motifs. For illustration we show the anti-CD19 CAR currently used in NCI clinical trials, which is a CD28-CD3-zeta second generation whose scFv is derived from the FM63 antibody.

The inclusion of this constant domain was not beneficial in any of the anti-CD22 CAR constructs tested and so it was not included in the final vector format. Thus, we extensively tested three different scFv specific for CD22 (BL22, HA22, M971), the optimal distance of the scFv from the T cell membrane by including the IgG CH2CH3 domain, and both second and third generation signaling packages, see ***Figure 1***. Of note, the most important variable turned out to be the actual binding site of the scFcv on CD22 itself. M971 differed from BL22 and HA22 in that it bound to a different portion of the CD22 molecule, and it was this attribute, more proximal binding to the membrane, that provided the most active CAR construct[73]. M971 has the further advantage of being entirely derived from human sequences. M971 was derived from a human B cell derived cDNA library and thus there is no need to "humanize" the sequence, which is often carried out for scFv derived from murine monoclonal antibodies. The relative binding sites of HA22, M971, and a comparison to CD19 are illustrated in ***Figure 2***.

Figure 2: CD22 scFv binding sites

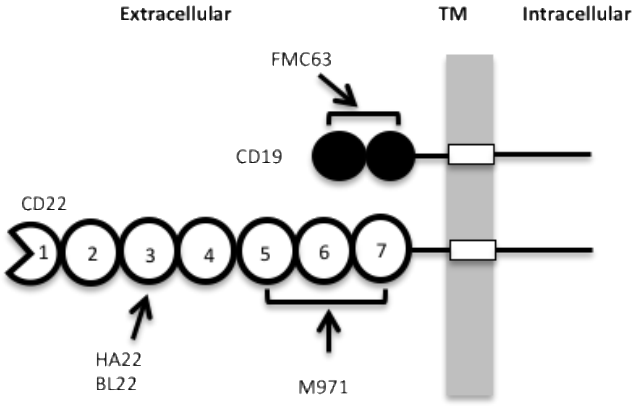


Figure 2. CD22 scFv binding sites. In this schema the relative positions of the HA22/BL22 and the M971 binding epitopes on the CD22 molecule are shown. For comparison, the CD19 molecule (filled circles), which has only 2 Ig-like structural domains as opposed to the 7 present in CD22 is shown. FMC63 is the scFv upon which the CD19-CAR is based.

To test for the anti-leukemic activity of CD22-CAR T cells, retroviral vector-containing supernatants were generated for each of the constructs described. The variables in domain structure included either two (second-generation CARs) or three (third-generation CARs) signaling domains, and either a "long" (containing the IgG CH2CH3 domain) or "short" (just the scFv) extracellular formats (***Figure 1***). Second-generation CARs were composed of either CD28 and CD3-zeta chain derived signaling domains or CD137 (4-1BB) and CD3-zeta chain motifs. Third generation CARs, which combined CD3-zeta, CD137, and CD28 motifs, always had lower surface expression on transduced T cells and provided no advantage in *in vitro* killing assays (using CD22+ leukemia cell lines as targets) or in cytokine production assays (not shown), and so efforts were concentrated on developing the two second generation formats. Our primary screening tool was to measure the ability of T cells transduced to express CARs to kill leukemia cells bearing CD22. Representative cytotoxic T lymphocyte (CTL) killing assays are shown in ***Figure 3***. For a positive control we used the CD19-CAR construct, and for a negative control we used non-transduced T cells that were cultured and activated in parallel (Mock). In this assay the superiority of the M971 construct is seen, as killing at equivalent effector-to-target ratios is superior to that of HASH 28z-CAR construct for all leukemia lines tested (see ***Figure 3***). T cells that were not CAR transfected (mock) did not kill leukemia cell lines. When cytokine production was measured by CAR-expressing T cells, the levels of interferon gamma uniformly matched the activity of that CAR in cell killing assays (not shown).

Figure 3: CTL Activity of CAR T cells

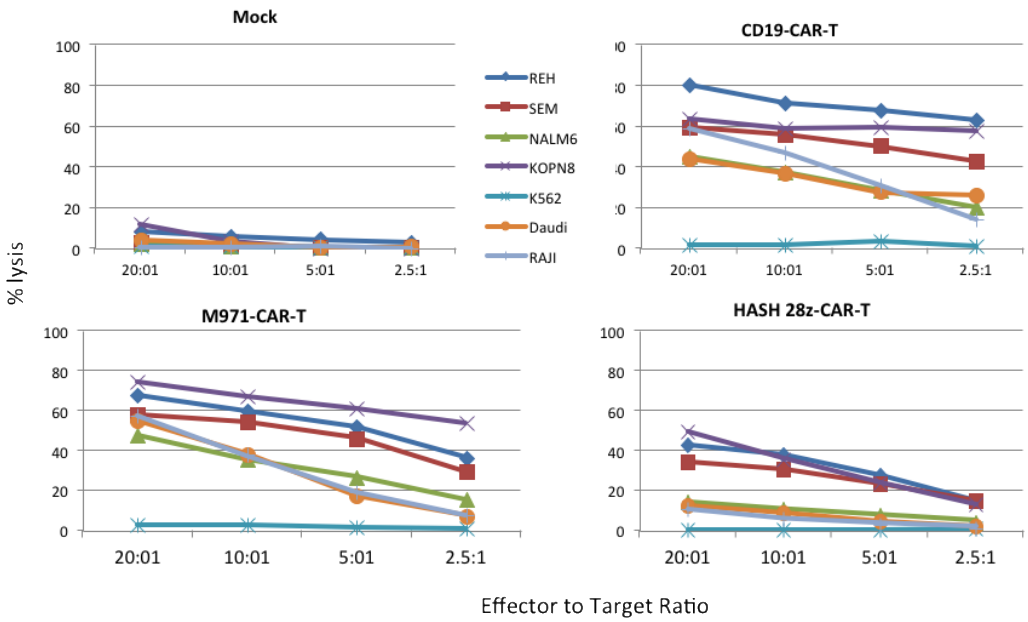


Figure 3. CTL Activity of CAR T cells. Human PBMC were activated in vitro then transduced 48 hours later with RV vector-containing supernatants encoding either CD19-CAR (upper right), the M971-CAR (lower left), or the CD22-specific HASH 28z-CAR (lower right). Cells were transduced for two consecutive days and then expanded 4-5 more days in IL-2 containing media. All vectors in this figure used second-generation, CD28-CD3 zeta chain, signaling motifs, and hence differed only in their extracellular binding domains. Also compared was a third generation HA22 vector and a second generation CD22 vector with 4-1BB signaling motifs. These did not have better killing than the HASH 28z construct shown here. Leukemia cell lines positive for both CD19 and CD22 (REH, SEM, NALM6, KOPN8, Daudi, Raji) and a control cell line (K562) were labeled with ^{51}Cr and used in a standard 5-hour Cr-release assay. Results are reported as percent lysis of targets versus the ratio of effector cells (CAR-Ts) to targets cells (leukemia cells) in triplicate wells.

The ability to transduce T cells and to function in *in vitro* assays of immune activity, such as in the CTL assay, was the first level of CAR construct evaluation. CD22-CAR constructs with the highest activity were then tested in *in vivo* assays. In these assays, NSG immunodeficient mice were injected intravenously (IV) with the NALM6 leukemia cell line that has been permanently transfected to express the luciferase gene (termed NALM6-GL, gift of S. Grupp and D. Barret, Children's Hospital of Pennsylvania). When NALM6-GL grows as a xenograft in the immunodeficient mice, the leukemia can be detected by imaging the mice with sensitive CCD camera that can measure photons produced by the luciferase enzyme following injection of the mice with the substrate luciferin. Although numerous experiments were carried out with the differing constructs, the optimal *in vivo* results were obtained when the leukemia-bearing mice were treated with a CAR that expressed the M971 scFv linked to the 4-1BB and CD137 signaling domains, ***Figure 4***. In this assay, human T cells were activated with anti-CD3/anti-C28 beads in the presence of 40 U/mL IL-2 and a transduction rate of 90% was achieved. A half-million NALM-6 human leukemia cells were injected on day 0, and three days later, CAR-expressing human T cells were injected (day 10 following initial activation). Mice were treated with non-transduced, or $1x10^5$, $1x10^6$, $5\ x10^6$, or $15\ x10^6$ CD22-CAR T cells. A clear dose response relationship was seen (***Figure 4***). Mice receiving $1\ x10^5$ transduced T cells showed no effect and were indistinguishable from mice treated with the non-transduced T cells. Mice receiving $15\ x10^6$ cells were completely cleared of disease.

Figure 4: CD22-CAR activity, dose escalation experiment

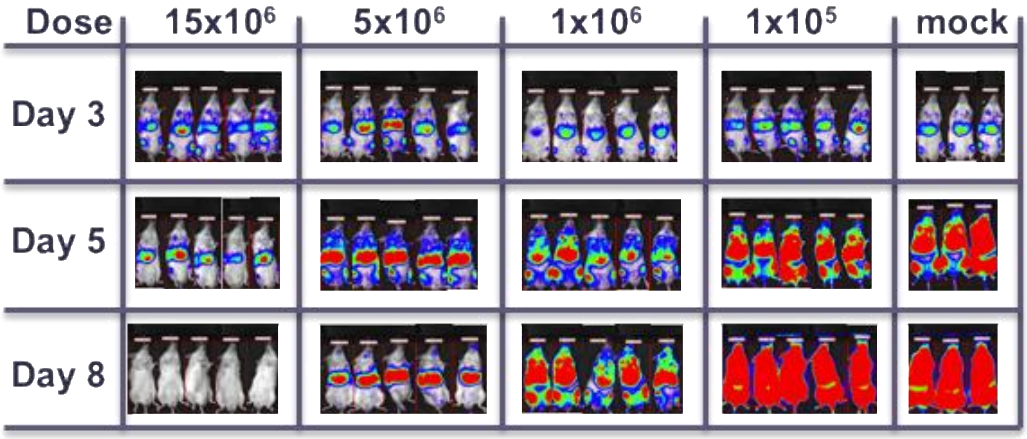


Figure 4. CD22 CAR activity, dose escalation experiment. NSG (immunodeficient) mice were injected with 0.5 x10^6 NALM6-GL cells on day 0, and imaging performed on day 3, using luciferin substrate, to demonstrate the presence of leukemia in all animals. On day 3, indicated groups of mice were injected IV. with 0.1, 1, 5, 10, or 15 x10^6 CD22-CAR transduced T cells. The CAR used expressed the m971 scFv and the CD3-zeta chain and CD137 signaling motifs. T cells were stimulated with anti-CD3/CD28 beads, cultured in 40 units/mL interleukin-2, and used 10 days after the initial stimulation. Mice were imaged again on day 5 and day 8, and complete elimination of the leukemia was seen with the highest dose level by day 8.

While there is no expectation that a LV-based CAR would function any differently in a transduced T cells than a RV-based CAR, a series of animal studies were carried out with LV constructs generated by the Mackall laboratory and using LV vector produced by the GMP-based protocol at Lentigen, Corp. ***Figure 5*** demonstrates the equivalency of both methods of T cell transduction.

Figure 5: Demonstrating equivalency of RV and LV vector transduction in disease-bearing mice

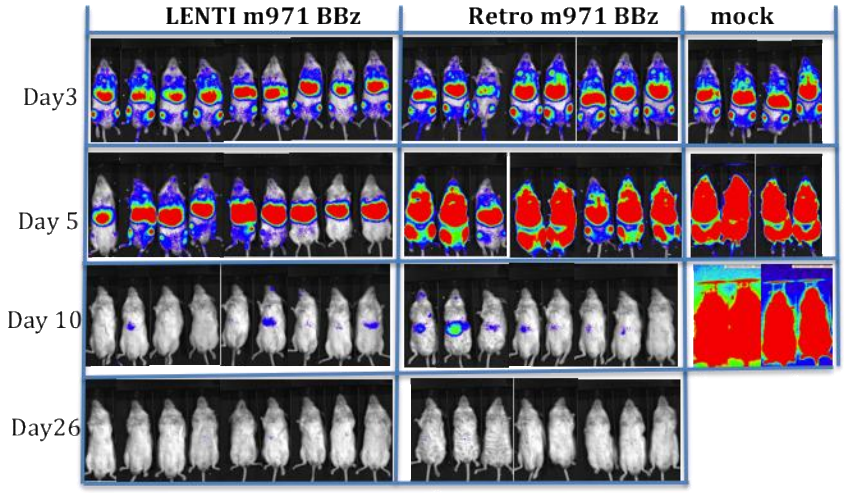


Figure 5. Demonstrating equivalency of RV and LV vector transduction in disease-bearing mice. Conditions identical to Figure 4 were used to analyze identical activated T cell populations transduced with either the anti-CD22 M971-BBz vector produced in a LV format or RV format. Mice treated with non-transduced T cells (mock) had to be sacrificed due to disease burden as per animal facility requirements prior to assessment on day 26.

2.2.5 CD22-CAR Therapy for B-ALL: Clinical Experience

The emergence of CD19 immune escape has prompted interest in the development of immunotherapies targeting alternative B-ALL cell surface molecules, such as the CD22-CAR. Clinical testing of this first chimeric antigen receptor targeting CD22 in children and young adults with R/R B-cell ALL up to the age of 30 years was recently reported by the NCI POB [37]. Doses of CD22-CAR T cells were administered in a standard 3 + 3 dose escalation design. The results from the first twenty-one subjects enrolled, including 17 who received prior CD19-CAR therapy, are encouraging. Lymphoblasts were CD19-negative or dim in 10 subjects at enrollment, and median blast percentage was 70.5% and all were CNS classification CNS1 (<5 WBC/mcL and no blasts, see **Appendix 13.3.1**). Median CD22 site density was 2839 molecules per cell (range 613-13452) at baseline.

Response varied with administered cell dose in this dose escalation study. The first dose level tested was 3 x 10^5 cells/kg; chosen to be lower than the current standard CD19-CAR dosing because this was a first-in-human (FIH) study. Six subjects received this dose, with one experiencing an MRD-negative CR. A second subject demonstrated signs of clinical activity as evidenced by a substantial reduction in circulating blasts, but the subject subsequently developed rapid disease progression and did not achieve a CR. At doses ≥ 1 x 10^6 cells/kg CD22-CAR T cells (dose level 2), CR occurred in 9/10 subjects who had received prior CD19-directed immunotherapy. There was no evidence that previous CD19-directed immunotherapy or diminished expression of CD19 impacted response to the CD22-CAR T cell therapy[37].

The primary toxicity reported was Grade 1 and Grade 2 cytokine release syndrome (CRS) with onset correlating with CAR T cell expansion on or after day 5. One subject experienced Grade 4 hypoxia, a dose limiting toxicity (DLT), at dose level 3 (3 x 10^6 cells/kg CD22-CAR T cells). Additional toxicities in the first 16 subjects included transient visual hallucinations (n=2), mild unresponsiveness (n=1), mild disorientation (n=1) and mild-moderate pain (n=2), which resolved to baseline by day 28 post-infusion. B-cell aplasia (< 50 cells/mcL) was noted in all subjects achieving remission, including subjects who were not previously B-cell aplastic. Subject #14 died in remission as a result of sepsis after resolution of CRS and resolution of treatment-induced neutropenia. Additional Grade 3-4 toxicities included: anemia, febrile neutropenia, sinus tachycardia, diarrhea, fever, multi-organ failure, catheter related infections, sepsis, altered liver function tests, expected toxicities of chemotherapy preparative regimen, electrolyte alterations, tumor lysis syndrome, hypotension and respiratory failure. Based on the single DLT at dose level 3 and evidence of clinical activity at dose level 2, 1 x 10^6 cells/kg CD22-CAR T cells was identified as the recommended phase 2 dose (RP2D) by NCI POB investigators.

From this small series, the investigators conclude that the CD22-CAR shows many characteristics that are similar to the CD19-CAR clinical experience. Response occurs rapidly within 28 days and correlates with CD22-CAR expansion. Cytokine release syndrome did occur, but the maximal severity was Grade 2. The CD22-CAR T cells traffic to the spinal fluid at levels similar to that observed with CD19-CAR. Although a cyclophosphamide/fludarabine based preparative regimen was used, neurotoxicity was limited to Grade 1 and observed in only 2 subjects. No evidence of unexpected toxicity was seen. As anticipated, since the CD22-CAR incorporates a 4-1BB costimulatory domain, subjects maintained CAR persistence and persistent B cell aplasia at 90 days, with some evidence for persistent CAR cells out to 9 and 18 months seen in those subjects who are in the longest remission.

NCI POB investigators have continued to treat children and younger adults with the CD22-CAR. As of June 22, 2018, a total of 44 patients have been treated (personal communication). After the initial publication[37],[102], the investigators altered their CD22-CAR product to include a T-cell selection (TCS) step. Following this shift in production method, an additional 7 patients were treated at the recommended 1 x 10^6 cells/kg dose. Three patients experienced DLTs (2 experienced disseminated intravascular coagulation [DIC]; one experienced macrophage activation syndrome). The investigators subsequently reduced the dose to 3 x 10^5 cells/kg using the TCS strategy. At this dose, three patients experienced DLTs (hypoxia, CRS, and one patient with capillary leak syndrome resulting in acute respiratory distress syndrome). Thirteen of the 17 patients treated with CD22-CAR with TCS manufacturing step experienced a bone marrow CR; all but one were MRD-negative complete remissions. In addition to CRS and neurotoxicity, the investigators noted that a side effect of the CD22-CAR product appears to be capillary leak pulmonary syndrome that is reversible with steroids. It is unclear whether this toxicity is related to CRS, but appears to occur without the typical CRS related hypotension. It is also unclear whether this side effect was related in part to the production method using TCS.

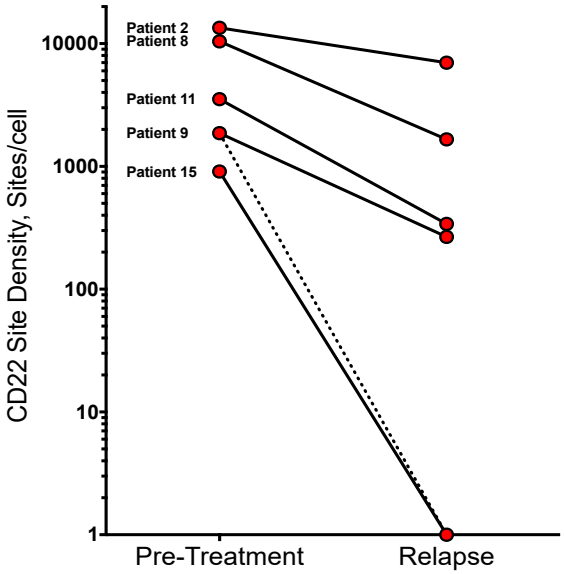
The experience at the POB NCI represents the first data to demonstrate that a chimeric antigen receptor targeting a molecule other than CD19 can also mediate potent antileukemic activity. This CD22-CAR is the first alternative to CD19 for CAR based immunotherapy for pediatric B-ALL. We now seek to test this CD22-CAR in an expanded population of adults with R/R CD22+ B-cell malignancies, including ALL and lymphoma. We do not plan to employ the TCS strategy used by the POB NCI in the most recent 17 patients treated. Therefore, we plan to use a CD22-CAR target dose of 1 x 10^6 cells/kg for subjects with ALL in this Phase Ib study. Because CD22-CAR T cells have not been previously administered to subjects with aggressive B-cell NHL, a Phase I dose escalation will be implemented to establish the recommended dose in this population.

2.2.6 CD22 Escape following CD22 targeted immunotherapy

Changes in CD22 surface expression has been observed at relapse following CD22-directed therapies. One notable example is seen following CD22-directed antibody therapy (inotuzumab), where several subjects emerged with partial loss of CD22 on leukemia blasts after this therapy. Similarly, in the CD22-CAR T cell trial reported by Fry, et al[37], alterations in CD22 site density were observed in a few of the relapsed subjects (see ***Figure 6***). Following CD22-CAR therapy, Subject #8 revealed a decrease in CD22 site density at the 6-month restaging evaluation from over 10,000 sites/cell prior to CD22-CAR therapy to approximately 1000 sites/cell at relapse in the presence of circulating CD22-CAR T cells. Subject #9, known to be CD19 negative at the time of CD22-CAR infusion, developed relapsed leukemia at 5 months post-CD22 CAR therapy that was partially positive for both CD19 and CD22. Subject #15, who was known to be CD19 negative prior to CD22-CAR therapy and who had received inotuzumab prior to receiving her CD22-CAR T cells, had a bimodal CD22 population noted in her peripheral blood flow with 89% of blasts expressing CD22; bone marrow flow cytometry showed complete CD22+ population at that time. Despite this, at 1-month post CD22 CAR therapy, she had a complete MRD negative remission with 41% circulating CD22-CAR T cells in her bone marrow. At her 2-month restaging visit, despite a high level of circulating CD22-CAR T cells, she relapsed with CD19-/CD22- ALL (see ***Figure 6***, Subject #15). Additionally, Subject #11 who was previously refractory to blinatumomab and CD19-CAR, attained a complete remission but had a decrease in CD22 site density from approximately 3,500 sites/cell prior to CD22 CAR therapy to less than 500 sites/cell at 1 month

post infusion. The mechanism for CD22 surface expression alteration is unknown at this time; however, constant CAR immune pressure as seen in CD19-CAR treated subjects may contribute to this phenomenon.

Figure 6: CD22 Site Density with CD22 CAR T cell Therapy



2.3 AUTOMATION OF CELLULAR THERAPY PRODUCT MANUFACTURING

Cell-based therapies, one of the latest advances in cancer immunotherapy, require complex manufacturing with significant regulatory implications. The U.S. Food and Drug Administration (FDA) has recently approved biologic license applications (BLA) for CAR T cell therapy from Kite Pharma, Inc. (Yescarta [axicabtagene ciloleucel) and Novartis (Kymriah™ [CTL019]) for CD19-CAR T cells based on remission rates in patients with refractory B-cell ALL and patients with large B cell lymphoma, respectively. These two new biologics offer an excellent option to many patients who previously did not have curative treatment options, but also unfortunately are accompanied by several limitations, such as: 1) costly therapy; 2) lengthy process for cell generation; and 3) CD19 immune escape, which has been described in **Section 2.2.2.**

The list price in the U.S. for Yescarta per dose is $373,000, while the price for Kymriah is $475,000[103]. As of April, 2018, the Centers for Medicare & Medicaid Services (CMS), said it would pay $395,380 to health care providers who use Yescarta on an outpatient basis (net price plus 6%)[104]. The agency set the co-payment for Yescarta at $79,076, but indicated that the patients' costs would be limited to the annual inpatient hospital deductible of $1,340 in 2018. Similarly, CMS stated their payment rate for Kymriah is $500,839[104]. While additional reimbursement has been requested for inpatient cell therapies, which may reach a total cost of as much as $1.5 million, CMS has not announced their final decision.

It has been suggested that the Miltenyi CliniMACS Prodigy® 'all-in-one' cell processing system can produce a cellular product meeting the same release criteria for safety and functional analysis as the FDA approved manufacturers, for less than one-third of the cost. This closed system completes the cell preparation, enrichment, activation, transduction, final formulation, and

sampling in one machine. Such a savings to the hospital, patient and insurance companies would be very attractive.

In addition, the time to treatment favors an in-house cell manufacturing process. Treating patients with Yescarta or Kymriah™ requires patients meet the stringent criteria for treatment, undergo apheresis at the treatment facility with shipment of the apheresis product to the pharmaceutical manufacturer. The cell therapy product is generated, tested and then returned to the treatment facility, which may take as long as a month or more. Upon receipt, the patient undergoes chemotherapy conditioning regimen followed by infusion of the cell product. The length of time from apheresis to cell infusion using the Miltenyi CliniMACS Prodigy® 'all-in-one' cell processing system may be as short as 10 days depending upon product testing time, a significant reduction in time to treatment, which may be a major advantage for patients with advanced disease. This study will utilize the Miltenyi CliniMACS Prodigy® 'all-in-one' cell processing system and determine its feasibility, defined as the rate of successful manufacture of the CD22-CAR T cells produced to satisfy the targeted dose level and meet the required release specifications.

2.3.1 Differences in Manufacturing CD22-CAR T cells between Stanford and NCI POB

The investigational CD22-CAR T cell product manufactured by Stanford under IND 19103 uses the identical CAR vector (Ef1a-BBZ-CD22-CAR) produced at Lentigen (referenced in BB-MF 13987) as tested in the NCI POB protocol #15-C-0029 described in **Section 2.2.5**. The manufacturing process for CD22-CAR T cells varies in that the product to be produced at Stanford University will be manufactured in the CliniMACS Prodigy® system, using the Miltenyi Biotech immunomagnetic beads for enrichment and TransAct T cell Reagent (Miltenyi Biotec) for activation. The NCI product in protocol #15-C-0029 is stimulated using Dynabeads and the transduction and expansion is performed in bags. In February 2017, NCI added a T cell selection step to their cell processing in the CliniMACS Plus System, while the Stanford process is completely undertaken in the CliniMACS Prodigy® system. In addition, the MOI used at NCI is 15, while testing at Stanford determined the best MOI for product manufacturing is 18. **Table 2** below summarizes the similarities and differences with the proposed manufacturing at Stanford and the current manufacturing at NCI on protocol #15-C-0029.

Table 2. Manufacturing Differences at Stanford and NCI for CD22-CAR T cells

Characteristic	**Stanford IND #19103** Protocols CCT5029 & CCT6003	**NCI IND #16180** Protocol #15-C-0029
Manufacturing System	CliniMACS Prodigy® system	In bags at the bench
T cell selection	CliniMACS Prodigy® system	CliniMACS Plus
CAR Construct	same	same
Expansion	CliniMACS Prodigy® system	In bags
End of process formulation	7 day manufacturing process, with cryopreservation of cell product	9 day manufacturing process, with ability to administer fresh or cryopreserved cell product

Since the manufacturing process is not identical for the two CD22-CAR T cell products, it is possible that the toxicities observed in NCI POB protocol #15-C-0029 will differ from the toxicities observed in this protocol and the corresponding protocol in pediatric and young adult subjects (CCT6003 / IRB-50878). For this reason, we have designed an early safety evaluation in 10 evaluable subjects with ALL before expanding the cohort, and will follow a 21-day treatment stagger for the first 3 to 6 subjects with ALL. CD22-CAR T cells have not previously been tested in subjects with aggressive B-cell NHL, so we will evaluate initial safety using a 3 + 3 dose escalation design using a 21-day treatment stagger. We have also included a Dose Level -1 in each disease cohort for dose de-escalation in the event of unexpected toxicity.

However, the manufacturing processes are quite similar, so we anticipate a similar bioactivity and toxicity profile for the CD22-CAR T cells produced under Stanford IND 19103 as tested previously at NCI POB in protocol #15-C-0029.

2.4 SAFETY CONSIDERATIONS FOR CD22_CAR THERAPY

2.4.1 Risk of Lymphodepleting Chemotherapy

Toxicities resulting from fludarabine and cyclophosphamide in the doses proposed in the current study are well known and are what have been used in the prior CD19-CAR and CD22-CAR protocols. Such a preparative regimen is designed to decrease the number of endogenous T cells, including T regulatory cells that may otherwise suppress CAR T cell cytotoxicity, and to induce increased availability of homeostatic cytokines thereby allowing for better engraftment of the transferred CAR T cells. The dose limiting toxicity for both fludarabine and cyclophosphamide is myelosuppression, however myelosuppressive effects are expected to be transient using the doses proposed. Other toxicities including fever, nausea, vomiting, stomatitis, diarrhea, anorexia, edema, skin rashes, myalgias, headache, agitation, and fatigue should be easily managed with appropriate supportive care. Sterile hemorrhagic cystitis occurs in about 20% of subjects who receive cyclophosphamide, but is unlikely given the relatively low dose administered in this trial and given that continuous intravenous infusion of normal saline will be used prophylactically as a uroprotective agent. Tumor lysis syndrome (TLS) following fludarabine and cyclophosphamide administration can occur in subjects with advanced bulky disease. To prevent this, subjects may be prescribed allopurinol at the investigator's discretion, and will be appropriately hydrated; with close monitoring for the development of TLS. Finally, opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed post-fludarabine and cyclophosphamide, especially in heavily pre-treated individuals. Subjects will receive appropriate antimicrobial prophylaxis (e.g., Bactrim for PCP and acyclovir for HSV and VZV prophylaxis) during and after treatment as per institutional standards.

Bendamustine hydrochloride (Bendeka®) in the dose proposed has excellent anti-lymphocyte activity and is FDA approved for treatment of chronic lymphocytic leukemia (CLL) and Indolent B-cell non-Hodgkin's lymphoma (NHL). Experience at Stanford using Bendamustine prior to CD19 CAR-T (commercial CAR therapy) for relapsed/refractory lymphoma shows a similar efficacy and possibly lower rated of neutropenia, infections and CRS, although a small sample size. Similar results have been reported recently with longer follow up based on 2-JULIET trial. Bendamustine causes myelosuppression including lymphopenia, anemia, leukopenia, thrombocytopenia and neutropenia. The most common non-hematologic adverse reactions include pyrexia, nausea and vomiting, diarrhea, constipation, anorexia, cough, headache, weight

decreased, dyspnea, rash and stomatitis.

Clofarabine (Clolar®) injection is a purine nucleoside metabolic inhibitor and is FDA approved for treatment of children 1 to 21 years of age with relapsed/refractory ALL after at least two prior regimens. Based on laboratory data clofarabine has similar anti-B and T-cell activity and increase antileukemic activity compared to fludarabine. Using clofarabine will allow continued use of the regimen with cyclophosphamide. Clofarabine causes myelosuppression. High-dose clofarabine is associated with fevers, capillary-leak syndrome, and SIRS, often requiring premedication and management with corticosteroids. It should be discontinued if hypotension develops during the 5 days of administration. The most common adverse reactions are nausea, vomiting, diarrhea, febrile neutropenia, headache, rash, pruritus, pyrexia, fatigue, palmar-plantar erythrodysesthesia syndrome, anxiety, flushing, and mucosal inflammation.

2.4.2 Risk of Autoimmunity

Autoimmune toxicity is a risk of adoptive cell therapy trials for cancer and can occur if the transferred cell populations recognize the target antigen on normal tissues. Thus far, on-target, off-tumor toxicity of both CD19-CAR and CD22-CAR T cells have been restricted to B cell aplasia, which can be managed with immunoglobulin replacement therapy. Given that the CD22-CAR T cells tested in this study incorporate scFvs that have already been tested in clinical trials, it is unlikely that unexpected autoimmune toxicity will occur. Subjects will be monitored closely for the occurrence of unexpected toxicity and, if it is observed, appropriate supportive care and modification to the clinical trial will be instituted.

2.4.3 Risks of Gene Therapy

Risks of gene therapy include insertional mutagenesis or emergence of replication-competent lentivirus (RCL). While insertional mutagenesis is theoretically possible using retroviral vectors, this has only been observed in the setting of infants treated for X-linked severe combined immunodeficiency using retroviral vector-mediated gene transfer into CD34+ bone marrow stem cells. In the case of retroviral or lentiviral vector-mediated gene transfer into mature T-cells, there has been no evidence of long-term toxicities associated with these procedures since the first NCI sponsored gene transfer study in 1989. Lentiviral vectors are thought to carry a lower risk of mutagenesis because lentiviral integration patterns favor sites away from cellular promoters[105]. The proposed protocol will test the cellular product for RCL and patient follow up will comply with all current FDA guidelines. As the viral vectors used have been engineered to minimize the risk of emergence of replication competent lentivirus, subjects will be tested for RCL according to 2018 FDA Draft Guidance ‘Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up’. All participants will be followed for several years following receipt of this therapy; and in the case of the development of a second malignancy, all efforts will be made to determine whether replication competent lentivirus has emerged.

2.4.4 Risk of Cytokine Release Syndrome

Dramatic and rapid expansion of CD19-CAR T cells is often associated with toxicity that can be extrapolated to therapy with other CAR agents, including cytokine release syndrome and neurotoxicity. Cytokine release syndrome (CRS) has been the topic of several reports and

reviews[8],[106]. In brief, CRS comprises a febrile, sepsis-like picture that results from hemodynamic and organ effects of supraphysiologic levels of inflammatory cytokines produced directly or indirectly by the activated T cells. Among the most important of these cytokines are IL-6 and IFN-gamma[10],[9],[8]. CRS can be safely managed with supportive care and, in some cases, immunosuppression using anti-IL6R mAbs therapy (e.g. tocilizumab) and corticosteroids[106]. Notably, CRS is limited in subjects with low tumor burdens, as the CAR T cell expansion appears to be largely driven by the magnitude of the tumor burden with contributions from any existing normal B cell population, and CRS severity correlates with the degree of CAR T cell expansion.

2.4.5 Risk of Neurotoxicity

Neurotoxicity is also observed in a significant minority (20-30% in most series) of subjects, with toxicity ranging from mild to severe[10],[9],[8]. The pathobiology of CD19-CAR associated neurotoxicity is not fully understood, but the clinical syndrome is associated with increased expansion of CD19-CAR T cells and identification of CD19-CAR T cells in the cerebrospinal fluid. Neurotoxicity may be seen more frequently in subjects with CNS leukemia[10], however subjects without documented CNS leukemia or lymphoma can also develop symptoms of neurotoxicity that range from mild to severe. Hence, any direct association of CNS disease burden to neurotoxicity severity is unclear[10] Neurotoxicity typically manifests clinically as confusion, aphasia and/or dysmetria, and occasionally seizures. Radiographic changes are typically not observed and the syndrome typically resolves in 1-2 weeks and appears fully reversible. The prevailing hypothesis regarding the pathophysiology of this syndrome is that it reflects non-specific neurotoxic effects of cytokines and/or activated T cells, rather than a direct on-target effect of CD19-CAR T cells. Indeed, the dose limiting toxicity of IL-6 when administered in a Phase 1 trial was neurotoxicity; and transient aphasia was observed[107]. In further support of this hypothesis, the Jensen laboratory at Seattle Children's Hospital has recently developed a rhesus model of neurotoxicity that utilizes a CD20-CAR rather than the CD19-CAR platform and the model appears to mirror the human syndrome well (ASH presentation, unpublished). This provides further evidence against a direct, on-target effect involving CD19 in brain tissue.

In the summer of 2016, three deaths were reported as a result of severe neurotoxicity in subjects treated with CD19-CAR T cells. Given that more than 750 subjects have been treated with these therapeutics, and only one previous death was reported and attributed to neurotoxicity associated with status epilepticus, these events were unexpected and work is underway to understand the basis for this phenomena. Subsequently, two additional deaths were noted in the same trial utilizing high dose cyclophosphamide (in the absence of fludarabine) and CD19-CAR T cells incorporating a CD28 costimulatory domain. Notably, CAR T cells that incorporate a 4-1BB costimulatory domain do not typically undergo as rapid an expansion as those using a CD28 costimulatory domain, so the risk of fatal or very severe neurotoxicity may be reduced with a 4-1BB containing CAR.

Cerebral edema is another potential cause of death associated with CD19-CAR T cells. In our own experience at Stanford, we had one case of fatal cerebral edema associated with CD19-CAR T cells incorporating a CD28 costimulatory domain in April 2017. Additionally, with respect to 4-1BB costimulation, a fatality occurred in February 2017 (personal communication - Seattle) in which a patient died from cerebral edema associated neurotoxicity following a CAR incorporating the 4-1BB costimulatory domain. The CAR being utilized in this study will incorporate a 4-1BB

costimulatory domain.

2.4.6 Risk of B-Cell Aplasia

The cumulative CD19-CAR experience affirms that prolonged B cell aplasia is an expected consequence of effective, persistent CD19-CAR therapy. Similar to the management of subjects with congenital absence of B cells and subjects treated with chronic rituximab, CAR-mediated B cell aplasia can be managed effectively with immunoglobulin replacement therapy[108],[109]. Thus far, significant increase in infection susceptibility or other toxicity related to chronic B cell depletion has not been noted in prior clinical studies[9].

2.4.7 Other Risks

2.4.7.1 Capillary Leak Syndrome

Additional, potentially unforeseen toxicities may arise with any investigational agent. One less-frequently identified toxicity was observed in the NCI POB clinical trial administering CD22-CAR T cells reported by Fry et al [102] described as capillary leak syndrome (CLS) characterized by rapid symptom onset, dry cough with shortness of breath, diffuse patchy appearance on chest imaging, drop in albumin, and tachycardia out of proportion to fever. Three patients developed Grade 3/4 CLS after receiving the cell infusion in the POB trial[110] (personal communication with N. Shah). The CLS in this study was not associated with typical CRS hypotension, and generally occurred prior to peak CAR expansion was evident.

Other investigational trials have also reported CLS. FDA put a hold on two Phase I trials in September 2017 when a 78-year-old male with blastic plasmacytoid dendritic cell neoplasm developed CRS and a Grade 4 CLS on day eight after receiving a gene-edited allogeneic CAR T-cell (UCART) therapy [UCART123], which despite treatment with corticosteroids and tociluzumab, was considered central in causing the patient's death. On a second study using UCART123, a 58-year old woman with acute myeloid leukemia developed Grade 3 CRS and reversible Grade 4 CLS nine days after cell infusion.

Some investigators have attributed CLS to be one of the several symptoms displayed in the constellation of CRS, along with fevers, hypotension, hypoxia, respiratory distress and neurological disturbances[111],[112]. CLS is listed in the boxed warning for CRS on the Drug Fact Sheet for blinatumomab, a bispecific CD19-directed CD3 T-cell engager.

2.4.7.2 Hemophagocytic lymphohistiocytosis (HLH)/ Macrophage Activation Syndrome (MAS)

Primary hemophagocytic lymphohistiocytosis (HLH) is a rare inherited disorder of immune regulation, whereas macrophage activation syndrome (MAS) is a form of secondary HLH triggered by immune-activating processes in patients who may have heterozygous mutations in the same genes that are mutated in primary HLH, leading to dysfunctions in the same immune regulatory processes[9]. Cytokines 1L-10, IL-6 and IFN-γ are elevated in both CRS and HLH/MAS, and may be triggered in predisposed patients by the transient cytokine activation seen in response to CAR therapies[113]. Symptoms include new or persistent cytopenias, rising ferritin levels and rising liver function tests (personal correspondence N. Shah). Up to 32.8% of patients receiving CD22 CAR T cells have been reported to have HLH-like manifestations prompting

utilization of anakinra[110].

2.4.7.3 Hemolytic Uremic Syndrome

Hemolytic uremic syndrome (HUS) was reported after treatment with moxetumomab pasudotox, a recombinant anti-CD22 immunotoxin, in a study conducted at the NCI in 5 of 55 patients (13%)[114]. In the CD22-CAR T cell trial conducted at NCI, HUS was characterized by ongoing 'hemolyzed' labs, significant hypertension and proteinuria, and low complement levels which was effectively treated with eculizmab, a C5-complement inhibitor in approximately 5% of patients [110].

2.4.7.4 Coagulopathy

Coagulopathies have been reported with CAR T cell therapy, associated with CRS and MAS and characterized by significant hypofibrinogenemia[115]. The coagulopathy observed in the NCI POB CD22-CAR T cell trial reported dramatic falls in fibrinogen levels, but also was characterized by bleeding which appeared out of proportion to the degree of thrombocytopenia, and a hypogranular appearance, 'grey' platelets upon exam (personal correspondence N. Shah). Treatment included platelet infusions, cryoprecipitate, topical thrombin administration and infusions of fresh frozen plasma.

2.4.7.5 Pancytopenia and Infection

Cytopenias after CAR therapies are well documented[116],[117]. In a report of patients with relapsed/refractory DLBCL receiving Axi-cel, the incidence of grade 3 or greater neutropenia, anemia and thrombocytopenia was 78%, 43% and 38% respectively [117], with persistent (3 months) neutropenia seen in 9% of patients, thrombocytopenia in 65% and anemia in 72% of patients. Filgrastim was required in 47% of patients to facilitate recovery of neutrophils [117]. A biphasic occurrence of cytopenias also appears to be characteristic of some CAR related toxicities, reported in 93% of patients' hematologic toxicities which occurred 21 days or later after cell infusion[116]. Given the extent of cytopenias post CAR therapy, it is assumed there also exists a higher risk of infection post CAR therapy. In a recent study of 83 patients younger than 26 years of age receiving CAR T cells, 33 (40%) patients had 37 incident infections in the initial period (28 days) after CAR infusion[118], while 54% of those patients had at least one infection prior to the CAR therapy. Of the 83 patients, 48 were followed post 28 days, with a median follow up of 51 (29-90) days, and there were 7 viral infections, 5 bacterial infections and no fungal infections. This study identified pre-CAR infusion risk factors for infection included prior hematopoietic cell transplantation (HCT), immunoglobulin G (IgG) level < 400 mg/dL, and lymphodepletion other than cyclophosphamide and fludarabine, and post-CAR infusion risk factors included higher-severity CRS and IgG level < 400 mg/dL[118]. Most patients with bacteremia (90%) were neutropenic, whereas viral infections occurred in similar numbers whether the patient was neutropenic or not. Institutions are addressing this CAR T cell therapy risk with prophylactic antibiotics and in some cases, prophylactic antiviral prophylaxis.

2.5 CORRELATIVE STUDIES BACKGROUND

CAR T cell therapy targeting a single tumor-associated antigen has mediated striking remissions in B cell leukemia and lymphoma. The clinical experience to date has demonstrated that anti-leukemic effects are associated with CAR T cell expansion[10],[41], and that sustained

remission is associated with CAR T cell persistence[119]. In subjects who have relapsed in the presence of persistent singular CD19-specific or CD22-specific CAR T cells, relapse has often been characterized by CD19 dim/negative or CD22 dim/negative phenotypes respectively. The complex interplay of tumor, T cell and intrinsic CAR properties that influence these outcomes are not well understood. We aim to utilize this safety study as an opportunity to collect correlative data that will permit extensive study of both the B cell and T cell compartment prior to and following CAR T cell therapy. We aim to integrate multi-dimensional technologies to permit complex analyses of the apheresis product, the CAR T cell product pre-infusion and *in vivo* expanded CAR T cells following antigen encounter. We additionally aim to investigate properties of B cell tumors that render them susceptible to CAR T cell cytotoxicity and study physiologic and malignant B cell remodeling under the pressure of CAR therapy.

2.5.1 Factors Impacting Loss of CAR T cells

Persistence of CAR T cells is associated with superior anti-leukemia effects in the setting of CD19-CAR T cell therapy for leukemia. We hypothesize that there are three distinct causes of T cell loss following CAR T cell therapy. First, in a subset of subjects, there is poor CAR T cell expansion following infusion of a product that appears to meet appropriate release criteria. We hypothesize that such products can be identified by the presence of exhausted or terminally differentiated cells that lack progenitor capacity. To explore the potential to identify such products *a priori*, we will analyze apheresis samples and manufactured products to enumerate the frequency of naïve, T stem cell memory, T central memory and T effective memory subsets. We will also analyze apheresis and manufactured products for the presence of markers of T cell exhaustion including PD-1, Tim3 and LAG-3 among others. Phenotypes will be correlated with the degree of expansion following CAR T cell infusion.

Second, we hypothesize that a subset of subjects will undergo efficient early T cell expansion but will demonstrate loss of CAR T cell persistence associated with the acquisition of a terminally differentiated phenotype and/or characteristics of T cell exhaustion. To test this, we will evaluate CAR-expressing T cells *in vivo* following adoptive transfer to identify characteristics associated with poor persistence. These phenotypic analyses will include flow cytometry and mass cytometry with a goal of identifying T cell phenotypes associated with poor persistence *in vivo*. We will also undertake epigenetic analyses to analyze correlations between T cell phenotype, persistence and enhancer profiles. Where possible, we will also compare the phenotype of cells harvested from blood, bone marrow, CSF and lymph nodes to analyze whether the tissue microenvironment has substantial impact on T cell exhaustion, phenotype or function.

Finally, we hypothesize that a subset of subjects lose their CAR T cell populations as a result of immune rejection. To test this, we will analyze for the presence or development of anti-CAR mediated T cell responses using cell based assays, as well as overlapping peptide libraries to localize the targets of anti-CAR reactivity.

2.5.2 Impact of Persistent CD22 Immune Pressure on B cell Differentiation

CD19 and CD22 are acquired during the process of normal and malignant B cell differentiation. Persistent immune based pressure targeting either of these antigens has the potential to induce profound changes on both normal and malignant B cell differentiation. In this study, we will use mass cytometry, flow cytometry and ATAC-Seq to monitor changes in normal and malignant B cell differentiation in the presence of persistent CAR T cells targeting CD22. We will explore loss

of antigen and whether such antigen loss is associated with a particular differentiation state, a particular genotype and/or are associated with increased or diminished fitness as evidenced by clinical behavior, or where possible growth patterns *ex vivo*. Similar analyses will focus on subjects with remission marrows, analyzing whether normal B cell development can proceed in the absence of CD22 subpopulations. This will provide a novel opportunity to characterize the impact of CD22 changes on normal lymphoid and/or myeloid cells development.

2.5.3 Utility of Minimal Residual Disease Assessment

The role of Minimal Residual Disease (MRD) testing by next-generation sequencing (NGS) in subjects with ALL treated with CAR-T cells has not been well studied. In this study we will prospectively collect MRD evaluations before and after CAR-T therapy in subjects with ALL with identifiable clones and analyze using NGS. MRD by NGS will be correlated with flow cytometry and with peripheral blood MRD when available.

2.6 STUDY DESIGN

2.6.1 Short Title for Study

CD22-CAR T cells in adults with B-cell malignancies

2.6.2 Interventional model

Two arm, open label single treatment. Phase I dose escalation study for aggressive B-cell NHL disease cohort; Phase Ib safety of previously selected target dose for ALL disease cohort.

2.7 PROTOCOL RATIONALE AND SUMMARY

Most aggressive cancers eventually relapse or become refractory to current conventional treatments, necessitating the immediate development of new treatment modalities. The most recent advances in cancer therapy involve the development of new immune stimulating agents designed to target T-cells to cancer cells and initiate cell death signaling pathways. However, limitations to this type of immunotherapy include lack of tumor specificity, insufficient T-cell recruitment/numbers, and the existence of endogenous immunosuppressive agents which significantly attenuate or altogether block T-cell activity.

The primary goals of this trial are threefold: 1) to evaluate the feasibility of generating CD22-CAR T cells from adults with B cell malignancies using the Miltenyi CliniMACS Prodigy® system to meet the manufacturing specifications; 2) to determine the MTD/RP2D for adult subjects with aggressive B-cell NHL, given that CD22-CAR T cells have not been previously administered to this disease group; and 3) to establish the safety of CD22–CAR T cells in adults with ALL at the recommended dose of $1x10^6$ cells/kg. The secondary aim is to assess the clinical activity of administering autologous CD22-CAR T cells that meet established release specifications in adults with R/R CD22+ B-ALL and R/R aggressive B-cell NHL following a lymphodepleting conditioning regimen. Correlative analyses on this protocol or a companion study will: 1) Analyze alterations in early B cell development induced by immune pressure exerted via CD22-CAR T cells; 2) Evaluate whether subjects receiving CD22-CAR T cells relapse with loss or diminished expression of CD22, when feasible; 3) Measure persistence of CD22-CAR T cells in the blood, bone marrow and CSF (when feasible), and explore correlations between CD22-CAR T cell properties and CAR T cell efficacy and persistence; and 4) Establish the utility of chromatin

structure and epigenomic technology to characterize CAR T cell therapies. The precedent for conducting clinical trials of this scope was established by the ongoing clinical trials CCT5001/IRB-41382 and CCT5007/IRB-41383 conducted by Stanford Center for Cancer Cell Therapy.

Subjects with B cell malignancies, including ALL, and aggressive B-cell NHL defined as DLBCL, PMBCL, and tDLBCL arising from Follicular Lymphoma and Marginal Zone Lymphoma, or Follicular Lymphoma Grade 3B are eligible. CD22 expression in ALL is well established and is a relevant indicator of CD22-CAR efficacy in this patient population, so all ALL subjects must demonstrate CD22 positivity for eligibility. Little has been reported about expression in aggressive B-cell NHL in relation to therapeutic response, so archival tissue or lymph node biopsies will be collected at the time of enrollment/screening from subjects and any expression of CD22 will be acceptable for enrollment (including no detected expression). If archival tissue is not available, a potential aggressive B-cell NHL subject must be willing to undergo a biopsy for tissue analysis.

Subject eligibility requires relapsed or refractory disease after standard therapies; previous immunotherapy will not render subjects ineligible. For subjects who have previously received CAR T cell therapy, the level of circulating CAR T cells must be < 5% of CD3+ cells that express the previous CAR at the time of leukapheresis, if a qualified assay exists. Subjects who have undergone autologous SCT will be eligible, and subjects who have received allogeneic SCT will be eligible if, in addition to meeting other eligibility criteria, they have no evidence of active GVHD and have been without immunosuppressive agents for at least 30 days.

In the only previous study using CD22-CAR T cells, conducted in children and young adults at the POB NCI, the MTD chosen for further study in subjects with ALL was 1 x 10^6 transduced T cells/kg (± 20%), despite not achieving the standard definition according to number of dose limiting toxicities.

Because CD22-CAR T cells have not been previously administered to subjects with aggressive B-cell NHL, a dose finding 3 + 3 dose escalation design will be used (see **Table 3**). Once the MTD/RP2D is determined in aggressive B-cell NHL subjects, an expansion cohort will also be treated to further explore safety of CD22-CAR T cells.

In addition, a preliminary evaluation of efficacy of the CD22-CAR T cells will be conducted in the two disease populations: subjects with R/R ALL and subjects with R/R aggressive B-cell NHL.

This study will be conducted at Stanford as a single-site study by the principal investigator Matthew Frank, M.D., Ph.D. The IND Sponsor of this study will be Stanford University.

2.7.1 Primary Outcome Type

Safety and Feasibility

2.7.2 Investigational Agent

Autologous T cells transduced with lentiviral vector (CD22.BB.Z) Chimeric Antigen Receptor (CD22-CAR); following Lymphodepleting Chemotherapy

2.7.3 IND number:

IND # 19103, Sponsor: Stanford University

2.7.4 Primary Outcome Measures

This study will be registered on ClinicalTrials.gov but is not subject to the results reporting requirement.

1. Title: Rate of successful manufacture of CD22-CAR T cells using the Miltenyi CliniMACS Prodigy ® system
   - Outcome Measure1: The percentage of apheresis samples (fresh or frozen) that are successfully processed and expanded to manufacture CD22-CAR T cells that satisfy the target dose level and meet release specifications will be determined for each dose cohort.
   - Outcome Timeframe1: 7-11 days from start of manufacturing.
2. Title: MTD/RP2D of CD22-CAR T cells in subjects with aggressive B-cell NHL
   - Outcome Measure2: Incidence and severity of dose limiting toxicities (DLTs) following chemotherapy preparative regimen and infusion of CD22-CAR T cells, as recorded and graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, at each dose level tested in subjects with aggressive B-cell NHL.
   - Outcome Timeframe2: 28 days after infusion of CD22-CAR T cells
3. Title: Safe dose of CD22-CAR T cells in subjects with ALL
   - Outcome Measure3: Incidence and severity of dose limiting toxicities (DLTs) following chemotherapy preparative regimen and infusion of CD22-CAR T cells, as recorded and graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, at each dose level tested in subjects with ALL.
   - Outcome Timeframe3: 28 days after infusion of CD22-CAR T cells

2.7.5 Secondary Outcome Measures

1. Secondary Measure 1: Clinical activity of CD22-CAR T cells in adults with relapsed/refractory CD22-expressing B-cell ALL at target dose

- Outcome Measure1: Clinical activity will be assessed by Guidelines for efficacy evaluation in Acute Lymphoblastic Leukemia (ALL) (based on NCCN Guidelines (2013, Appelbaum, ASH and IWG guideline, Cheson 2003.) (see Appendix C, **Section 13.3.1**). Results will be reported as best response (i.e. complete remission [CR], Complete remission with incomplete blood count recovery [Cri], No response, or Relapsed disease or Unknown) at Day 28 for adult subjects with ALL treated at the target dose.
  - Timeframe1: 28 months after infusion of CD22-CAR T cells

2. Secondary Measure 2: Clinical activity of CD22-CAR T cells in adults with relapsed/refractory aggressive B-cell NHL at MTD/RP2D
   - Outcome Measure2: Clinical activity will be assessed by Lugano response criteria for lymphoma (see Appendix C, **Section 13.3.2**). Results will be reported as best response (i.e. complete response [CR], partial response [PR], stable disease [SD], or progressive disease [PR]) at Month 3 for adult aggressive B-cell NHL subjects treated at MTD/RP2D.
   - Timeframe2: 3 months after infusion of CD22-CAR T cells

2.7.6 General Study Design

This is a Phase 1/1b study in adults with B cell malignancies (ALL and aggressive B-cell NHL) who have relapsed or refractory disease after standard treatment options. Autologous PBMC will

be obtained by leukapheresis and transduced with CD22-CAR lentiviral vector. Cryopreserved PBMC stored from participation in other institutional cell therapy or cell collection studies or standard of care may be used to generate the cellular product on this study as long as they meet the criteria established in this IND. Cryopreserved PBMC will be transported to the manufacturing facility [Miltenyi Biotech Inc (MBI) or Stanford's Laboratory for Cell and Gene Medicine (LCGM)], where they will undergo selection, activation, transduction with the lentiviral vector, expansion, and formulation using the Miltenyi CliniMACS Prodigy® system for the manufacture of CD22-CAR T cells. The product will be cryopreserved and returned to Stanford's Cell Therapy Facility (CTF), from which the product will be distributed to the patient care unit for infusion.

Subjects will receive a lymphodepleting preparative regimen prior to infusion of CD22-CAR T-cells. Subjects will be evaluated sequentially after treatment for toxicity, antitumor effects and for persistence of CAR in blood samples and functionality of transduced T cells. Subjects with ALL will be enrolled and evaluated separately from subjects with aggressive B-cell NHL. Additional blood, lymph node and bone marrow samples may be collected to complete correlative study analysis.

Table 3. CD22-CAR Dose Levels

Level	**Dose CD22-CAR transduced T cells†**	**R/R ALL Cohort**	**R/R aggressive B-cell NHL Cohort**
Dose Level -1	3 x 10^5 cells/kg (± 20%)	*De-escalate if DLT rate >30% at Level 1*	*Deescalate if DLT rate >30% at Level 1*
Dose Level 1	1 x 10^6 cells/kg (± 20%)	Target dose	3+3 Dose escalation design until MTD/RP2D determined (see **Section 5.4.4**)
Dose Level 2	3 x 10^6 cells/kg (± 20%)	*May escalate if no clinical activity and <30% DLT rate observed at Level 1*	
Dose Level 3	1 x 10^7 cells/kg (± 20%)	*Not tested in this cohort*	
† Dose based on weight measured within 14 days of manufacturing start date, capped at 100 kg.			

The feasibility of generating this product using the Miltenyi CliniMACS Prodigy® 'all-in-one' cell processing system will be evaluated across both disease groups. If initial feasibility of cell production is not met (i.e. 3 of the first 6 aggressive B-cell NHL subjects' cells or 5 of the first 10 ALL subjects' cells cannot be produced to meet the established release criteria) further enrollment will be paused pending evaluation of the manufacturing process, and modifications made appropriate to improving feasibility prior to continuing enrollment. This may require a protocol or IND amendment. In any event, if 3 (or 5) of the first 6 (or 10) cell products manufactured do not meet established criteria, it will be reported to the IRB and APB and no manufacturing changes will be implemented until IRB and APB approval is obtained.

The expected DLT rate is 20%, however, the maximum acceptable rate for DLT is 30% in each of the two disease cohorts. Using a standard 3 + 3 dose escalation design to evaluate three dose levels, subjects with aggressive B-cell NHL will be sequentially enrolled to Dose Level 1 to 3. The MTD for subjects with aggressive B-cell NHL is declared as the feasible dose achieving 0

DLTs out of 3 subjects or 1 DLT out of 6 subjects (possible to be 0 out of 6 due to feasibility). Once the MTD/RP2D is established in this disease population, additional aggressive B-cell NHL subjects will be enrolled to further evaluate the safety of this regimen as outlined in **Section 12.1.2.1**. Additional safety monitoring for possible de-escalation will be performed after 17 evaluable aggressive B-cell NHL patients have been treated at the established MTD/RP2D and every 5 thereafter, until a total of 36 evaluable subjects with R/R aggressive B-cell NHL are treated. Initially, 10 subjects with ALL will receive the target dose of 1 x 10^6 transduced T cells/kg (± 20%) and be evaluated for safety. Additional safety monitoring for possible de-escalation will be performed every 5 patients thereafter, until a total of 28 evaluable subjects with R/R ALL are treated.

A futility analysis will be conducted to determine the secondary endpoint for efficacy in each of the disease groups (subjects with ALL and subjects with aggressive B-cell NHL treated at the MTD/RP2D). For subjects with B-ALL, the analysis will be conducted when 15 evaluable subjects have reached Day 28 bone marrow assessment (primary efficacy endpoint); and if 7 or fewer subjects have a CR, further enrollment to that disease group will stop. In addition, a futility analysis will be conducted when 17 evaluable subjects with aggressive B-cell NHL treated at MTD/RP2D have reached month 3 disease assessment (primary efficacy endpoint); and if 4 or fewer subjects have a CR or PR, further enrollment to that disease group will stop. **See Section 12.3 for comprehensive details of study stopping rules.** In the event a stopping rule is met, the Safety Monitoring Committee (SMC) will be notified. SMC findings and recommendations will be communicated to IRB and APB promptly via a safety report or protocol amendment.

2.7.7 Study Duration

2.7.7.1 Primary Completion

Up to 1-2 subjects per disease group will be accrued per month, and therefore this study may require up to 2- 3.5 years to complete accrual. The study primary and secondary objectives will be complete approximately 3 years after opening to accrual.

2.7.7.2 Study Completion

Subjects will be followed after treatment to evaluate toxicities, track disease progression, and to monitor for gene therapy effects. Short term follow-up includes evaluations up until disease progression or subsequent alternative disease-directed therapy is initiated. In addition, long term follow up for gene therapy according to the U.S. Food and Drug Administration (FDA) ***Guidance for Industry: Gene Therapy Clinical Trials – Observing Participants for Delayed Adverse Events*** will be conducted on every infused subject for the required 15 years post infusion of gene-edited cells on this study or an alternative long term follow up protocol. The study is expected to take 17-18.5 years to complete all enrollment and follow-up. The study may be concluded earlier if subjects are transitioned to a Long-Term Gene Therapy Follow Up protocol.

3 PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

All subjects must sign and date the Institutional Review Board (IRB) and Administrative Panel on Biosafety (APB) approved consent form(s) before initiating any study specific procedures or activities that are not part of a subject's routine care.

The Screening Participant Eligibility Checklist on the following page must be completed in its entirety for each subject prior to registration. If patient or manufacturing scheduling requires, consenting and screening may be completed in two stages: 1) for leukapheresis, and 2) for lymphodepleting chemotherapy and CD22-CAR T cell infusion, as outlined with checklists for each stage in Section 13.8, Appendix H.

The completed, signed, and dated checklist(s) must be retained in the subject's study file and the study's Regulatory Binder or an electronic version completed within the subject's medical record.

The study coordinator, Stanford co-investigator, principal investigator, or treating physician, and a third reviewer must verify that the participant's eligibility is accurate, complete, and legible in source records, as required by the CCTO SOP 'Confirmation of Participant Eligibility in Clinical Trials'. A description of the eligibility verification process should be included in the EPIC or other Electronic Medical Record progress note.

The protocol-specific checklist is **required** by the SRC and must be approved by the IRB.

3.1 SCREENING PARTICIPANT ELIGIBILITY CHECKLIST

Protocol Title:	**Phase I/Ib Clinical Trial of Autologous CD22 Chimeric Antigen Receptor (CAR) T cells in Adults with Recurrent or Refractory B Cell Malignancies**
Protocol Number:	**CCT5029 / IRB-50836**
Protocol Director:	**Matthew Frank, M.D., Ph.D.**

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

III. Study Information:

SRC Approved ☐ IRB Approved ☐ Contract signed ☐

IV. Inclusion/Exclusion Criteria

INCLUSION CRITERIA	Yes	No	Supporting Documentation†
1. Disease Status a. <u>Disease Status of ALL</u> • Must have chemotherapy refractory disease defined as progression or stable disease after a standard line of therapy, or relapsed disease after achieving CR. • Subjects with persistent or relapsed minimal residual disease (MRD) (by flow cytometry, PCR, FISH, or next generation sequencing) require verification of MRD on two occasions at least 2 weeks apart. • Subjects with Philadelphia Chromosome positive acute lymphoblastic leukemia (Ph+ALL) are eligible if they progressed after receiving a tyrosine kinase inhibitor (TKIs). • Subjects with recurrence of isolated CNS relapse after achieving complete remission (CR) are eligible. b. <u>Disease Status of aggressive B-cell NHL</u> • Histologically confirmed aggressive B cell NHL including the following types defined by WHO 2008: o DLBCL not otherwise specified; T cell/histiocyte rich large B cell lymphoma; DLBCL associated with chronic inflammation; Epstein-Barr virus (EBV)+ DLBCL of the elderly; OR	☐	☐	☐ **NA** ☐ **NA**

INCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
○ primary mediastinal (thymic) large B cell lymphoma; OR ○ transformation of follicular lymphoma, marginal zone lymphoma or chronic lymphocytic leukemia/small lymphocytic lymphoma to DLBCL; OR ○ Follicular Lymphoma Grade 3B • Subjects with DLBCL, Follicular Lymphoma Grade 3B –or– Subjects with transformed FL, MZL, or CLL/SLL who HAVE NOT received chemotherapy prior to transformation: ○ Must have received an anthracycline regimen and an anti-CD20 monoclonal antibody (unless documented CD20-negative) and be refractory or relapsed after second line of DLBCL treatment. Subjects with a partial response to second line therapy must be ineligible for autologous transplant. • Subjects with transformed FL, MZL, or CLL/SLL who HAVE received anthracycline-containing chemotherapy prior to transformation: ○ Must have progressed, had SD or recurred with transformed disease after initial treatment for DLBCL.			
2. Measureable Disease • <u>Subjects with ALL:</u> must have evaluable or measurable disease (MRD positive by flow cytometry, NGS, or PCR is acceptable). • <u>Subjects with aggressive B-cell NHL</u>: must have evaluable or measurable disease according to the revised IWG Response Criteria for Malignant Lymphoma[38]. Lesions that have been previously irradiated will be considered measurable only if progression has been documented following completion of radiation therapy.	☐ ☐	☐ ☐	
3. CD22 expression • <u>Subjects with ALL</u>: CD22 positive expression on malignant cells is required and must be detected by immunohistochemistry or flow cytometry. The choice of whether to use flow cytometry or immunohistochemistry will be determined by what is the most easily available tissue sample in each subject.	☐	☐	

INCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
CD22 expression must be demonstrated subsequent to any anti-CD22 targeted therapy (e.g. Moxetumomab pasudotox or inotuzumab ogozamicin) in subjects with ALL. • Subjects with aggressive B-cell NHL: CD22 expression at any level, including undetectable, will be acceptable. Subjects must have archival tissue available for analysis of CD22 expression or must be willing to undergo a biopsy of easily accessible disease.	☐	☐	
4. Prior Bone Marrow-Stem Cell Transplant Subjects who have undergone autologous SCT with disease progression or relapse following SCT are eligible. Subjects who have undergone allogeneic SCT will be eligible if, in addition to meeting other eligibility criteria, they have no evidence of GVHD and have been without immunosuppressive agents for at least 30 days.	☐	☐	
5. Prior Therapy Wash-out At least 2 weeks or 5 half-lives, whichever is shorter, must have elapsed since any prior systemic therapy at the time the subject is planned for leukapheresis, except for systemic inhibitory/stimulatory immune checkpoint therapy, which requires 5 half-lives. Exceptions: a. There is no time restriction with regard to prior intrathecal chemotherapy provided there is complete recovery from any acute toxic effects of such; b. Subjects receiving hydroxyurea may be enrolled provided there has been no increase in dose for at least 2 weeks prior to starting apheresis; c. Subjects who are on standard ALL maintenance type chemotherapy (vincristine, 6-mercaptopurine or oral methotrexate) may be enrolled provided that chemotherapy is discontinued at least 1 week or 5 half-lives, whichever is shorter, prior to apheresis. d. Subjects receiving steroid therapy at physiologic replacement doses only are allowed provided there has been no increase in dose for at least 2 weeks prior to starting apheresis; e. For radiation therapy: Radiation therapy must	☐	☐	

INCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
have been completed at least 3 weeks prior to apheresis, with the exception that there is no time restriction if the volume of bone marrow treated is less than 10% and also the subject has measurable/evaluable disease outside the radiation port or the site of radiation has documented progression.			
6. Prior CAR Therapy Subjects who have undergone prior CAR therapy must be at least 30 days post CAR infusion and have < 5% of CD3+ cells express the previous CAR if a validated assay is available.	☐	☐	
7. Toxicities due to prior therapy must be stable or resolved (except for clinically non-significant toxicities such as alopecia or cytopenias covered in *footnote to #10)	☐	☐	
8. Age greater than or equal to 18 years of age	☐	☐	
9. Eastern cooperative oncology group (ECOG) performance status of 0, 1, or 2; or Karnofsky ≥ 60% (See section 13.1, Appendix A)	☐	☐	
10. Normal Organ and Marrow Function (supportive care is allowed per institutional standards, i.e. filgrastim, transfusion) • ANC ≥ 750/uL* • Platelet count ≥ 50,000/uL* • Absolute lymphocyte count (ALC) ≥ 150/uL* Adequate renal, hepatic, pulmonary and cardiac function defined as: • Creatinine ≤ 2 mg/dL OR creatinine clearance ≥ 60 mL/min • Serum ALT/AST ≤ 10x Upper limit of normal (ULN) (Elevated ALT/AST related to leukemia involvement of the liver will not disqualify a subject). • Total bilirubin ≤ 1.5 mg/dL, except in subjects with Gilbert's syndrome. • Cardiac ejection fraction ≥ 45%, no evidence of physiologically significant pericardial effusion as determined by an ECHO, MUGA or Cardiac MRI [performed **within 180 days or after most recent anthracycline based treatment or mediastinal radiation therapy** (whichever is most recent)]	☐ ANC Platelet ALC Creatinine AST/ALT Bilirubin LVEF	☐	

INCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
• No clinically significant ECG findings • No clinically significant pleural effusion • Baseline O_2 saturation > 92% on room air * ALL subjects will not be excluded because of pancytopenia ≥ Grade 3 if it is felt by the investigator to be due to underlying leukemia.	ECG Pleural SaO_2		
11. CNS Status Subjects with CNS involvement are eligible as long as there are no overt signs or symptoms that in the evaluation of the investigator would mask or interfere with the neurological assessment of toxicity.	☐	☐	
12. Females of childbearing potential must have a negative serum or urine pregnancy test within 7 days prior to leukapheresis (females who have undergone surgical sterilization or who have been postmenopausal for at least 2 years are not considered to be of childbearing potential)	☐	☐	
13. Contraception Subjects of child-bearing or child-fathering potential must be willing to practice birth control from the time of enrollment on this study and for four (4) months after receiving the preparative lymphodepletion regimen.	☐	☐	
14. Ability to give informed consent. Must be able to give informed consent. Legal authorized representative (LAR) is permitted if subject is cognitively able to provide verbal assent.	☐	☐	

Subjects meeting any of the following criteria are not eligible for participation in the study:

EXCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
1. Recurrent or refractory ALL limited to isolated testicular disease.	☐	☐	
2. Hyperleukocytosis (≥ 50,000 blasts/μL) or rapidly progressive disease that in the estimation of the investigator and sponsor would compromise ability to complete study therapy.	☐	☐	
3. History of other malignancy, unless disease free for at least 3 years. At the discretion of the Principal Investigator, subjects in remission for	☐	☐	

EXCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
1-2 years prior to enrollment may be deemed eligible after considering the nature of other malignancy, likelihood of recurrence during one year following CAR therapy, and impact of prior treatment on risk of CD22-CAR T cells. Subjects in remission <1 year are not eligible. • Exception: Nonmelanoma skin cancer or carcinoma in situ (e.g. cervix, bladder, breast) is eligible. • Hormonal therapy in subjects in remission > 1 year will be allowed.			
4. Presence of active fungal, bacterial, viral, or other infection requiring intravenous antimicrobials. Simple UTI and uncomplicated bacterial pharyngitis are permitted if responding to active treatment.	☐	☐	
5. No knowledge of: • HIV, • Hepatitis B (HBsAg positive) or • Hepatitis C virus (anti-HCV positive) infection. A history of hepatitis B or hepatitis C is permitted if the viral load is undetectable per quantitative PCR and/or nucleic acid testing.	☐ HIV HBV HCV	☐	
6. Presence of cerebrovascular ischemia / hemorrhage, dementia, cerebellar disease, or any autoimmune disease with CNS involvement that in the judgment of the investigator may impair the ability to evaluate neurotoxicity.	☐	☐	
7. History of myocardial infarction, cardiac angioplasty or stenting, unstable angina, or other clinically significant cardiac disease within 12 months of enrollment.	☐	☐	
8. Any medical condition that in the judgement of the sponsor investigator is likely to interfere with assessment of safety or efficacy of study treatment.	☐	☐	
9. History of severe immediate hypersensitivity reaction to any of the agents used in this study.	☐	☐	
10. Women who are pregnant or breastfeeding.	☐	☐	
11. In the investigator's judgment, the subject is unlikely to complete all protocol-required study	☐	☐	

EXCLUSION CRITERIA	Yes	No	Supporting Documentation†
visits or procedures, including follow-up visits, or comply with the study requirements for participation.			
12. Primary immunodeficiency or history of autoimmune disease (e.g. Crohns, rheumatoid arthritis, systemic lupus) requiring systemic immunosuppression/systemic disease modifying agents within the last 2 years.	☐	☐	

†All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

IV. Statement of Eligibility

By signing this form of this trial I verify that this subject is [☐ **eligible** / ☐ **ineligible**] for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Treating Physician Signature:	Date:
Printed Name:	
Secondary Reviewer Signature:	Date:
Printed Name:	
Study Coordinator Signature:	Date:
Printed Name:	

3.2 INFORMED CONSENT PROCESS

All participants must be provided a consent form (or in the event that two stages of enrollment are planned, two consent forms as outlined in Section 13.8, Appendix H) describing the study with sufficient information for participants to make an informed decision regarding their participation. The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and potential benefits, and alternative therapies will be carefully explained to the subject, and the subject/LAR asked to review the consent form and to ask questions prior to agreeing to participate in this protocol. The subject is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences.

The original signed copy of the consent document(s) must be retained in the research file and a copy placed in the medical record.

3.3 SUBJECT SCREENING ASSESSMENTS AND REGISTRATION

3.3.1 General considerations for Subject Screening

The screening period begins on the date the subject signs the IRB/APB approved consent form and continues through date of enrollment (the date triple review eligibility sign off is completed to verify that the participant's eligibility is accurate, complete, and legible in source records). Informed consent must be obtained before completion of any non-standard of care study specific procedures. Procedures that are part of standard of care are not considered study specific procedures and may be performed prior to obtaining consent and used to confirm eligibility. Confirmation of this data must occur within 28 days of enrollment, unless specified otherwise. If the subject has received anti-CD22 targeted therapy (e.g. Moxetumomab pasudotox or inotuzumab ogozamicin), then CD22 expression must be demonstrated subsequent to this therapy in subjects with ALL. Subjects with aggressive B-cell NHL must have archived tissue available for analysis or be willing to undergo biopsy prior to starting conditioning lymphodepletion regimen.

After written informed consent has been obtained, subjects will be screened to confirm study eligibility and participation. Only subjects who meet the eligibility criteria listed in the Eligibility Checklist **Section 3.1** will be enrolled in the study. If at any time prior to enrollment the subject fails to meet the eligibility criteria, the subject should be designated as a screen failure on the subject screening log with the reasons for failing screening.

3.3.2 Study Enrollment

Before enrollment of a subject into the study, the responsible physician must ensure the subject meets all eligibility criteria using the Study Screening Procedures outlined in **Section 5.1**. Eligibility criteria will be reviewed and confirmed by the Principal Investigator or designee prior to any subject being enrolled into the study.

Enrollment will be defined as the date triple review eligibility sign off is completed to verify that the participant's eligibility is accurate, complete, and legible in source records. At time of enrollment, each subject will receive a unique subject identification number. This number will be used to identify the subject throughout the study and must be used on all study documentation related to the subject. Furthermore, the subject identification number must remain constant throughout the entire clinical study, it must not be changed after enrollment or if the subject is

rescreened or retreated.

Screening and enrollment for subjects enrolling in two stages due to patient or manufacturing scheduling will be completed as above but appropriate screening evaluations will be completed for each stage of eligibility as outlined in **Section 13.8, Appendix H**; subject identification number will remain constant throughout both stages of the protocol.

4 TREATMENT PLAN

4.1 OVERVIEW

This is a Phase I/Ib study in adult subjects with B cell malignancies, including ALL and aggressive B-cell NHL who have relapsed or refractory disease. In light of the previous experience of CD22-CAR T cells in subjects with ALL, subjects with ALL will receive 1 x 10^6 cells/kg CD22-CAR transduced T-cells. Simultaneously, a standard 3 + 3 dose escalation design will be tested in subjects with aggressive B-cell NHL to establish MTD/RP2D using 3 dose levels of CD22-CAR T-cells (see **Table 3**). Autologous PBMC will be obtained by leukapheresis and transduced with CD22-CAR lentiviral vector. Cryopreserved PBMC stored from participation in other institutional cell therapy or cell collection studies or standard of care may be used to generate the cellular product on this study as long as the cryopreserved cells meet the criteria established in this IND.

Subjects will receive a lymphodepleting chemotherapy preparative regimen followed by infusion of CD22-CAR transduced T-cells. The DLT assessment period is defined as 28 days. Subjects will be evaluated sequentially after treatment for toxicity, antitumor effects and for persistence of CAR in blood samples and functionality of transduced T cells. Safety evaluations will also be conducted in each disease group separately: subjects with ALL and subjects with aggressive B-cell NHL.

Although a secondary endpoint, efficacy will be important in determining if a Phase 2 study is warranted with this CAR T cell therapy. Efficacy analysis will be conducted in each disease cohort and will be used to establish a preliminary assessment of clinical response, overall survival (OS), and progression-free survival (PFS) as described in **Section 12.2**. Stopping rules are defined in **Section 12.3**.

4.1.1 Treatment Plan for Adults with ALL

Initially 10 subjects with ALL will receive 1 x 10^6 cell/kg (± 20%) of CD22-CAR transduced T-cells to explore safety of CD22-CAR T cells manufactured using the Miltenyi Biotech CliniMACS Prodigy® system. Treatment will be staggered as follows: at least 21 days will elapse between cell infusion for each of the first 3 subjects with ALL, followed by a pause while the third ALL subject completes the 28-day DLT observation period. If no DLTs have been observed in the first 3 subjects with ALL, no further treatment stagger is necessary. If a DLT is observed in any of the first 3 subjects with ALL, the 21-day treatment stagger will continue for another 3 subjects. If Dose Level -1 or Dose Level 2 is explored in subjects with ALL, the same 21 day treatment stagger for the first 3 subjects will be followed.

Given the expected toxicity rates associated with CAR T-cell therapies, the maximum acceptable rate for dose limiting toxicity (DLT) in the ALL group and the aggressive B-cell NHL group

treated is 30% for each disease group. The dose will be reduced if the observed rate for DLT meets the criteria described in **Section 12.1.2.2**, with DLT evaluations after 10 subjects with ALL complete the 28-day DLT observation period, then after every 5 subjects up to 28 subjects with ALL.

Alternatively, if there are 15 subjects with ALL treated at Dose Level 1 without sufficient evidence of activity and the DLT rate <30% at Dose Level 1, the Safety Monitoring Committee (SMC) may recommend dose escalation to Dose Level 2, as described in **Section 10.3.3** and **Section 12.2.2**.

4.1.2 Treatment Plan for Adults with Aggressive B-cell NHL

This CD22-CAR has not been previously administered to subjects with aggressive B-cell NHL. Therefore subjects will be treated in a standard 3 + 3 dose escalation design using 3 dose levels of CD22-CAR T-cells, with a potential Dose Level -1, (see **Table 3**) to establish MTD/RP2D. Treatment will be staggered as follows for each dose level: at least 21 days will elapse between infusion of each subject during dose escalation. The final subject with aggressive B-cell NHL in a dose cohort must complete the 28-day DLT observation period before the decision is made whether to treat additional subjects at the current dose level or to dose escalate (as per **Table 6**). The SMC will review and confirm any decision to escalate dose of CD22-CAR T cells to the next level, based on the dose escalation plan described in Section **5.4.4**.

The MTD is defined in **Section 5.4.4.1**. Once the MTD/RP2D is established, and expansion cohort will enroll additional subjects to further evaluate this regimen in subjects treated at MTD/RP2D, for safety (described in **Section 12.1.2.1**) and initial efficacy (described in **Section 12.2.1**). Evaluation of subjects in the aggressive B-cell NHL at MTD/RP2D will include those from the dose escalation cohort treated at MTD/RP2D, for a total of up to 36 subjects.

4.2 General Concomitant Medication and Supportive Care Guidelines

4.2.1 Infection Prophylaxis

Any temperature of >38° C will require initiation of the fever work-up and treatment according to institutional standards.

4.2.1.1 Viral Prophylaxis

All subjects will be treated as high risk and will receive viral prophylaxis according to institutional standards.

4.2.1.2 Fungal Prophylaxis

All subjects will be treated as high risk and will receive fungal prophylaxis according to institutional standards.

4.2.2 B-cell Depletion

Since B cell antigen-targeted immunotherapy has been demonstrated in other studies to also eliminate normal B cells, serum IgG levels will be monitored before cell infusion and every 3-12 weeks after infusion. If IgG < 400, intravenous IgG (IVIG) will be administered with the appropriate premedications as per institutional guidelines at the discretion of the treating physician.

4.2.3 Blood Product Support for Anemia and Thrombocytopenia

Using complete blood count (CBC) as a guide, the subject will receive platelets and packed red blood cells as needed. Attempts will be made to keep hemoglobin (Hgb) > 7.0 gm/dl, and platelets > 10,000/mm^3, or as per institution guidelines. All blood products with the exception of the lymphocyte product will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused white blood cells (WBCs) and decrease the risk of cytomegalovirus (CMV) infection.

In subjects with coagulopathy, attempts will be made to keep platelets > 50,000/mm^3 and fibrinogen ≥ 100 mg/dL.

4.2.4 Tumor Lysis Syndrome

Subjects at risk for tumor lysis syndrome, as per the investigator's discretion, will receive allopurinol and hydration prophylactically as per the institutional guidance/managing team's determination.

4.2.5 Anti-Neoplastic Therapy

Subjects must have evaluable disease at the time of protocol enrollment or enrollment for Stage 2. Every effort will be made to initiate cell culture within 30 days of apheresis so minimal time transpires for a subject's disease to progress significantly. In the event that circumstances external to this study arise , subjects may receive disease-directed therapy (standard or investigational) prior to moving to the treatment phase of this study if the investigator determines there is at significant risk of disease progression during the delay. Since the toxicity of CAR-based therapy is related to disease burden (more disease associated with more CAR-related toxicity) and because the time to initiation of conditioning lymphodepletion regimen may be delayed due to multiple reasons, our experience to date indicates that patients may have rapid progression of their disease in this interval risking increased toxicity from either CAR therapy or disease progression. Treatment data and unresolved toxicities will be captured in baseline assessment case report forms (CRFs).

Data related to anti-neoplastic bridging therapy administered to subjects after enrollment, will be captured on study CRFs including dates of therapy, the agent and doses administered, the dose and schedule of radiation therapy, and response to therapy (if assessed prior to start of lymphodepleting chemotherapy).

In order to initiate the conditioning lymphodepletion regimen**,** subjects must meet criteria established in **Section 5.3**, or if enrolling in two stages, the eligibility criteria outlined in **Section 13.8,** Appendix H. A disease evaluation (methodology per PI discretion) may be performed at the investigator's discretion, based on the duration of time from initial screening to start of chemotherapy.

4.2.6 Concurrent Therapy for Extramedullary Leukemia or CNS Leukemia

Subjects may have CNS disease without overt evidence of signs and symptoms that may mask the development of neurotoxicity. Concurrent craniospinal radiation will not be allowed. Concurrent therapy or prophylaxis for CNS leukemia consisting of standard intrathecal chemotherapy will be allowed as clinically indicated in subjects with ALL at times of re-staging lumbar punctures. Intrathecal chemotherapy will NOT be given from Day 0 until at least Day 24 (i.e., during the first

re-staging evaluation performed at Day 28 +/- 4). Subsequent intrathecal therapy will be given no more frequently than monthly. Intrathecal chemotherapy will NOT be administered in any subject who develops CNS toxicity until at least 2 weeks after complete resolution of neurologic toxicity.

4.2.7 Cytokine Release Syndrome

Cytokine release syndrome, a toxicity associated with infusion of CAR T cell therapy, has been described in **Section 2.4.4.** Grading and management of CRS in this protocol will follow the guidelines in **Section 13.2**, Appendix B[10], which includes diligent supportive care and search for infection, with immunosuppression using anti-IL6R mAbs (e.g. tocilizumab) and/or corticosteroids reserved for more severe cases. Use of anakinra, an IL-1 receptor antagonist, currently approved by the US Food and Drug Administration for the treatment of patients with rheumatoid arthritis and neonatal-onset multisystem inflammatory disease, has been used off label for the treatment of secondary hemophagocytic lymphohistiocytosis (HLH), a condition in the spectrum of CRS potentially associated with chimeric antigen receptor (CAR) T-cell therapy[120],[121]. Anakinra may be considered for management of CRS related toxicities, at the investigator's discretion. Because understanding of the constellation of symptoms defining CRS is evolving, AE CRFs will capture both the syndrome and the individual symptomatology of CRS. Investigators should follow the protocol's CRS management guidelines whenever possible, but deviation from the guidance will not be considered a protocol deviation as all toxicity management should be at the treating physician's discretion.

4.2.8 Neurotoxicity

Neurotoxicity (e.g., encephalopathy, somnolence, aphasia) has been observed with CAR T cell therapies and will be scored using CTCAE 5.0 and the tools available in Appendix B, **Section 13.2**, using a combination of the the ASBMT Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS) Consensus Grading for Adults with the Immune effector Cell-associated Encephalopathy (ICE) assessment tool. All subjects will receive levetiracetam (Keppra®) 500- twice a day (BID) or per institutional guidelines beginning the day before cell infusion. For good clinical practice, clinicians and staff will evaluate the ICE score every shift (± 2 hours) after cell therapy while hospitalized or up to Day 14, whichever happens first. The maximum ICE score for each day should be documented, and any drop in ICE score below 7 should be urgently communicated to attending physician. Neurologic evaluations will be assessed daily up to Day 14. Evaluation of any new onset of neurotoxicity should consider recommended interventions in Appendix B (**Section 13.2.3.2**, ASBMT Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS) Consensus Grading for Adults)

These recommendations should serve as guidance for toxicity management, but deviation from the guidance will not be considered a protocol deviation, as all toxicity management should be at the treating physician's discretion.

Medications with sedative properties should be avoided if possible unless required to manage seizures, e.g. benzodiazepines. Leukoencephalopathy has been observed on MRI in the setting of neurotoxicity. Follow-up MRI is recommended to monitor the course of leukoencephalopathy to potential resolution.

Subjects and their families/caregivers should be warned of the risk of late neurotoxicity through Day 28 and told to seek immediate medical attention for any new symptoms of neurotoxicity. In

addition, subjects should be advised not to drive or operate heavy machinery for the first month after discharge and/or until 1 month after complete resolution of neurotoxicity symptoms.

4.3 Criteria for Removal from Protocol Therapy and Off Study Criteria

4.3.1 Criteria for removal prior to CD22-CAR T cell infusion

Subjects will be taken off treatment and followed until effects of chemotherapy and all toxicities are resolved to Grade 1 or baseline for any of the following:

- ✓ General or specific changes in the subject's condition that render the subject unacceptable for cell infusion on this study in the judgment of the investigator, including inability to meet eligibility criteria for Stage 2: Lymphodepleting Chemotherapy and CD22-CAR T cell infusion, if enrolling in two stages.
- ✓ Pregnancy in a female of child-bearing potential.
- ✓ Cells do not meet release criteria.

Once toxicities and effects of chemotherapy resolve, subjects who are unable to undergo cell infusion will be removed from this study.

4.3.2 Criteria for removal from the option for a 2nd cell infusion

Subjects will not be eligible for further therapy (2^{nd} cell infusion) and will be followed until off-study criteria are met for the following:

- ✓ Dose limiting toxicity (DLT) after first infusion. The definition of DLT is in **Section 5.4.7.1**
- ✓ Pregnancy in a female of child-bearing potential.
- ✓ No cells available for re-treatment.

4.3.3 Criteria for transition to Long Term Follow-up

Subjects will move directly to Long Term Follow-up for gene therapy and followed until off study criteria are met for the following:

- ✓ Did not respond to CD22-CAR treatment (did not achieve a CR or PR) or progresses following response, and is either not eligible or chooses not to pursue re-treatment
- ✓ Proceeds to alternative therapy after CAR treatment

Subjects on Long Term Follow-up will be followed for survival, subsequent therapy, long-term gene therapy follow up (including RCL blood sampling), as described in **Section 5.5.5**. At the discretion of the investigator, subjects may continue correlative sample collection.

4.3.4 Off-Study Criteria

- ✓ Subject withdrawal of consent (in which case the reason will be documented, if possible). Subjects who withdraw consent for additional procedures may still participate in long-term follow up.
- ✓ Subject who meet any of the withdrawal criteria listed in Section **4.3.1** and who

have recovered from all study-induced toxicity.

- ✓ Subject lost to follow-up. Should a subject fail to return to the clinic for a scheduled protocol specific visit, site will need to make 2 attempts by a combination of telephone and mail to contact the subject. Site 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.
- ✓ Death
- ✓ Conclusion of the 15 years of follow up, or subject enrolls in a separate long-term follow up protocol for subjects receiving gene transfer.

4.3.5 Off-Study Procedure

Off study date and reason should be documented in the study CRFs. For subjects withdrawing consent, the investigator should inquire whether the subject agrees to allow chart review of normal medical care procedures and/or long term follow-up of gene therapy research participants. Cell products and biological samples from off-study subjects may continue to be used for process development and correlative studies, as outlined in the consent form.

5 SUBJECT STUDY PROCEDURES

5.1 SUBJECT SCREENING

The screening period begins on the date the subject/LAR signs the IRB and APB approved consent form and continues through confirmation of enrollment. Procedures that are to be performed as part of standard medical care, such as for diagnosis or treatment of a disease or medical condition, may be performed and the results subsequently used for determining study eligibility without first obtaining consent. Informed consent must be obtained prior to initiation of any clinical procedures that are performed solely for the purpose of determining eligibility for research, e.g. withdrawal from medication (wash-out period). Only subjects who meet the eligibility criteria listed in **Section 3.1** will be enrolled in the study.

All subjects will undergo the screening procedures, which include a comprehensive history and physical exam performed by a study physician as outlined below. Imaging, bone marrow biopsy and aspiration, and organ specific studies will be performed as per institutional guidelines. The following screening tests must be performed within 28 days prior to enrollment unless specified otherwise.

If patient or manufacturing scheduling requires, screening may be completed in two stages: 1) for leukapheresis, and 2) for lymphodepleting chemotherapy and CD22-CAR T cell infusion, as outlined for each stage in **Section 13.8, Appendix H**.

(a) CD22 expression

For R/R ALL subjects: Expression will be evaluated by immunohistochemistry or by flow cytometry in a CLIA approved laboratory and documentation at screening will be required (testing is permitted to be conducted at any time since diagnosis, unless subject has received anti-CD22 targeted therapy, such as Moxetumomab pasudotox or inotuzumab ozogamicin, in

which case the specimen must be collected after such therapy).

For R/R aggressive B-cell NHL: CD22 expression at any level, including undectable, will be acceptable. Subjects must have archival tissue available for analysis of CD22 expression or must be willing to undergo a biopsy of easily accessible disease.

(b) Medical history and concomitant medication

The subject's complete history through review of medical records and by interview will be collected and recorded. Concurrent medical signs and symptoms must be documented to establish baseline severities. A disease history, including the date of initial diagnosis and list of all prior treatment, responses, and duration of response to the prior treatment also will be recorded. Most current disease status will be determined to include presence and location of current disease. Prior therapy washout periods will be calculated. History of known infection with HIV, hepatitis B and hepatitis C will be elicited, but viral testing is not necessary, as this will be performed standard of care prior to the leukapheresis procedure, as per institutional procedures.

(c) Physical examination

A complete physical examination will be performed. The exam will include general appearance of the subject, height and weight, examination of the skin, eyes and ears, nose, throat, lungs, heart, abdomen, extremities, musculoskeletal system, and nervous system.

(d) Vital signs, including blood pressure, heart rate, oxygen saturation and temperature

(e) Performance status (ECOG or Karnofsky) see Appendix A, **Section 13.1**

(f) Electrocardiogram (ECG)

(g) ECHO, MUGA or Cardiac MRI for LVEF and pericardial effusion assessment [performed **within 180 days or after most recent anthracycline based treatment or mediastinal radiation therapy** (whichever is most recent)]

(h) Creatinine clearance: A measured 24 hour urine creatinine clearance test may be performed if the serum creatinine is elevated, and the measured value will be recorded in the CRF and may be used to qualify the subject for study participation.

(i) β-HCG pregnancy test on all women of child-bearing potential

(j) Coagulation tests including prothrombin time (PT)/ partial thromboplastin time (PTT)

(k) General Laboratory Tests: The following will be obtained during the screening process:

- Chemistries: sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), creatinine, glucose, calcium, AST, ALT, alkaline phosphatase, bilirubin, albumin, total protein
- Phosphorus and magnesium
- CBC with differential
- C-reactive protein (CRP), ferritin
- Urinalysis

(l) Additional Tests:

The investigator may order additional tests in some subjects if needed to fully assess clinical status and obtain baseline results. Examples of such tests include: haptoglobin, soluble IL2R, immunoglobulin levels, and viral serology or PCR for cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV), and Epstein-Barr virus (EBV).

(m) Disease Evaluation (methods will be determined by investigator based on subjects' location of disease, not all are required on all subjects)

- Imaging Studies appropriate to sites relevant to subject's disease: subjects with aggressive B-cell NHL will undergo PET/CT; other imaging studies (e.g. MRI of the brain) will be performed as determined by investigator
- Bone marrow aspirate/biopsy: subjects with ALL will undergo bone marrow biopsy, subjects with bone marrow involvement prior to therapy or if new abnormalities in the peripheral blood counts or blood smear cause suspicion of bone marrow involvement will undergo bone marrow aspirate and biopsy
- Lumbar puncture, if determined as clinically necessary by the investigator

5.2 LEUKAPHERESIS FOR CELL ACQUISITION:

Leukapheresis (apheresis) will be performed according to institutional standards with the goal of obtaining a minimum of 1×10^8 CD3+ T cells for transduction. If PBMCs have been cryopreserved for a different cell therapy or cell collection study or standard of care, they may be used to generate CD22-CAR T cells on this study if they meet criteria outlined in this IND.

Target cell number will be based upon the expected expansion and planned cell doses of transduced T cells/kg. The target will be 14-20 L apheresis in adults (target 2×10^9 mononuclear cells). Prophylactic intravenous $CaCl_2$ and $MgSO_4$ infusions may be administered by the apheresis clinical team per standard operating procedures. Institutional guidelines will be followed for venous access and apheresis procedures.

If the patient is unable to meet the required number of cells and is unable (by investigator's discretion or scheduling constraints) to undergo further apheresis collections, the patient will be discontinued from the study. If, after enrollment and leukapheresis, the decision is made to not proceed with CD22-CAR T cell manufacturing, the collected apheresis product will be made available to the subject for alternative CAR-T cell production, if requested.

Eligibility criteria to undergo leukapheresis is as follows all subjects, unless undergoing two stage enrollment (see **Section 13.8, Appendix H**):

- Subjects must have no evidence of a clinically significant uncontrolled infection prior to leukapheresis
- Absolute lymphocyte count ≥ 150/uL within last 7 days (unless lower count attributed to disease status)
- No new signs or symptoms of kidney or liver dysfunction outside eligibility criteria within last 7 days
- Females of child-bearing potential must not be pregnant.

Systemic Corticosteroid therapy at dose ≥ 5 mg/day of prednisone (or equivalent doses of other corticosteroids) and other short-acting cytotoxic/antiproliferative drugs drugs must be avoided for 3 days prior to leukapheresis.

The following procedures/requirements will occur on the leukapheresis collection day (unless otherwise specified) and as outlined in the **Section 9** Study Calendar:

- Vital signs, including blood pressure, heart rate, respiratory rate, oxygen saturation, and temperature
- Weight
- Labs (need not be repeated if screening labs were performed within 7 days prior to leukapheresis)
    - Chemistry panel (sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, AST, ALT, alkaline phosphatase, bilirubin, albumin, total protein)
    - Phosphorus and magnesium
    - CBC with differential
    - CRP
    - Pregnancy test (females of child-bearing potential only)
- Leukapheresis
- Correlative studies sample collection, as detailed in Appendix G, Section **13.7**
- Adverse/Serious Adverse Event reporting – record only unexpected serious adverse events considered related or possibly related to study procedures.
- Concomitant medications documentation

Subjects may receive disease-directed therapy (standard or investigational) prior to initiating conditioning lymphodepletion chemotherapy if the investigator determines there is at significant risk of disease progression if all treatment is stopped while waiting for cell manufacture (see **Section 4.2.5)**.

Any change to baseline toxicities ("Course Zero") will be captured in baseline assessment CRFs, but adverse events related only to anti-neoplastic bridging therapy will not be collected in study CRFs (see **Section 7.3**).

- , urea nitrogen, albumin, alkaline phosphatase, ALT and/or AST, total bilirubin).

5.3 CONDITIONING LYMPHODEPLETION CHEMOTHERAPY REGIMEN

Subjects will receive a conditioning lymphodepletion chemotherapy regimen in order to induce lymphocyte depletion and create an optimal environment for expansion of CD22-CAR T cells *in vivo*. Conditioning lymphodepletion chemotherapy regimen has been chosen based on the clinical site's standards of practice and institutional procedures to minimize deviations and risks to subjects. Routine anti-emetic prophylaxis and treatment should be employed. Corticosteroids may not be used (except for physiologic replacement).

5.3.1 Criteria for Initiating Conditioning Lymphodepletion Chemotherapy Regimen

In order to initiate the conditioning lymphodepletion chemotherapy regimen, all subjects must meet the following criteria within 7 days prior to starting chemotherapy:

- There must be 14 days or more than five half-lives from the last dose of standard chemotherapy (7 days from ALL maintenance therapy), with the exclusion of steroids, which may be continued until starting conditioning lymphodepletion regimen.
- No evidence of uncontrolled infection,
- No clinically significant cardiac dysfunction,
- Serum creatinine must be < 2 x ULN,
- No acute neurological toxicity > Grade 1 (with the exception of peripheral sensory neuropathy),
- Negative pregnancy test in child-bearing females within 7 days of starting chemotherapy.
- Repeat disease evaluation, at the discretion of the investigator, if the subject received any antineoplastic therapy after enrollment (per **Section 4.2.5**), if greater than 28 days has elapsed between date of enrollment and start of conditioning lymphodepletion chemotherapy regimen, or if investigator believes disease may have significantly advanced since enrollment.

Should an event exceed these criteria immediately prior to conditioning chemotherapy, conditioning chemotherapy must be delayed until the event resolves to ≤ Grade 1 or baseline.

5.3.2 Conditioning Lymphodepletion Chemotherapy Regimen Procedures

Subjects will initiate one of the conditioning chemotherapy regimens as listed in **Table 4**, at the discretion of the investigator. The conditioning chemotherapy regimen may be administered in an outpatient setting per investigator's discretion. The dose calculation for the chemotherapy administration will be based on the height (measured within the past 30 days) and weight measured on Day -6 or -5 of the start of lymphodepletion. At the investigator's discretion, the timing of the start and ending of the regimen of conditioning lymphodepletion chemotherapy may be adjusted based on the best interest of the patient.

The following procedures will be completed daily during chemotherapy, unless otherwise specified:

- Height (taken within 30 days prior) and weight (Day -6 or Day -5 only)
- Vital signs, including blood pressure, heart rate, respiratory rate, oxygen saturation, and temperature
- Pregnancy test (females of child-bearing potential only), performed within 7 days prior to the start of chemotherapy
- Labs
    - Chemistry Panel (sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, AST, ALT, alkaline phosphatase, bilirubin, albumin, total protein)
    - Phosphorus, magnesium, and LDH, uric acid (on Day -5 or -6 only)
    - CBC with differential
    - CRP and ferritin (on Day -5 or -6 only)
- Chemotherapy administration
- Correlative studies sample collection, as detailed in Appendix G, Section **13.7**

- Adverse/Serious Adverse Event reporting
- Concomitant medications documentation
- Hydration: Subjects will receive Home IV fluid infusion per institutional guidelines for the agents administered.

Each subject will receive one of the lymphodepleting regimens, at the investigator's discretion as follows:

Table 4. Conditioning Lymphodepletion Chemotherapy Regimen

Regimen #1		
Cyclophosphamide	500 mg/m^2 per day IV infusion over at least 60 minutes or as tolerated, on Day -5, -4 and -3	-5, -4, -3
Fludarabine	30 mg/m^2 per day IV infusion over at least 30 minutes or as tolerated, daily for 3 days.	-5, -4, -3
Regimen #2		
Cyclophosphamide	500 mg/m^2 per day IV infusion over at least 60 minutes or as tolerated, on Day -6 and -5	-6, -5
Clofarabine	40 mg/m^2 per day IV infusion over at least 2 hours or as tolerated, daily for 4 days. (Reduce dose by 25% of the usual dose if CrCl 60-80 mL/min; Reduce dose by 50% if CrCl 30-60 mL/minute)	-6, -5, -4, -3
Regimen #3		
Bendamustine	90 mg/m^2 per day IV infusion over 10 minutes or as tolerated, daily for 2 days. (Adjust or hold dose for CrCl Less than 30 mL/minute)	-4, -3

Hydration may be adjusted based on clinical need (e.g. over hydration or dehydration). There will

be no dose adjustment of chemotherapy agents for weight considerations or abnormal lab values. If subjects are eligible for the trial, then the full dose of lymphodepletion agents will be administered.

5.4 INVESTIGATIONAL AGENT ADMINISTRATION AND RESEARCH PROCEDURES

5.4.1 Cell Processing:

Cellular Product: Autologous T cells; transduced with lentiviral vector (CD22.BB.Z) Chimeric Antigen Receptor (CD22-CAR); following Lymphodepleting Chemotherapy

Autologous CD22-CAR T cells will be generated from fresh or cryopreserved PBMCs under GMP conditions in the manufacturing facility using Miltenyi CliniMACS Prodigy® system. Apheresis products from participating subjects will be sent to the manufacturing facility through Stanford Bone Marrow Transplant Department's Cellular Therapeutics Facility (CTF); final cell products will be received and final formulated product distributed by the CTF.

Any prepared cells not required for the first infusion or for research or regulatory purposes (including sufficient QA retention vials for testing cryopreserved product stability at time of additional infusions) will be cryopreserved by standard techniques and will be made available should the subject be eligible for a second infusion as outlined in **Section 5.6**. Additional product in excess of that needed for infusion may be utilized for research.

Fresh or cryopreserved PBMCs (depending on the timing of apheresis relative to cell culture, subject condition, and scheduling availability) will be used for cell manufacturing. Cells will be required to meet standard release criteria including transduction efficiency ≥ 10%, T cell content ≥ 70%, sterility and minimum levels of lipopolysaccharides (LPS), as well as no evidence for replication competent lentivirus. All procedures will take place using good manufacturing process guidelines.

The release criteria will be based upon analyses of CD22-CAR lenti-transduced autologous PBL and will include:

Table 5. CD22-CAR Product Rapid Release Criteria

Test	Criteria
Cell viability[1]	≥ 70%
Total Cell Count[1]	within 20% of planned dose level
CD3+%	≥ 70%
CAR+ %	≥ 10%
Endotoxin[1]	< 5 EU/kg
Mycoplasma by PCR[1]	Negative
RCL[2]	Negative
Sterility testing[3]	Negative

[1]Performed on sample from final product, results available at the time of infusion.

[2]Two samples will be sent to Indiana University GTTL: 1) Real-time PCR testing, results available at the time of infusion; 2) culture-based testing; results not available prior to infusion.

[3]Gram stain is performed on final product prior to infusion and is available at the time of infusion; 14-Day cultures will be sent from the harvest (Day 7, or 11) drug product. Sample results collected from the final product will not be available before cells are infused into the subject. Although cells will not be infused prior to a preliminary negative sterility reading from Day 3 to 5 of the culture.

5.4.2 CD22-CAR T cell Infusion

5.4.2.1 Cell Infusion Criteria

Subjects will be hospitalized to receive treatment with CD22-CAR T cells, if not previously hospitalized, and will remain hospitalized for approximately 7 (±2) days. Patients may be discharged once all AEs have resolved to Grade 1 or better, or at the discretion of the treating physician. Subjects may be discharged with non-critical and clinically stable or slowly improving toxicities (e.g., renal insufficiency, cytopenias) even if > Grade 1, if deemed appropriate by the investigator. Subjects must meet the following criteria in order for cells to be infused (based on labs obtained within 24 hrs of cell infusion):

- ✓ CD22-CAR T cells must have met release criteria (section **5.4.1**)
- ✓ Subject has no evidence of hemodynamic instability
- ✓ Subject has not developed a new requirement for supplemental oxygen therapy
- ✓ Subject has not developed symptoms concerning for new, systemic infection or condition that in the opinion of the PI may pose an unacceptable risk to the subject
- ✓ There is no evidence of clinically significant cardiac dysfunction, uncontrolled, significant tumor lysis syndrome, serum creatinine > 2 x ULN, and no acute neurological toxicity > Grade 1 (with the exception of peripheral sensory neuropathy).

If these criteria are not met, measures will be taken to resolve the underlying condition(s) and the cell infusion must be delayed until the event resolves. Fresh, uncryopreserved CD22-CAR T cells should be infused within 72 hours of planned infusion or frozen for later use. If the CD22-CAR T cells infusion is delayed > 2 weeks, conditioning chemotherapy must be repeated**, unless investigator deems this unnecessary**.

Corticosteroid therapy at a dose ≥ 5 mg/day of prednisone (or equivalent doses of other corticosteroids) and other short-acting cytotoxic/antiproliferative drugs must be avoided for 5 days prior to CD22-CAR T cell administration.

5.4.2.2 Premedications

Adult subjects will receive levetiracetam 500 mg orally twice a day as per institutional guidelines, at the physician's discretion (maximum dose of 750 mg/dose) beginning the day before cell infusion (Day -1).

Subjects at high risk of TLS, per investigator's discretion, may receive allopurinol 300 mg orally once daily or at 100 mg/m^2/dose TID beginning the day prior to cell infusion (Day -1) or at the time of the conditioning lymphodepletion chemotherapy regimen, as determined by investigator.

Subjects will receive the following medications 30-120 minutes prior to cell infusion:

1) Diphenhydramine: 25-50 mg/dose orally (PO) or IV;
2) Acetaminophen: 650 mg/dose PO or IV

5.4.2.3 Cell Infusion

Cells are delivered to the subject care unit by a staff member from CTF. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN) and an identification of the product and documentation of administration are entered in the subject's chart, as is done for blood banking protocols. Cell products should NOT be infused unless the product identification matches the subject's identification.

Cells are to be infused intravenously (IV) over 10-30 minutes (or as tolerated based on volume and/or DMSO toxicity) via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping. After infusing cells, rinse the infusion tubing and empty bag with a backflush of normal saline. Infuse the remaining cells using the backflush method. Documentation in the medical record should include the volume of cell infusion, the thaw start/stop time (if cells are cryopreserved), and cell product infusion time start/stop times.

5.4.3 Determination of Cell Dose

Cell dose will be body weight-based; the dose of CD22-CAR transduced T cells will be calculated using actual body weight, capped at 100 kg, measured within 14 days of cell manufacture start date.

If cell growth limitations preclude administration of the number of cells targeted for the assigned cohort level, the subject will receive as many cells as possible, as the efficacious dose is not yet known, and the subject will be evaluable for feasibility of manufacturing but will not be evaluable for safety in that dose cohort, although toxicities will be assessed and reported separately. An additional three subjects per disease group may be enrolled due to cell growth limitations.

If cell growth limitations preclude administration of the number of cells targeted for the assigned cohort level in 3 subjects (out of 6), manufacturing of the cell product will be considered not feasible as there will be inadequate number of subjects to evaluate safety at the targeted dose level.

5.4.4 Dose Escalation in Subjects with Relapsed/Refractory Aggressive B-cell NHL

There will be a Phase 1 dose-escalation design with three dose cohorts in subjects with aggressive B-cell NHL to determine the MTD/RP2D (see **Table 3**). Each dose cohort will initially include a minimum of 3 subjects. Treatment will be staggered as follows for each dose level: at least 21 days will elapse between infusion of each subject during dose escalation. The final subject with aggressive B-cell NHL in a dose cohort must complete the 28-day DLT observation period before the decision is made whether to treat additional subjects at the current dose level or to dose escalate to allow for safety assessment of DLTs (as defined in **Section 5.4.7.1**) as per **Table 6**. If Dose Level 3 is completed without DLTs, an MTD may not be determined. This will be considered the 'highest cell dose' studied, and will be the dose level that will be studied further in the expansion cohort or recommended phase 2 dose (RP2D).

Dose escalation will follow the rules outlined in the Table below.

Table 6. Dose Escalation Rules for Subjects with Aggressive B-cell NHL

Number of Subjects with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter up to 3 subjects at the next dose level. If 0 out of 3 occur, dose may escalate.
1 out of 3	If DLT develops in one of first 3 subjects at a Dose Level, the cohort will be expanded to 6 subjects. If no additional subjects develop DLT, MTD will not have been exceeded and the next dose level can be administered after the four week safety assessment period of the last subject at this dose level. If DLT develops in any subject at Dose Level -1, accrual will be temporarily stopped while consultation with the IRB and FDA occurs.
2 out of 6	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Up to three (3) additional subjects will be entered at the next lowest dose level if only 3 subjects were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is the MTD and is generally the RP2D. At least 6 subjects must be entered at this dose level.

5.4.4.1 Definition of Maximal Tolerated Dose (MTD)

The maximum tolerated dose (MTD) will be evaluated and reported separately in each disease group (ALL and aggressive B-cell NHL). During dose escalation of subjects with aggressive B-cell NHL, MTD is defined as the dose level below that at which 2/6 subjects develop DLTs. Subjects with ALL treated at the established dose of 1 x 10^6 transduced T cells/kg and subjects with aggressive B-cell NHL treated at the MTD/RP2D, the maximum acceptable rate of DLTs for each group is 30%.

5.4.5 Dose Expansion Cohort for Subjects with Aggressive B-cell NHL

After the MTD/RP2D is established for aggressive B-cell NHL subjects, a dose-expansion cohort will enroll up to 33 evaluable subjects (for a total of 36 evaluable subjects treated at the final MTD/RP2D) to monitor safety and indicate efficacy. The sample size for the aggressive B-cell NHL dose-expansion cohort is determined to indicate efficacy (see **Section 12.2.1** for more details).

The trial will continue to evaluate safety after the first 11 (or 14) subjects in the dose-expansion cohort, corresponding to a total of 17 evaluable subjects treated at the MTD/RP2D with either 6

(or 3) subjects from the dose-escalation cohort(s), and every 5 subjects thereafter (see **Section 12.1.2.1**). If de-escalation occurs, new safety boundaries will be generated based on the number of currently enrolled subjects and will take place after every 5 additional subjects. If Dose Level -1 is found to be unsafe, the trial will stop enrollment for this disease population.

5.4.6 Dose De-escalation Subjects with Relapsed/Refractory ALL

Subjects with relapsed/refractory ALL will be treated at Dose Level 1 (1×10^6 transduced T cells/kg), as previous clinical studies support this dose level. The dose will de-escalate to Dose Level -1 if the safety boundaries for the number of DLTs presented in **Section 12.1.2.2** are met.

5.4.7 Safety Assessment in Both Populations: in Subjects with Relapsed/Refractory B-cell ALL and Subjects with Relapsed/Refractory Aggressive B-cell NHL

Dose limiting toxicities (DLTs) will be evaluated in each disease group (ALL and aggressive B-cell NHL) and reported separately. The period of observation to determine DLT is 28 days after cell infusion. Only adverse events (AEs) assessed by investigator to be possibly, probably or definitely related to pre-infusion chemotherapy and/or CD22-CAR T cells (any component of the treatment regimen) will be used in the definition of DLT (See **Section 7.2.2** for definition of 'suspected' adverse reaction). Toxicities occurring after initiation of the conditioning lymphodepletion chemotherapy regimen but prior to CD22-CAR T cell infusion will primarily be attributable to the chemotherapy administration or disease, if not extraneous causes. After cell infusion, toxicities will be evaluated for temporal and causal relationship to chemotherapy versus cell infusion. Some symptoms may overlap and attribution will not be clearly definable, in which case, toxicities will be attributed as possibly related to both preparative regimen and cell infusion. Toxicities will be attributed to the T cells if: 1) they were NOT present before T cell infusion; OR 2) they increase in grade in temporal association with the T cell infusion; AND 3) they are not clearly explained by other factors.

Subjects who are fully assessable for DLT are those that have completed the conditioning lymphodepletion chemotherapy regimen and received the CD22-CAR T cell infusion. Chemotherapy-related toxicities experienced by subjects who are unable to receive CD22-CAR T cells *will not* be considered in the definition of DLT.

5.4.7.1 Definition of DLT

Adverse events that are at least possibly related to the treatment regimen (conditioning lymphodepletion chemotherapy regimen and/or CD22-CAR T cells) with onset within the first 28 days following CD22-CAR T cell infusion will be considered DLTs as follows:

- Hematologic toxicity will <u>not</u> be considered DLTs, as these are common after CAR T therapy and have been successfully managed with standard supportive therapies.

- Grade 4 CRS (of any duration) or Grade 3 CRS that lasts greater than 7 days.
- Grade 4 neurotoxicity (of any duration) or Grade 3 neurotoxicity that lasts >72 hours (severity as defined in Appendix B, **Section 13.2.3**.).
    - Infusion reactions ≥ Grade 2 in severity lasting more than 24 hours despite standard supportive care.

- Any other Grade 3 or greater, non-hematological toxicity will be considered a DLT with the following **exceptions**:
    - Tumor lysis syndrome, including associated abnormalities (e.g., electrolytes, uric acid, renal function);
    - Grade 3 diarrhea that resolves to ≤ Grade 2 within 4 days;
    - Grade 3 low electrolyte levels that are correctable and asymptomatic, Grade 3 hypoalbuminemia;
        - Hypocalcemia toxicity grade should be assigned based on the calcium level corrected for degree of hypoalbuminemia according to the following (or comparable) formula: For every albumin decrease of 1 gm/dL a total calcium increase of 0.2 mmol/L is to be made;
    - Abnormal coagulation parameters in subjects on anticoagulant therapy or with pre-existing coagulopathy;
    - Grade 3 transaminase, alkaline phosphatase, bilirubin or other liver function test elevation, provided there is resolution to ≤ Grade 2 or baseline within 14 days. Grade 4 transaminitis lasting < 72 hours will also not be considered DLT;Grade 3 nausea and/or anorexia.Grade 3 or 4 fever lasting ≤ 14 days, as this is common and expected with CAR therapy and may be a biomarker for an effective immunotherapeutic regimen;
    - Grade 3 or 4 infection or neutropenic fever unless subjects have normal blood counts at baseline, and infection is not considered likely related to lymphodepleting chemotherapy and relationship to CD22-CAR T cells is suspected (Note: Grade 4 infection uncontrolled for > 7 days will be considered DLT.);
        - Toxicities occurring within 24 hours post cell infusion related to cell infusion, (including Grade 3 and 4 allergic reaction) that are reversible to a grade 2 or less within 8 hours with up to two doses of acetaminophen 15 mg/kg/dose (to a max adult dose of 650 mg) or up to two doses of diphenhydramine 1 mg/kg up (to a max adult dose of 50 mg);
- In patients with history of prior SCT, any histologically proven acute GVHD grade 3 or higher within 30 days of receiving the CD22-CAR T cells will be considered DLT.

Adverse events will be graded according to NCI's Common Terminology Criteria for Adverse Events (CTCAE v5.0). As noted in **Section 2.4.4** CRS will be graded according to a revised grading system (See Appendix B, **Section 13.2.1**). Adverse events attributed to CRS will be mapped to the overall CRS grading assessment for the determination of DLT.

5.5 EVALUATIONS AND FOLLOW UP

Subjects will be hospitalized prior to receipt of CD22-CAR T cells, if not before administration of conditioning lymphodepletion chemotherapy regimen, and may remain in the hospital for approximately 7 (±2) days. Subjects should remain hospitalized for ongoing cell-related fever, hypotension, hypoxia, or ongoing central neurological toxicity > Grade 1, or if deemed necessary by the treating investigator. Subjects may be discharged with non-critical and clinically stable or

slowly improving toxicities (e.g., renal insufficiency) even if > Grade 1, if deemed appropriate by the investigator.

Given the possibility that a subject could develop CRS or neurotoxicity after discharge from the hospital, subjects will be asked to remain within commuting distance to the clinic for the first 28 days post cell infusion, and subjects and their family members/caregivers will be educated on potential symptoms such as fever, dyspnea, confusion, aphasia, dysphasia, somnolence, encephalopathy, ataxia, or tremor. If subjects develop these symptoms, they will be instructed to immediately contact the investigator or seek immediate medical attention.

During this post cell infusion period, the following procedures will be completed at the time points outlined in the Section **9** Study Calendar:

5.5.1 Evaluation Prior to Cell infusion

Prior to cell infusion subjects will undergo safety evaluations with general laboratory tests, physical exam, neurologic exam, vital signs, adverse event collection, concomitant medication monitoring, and correlative sample collection.

Thyroid stimulating hormone (TSH) levels should be measured once within 28 days prior to cell infusion.

Once, any time between enrollment and start of cell infusion, a blood sample for replication competent lentivirus (RCL) testing will be collected and sent to Indiana Gene Therapy Testing Laboratory (IU GTTL), as described in **Section 13.4**.

Please refer to the **Section 9** Study Calendar for a list of all procedures.

5.5.2 Monitoring During and Immediately After Cell Infusion

Monitoring will include vital signs (blood pressure, heart rate, respiratory rate, oxygen saturation, and temperature) prior to infusion, at the conclusion of cell infusion (± 10 min), and at 60 (± 10 min), and as clinically indicated.

- ✓ Supplemental oxygen will be available at the bedside.

If an allergic or other acute reaction occurs, studies appropriate for investigation of a transfusion reaction will be performed (e.g., urinalysis, CBC, Coomb's test). Acute reactions will be treated according to institutional standards of care.

5.5.3 Evaluations Post Treatment Assessment Period Day 0 to Month 3

After completing CD22-CAR T cell infusion all subjects will be followed in the post treatment assessment period. Counting from Day 0 (CD22-CAR T cell infusion), subjects will undergo the following evaluations (during hospitalization or will return to the clinic):

- From Day 0 to Day 14, evaluations 5 times per week, with no more than 48 hours between evaluations
- From Day 15 to Day 27, evaluations twice per week (± 4 days)
- Day 28 (± 4 days)
- Month 2 (± 2 weeks)

- Month 3 (± 2 weeks)

Please refer to the Section **9** Study Calendar for a list of all procedures at each visit.

Procedures will include safety evaluations with general laboratory tests, targeted physical exams (as clinically indicated), neurologic exams, adverse event collection, concomitant medication monitoring, and correlative sample collection. Vital signs monitoring (including blood pressure, heart rate, oxygen saturation, respiratory rate, and temperature) should be performed every shift (± 2 hours) during hospitalization and with each visit after discharge. Neurotoxicity evaluations in the form of ICE score/ CAPD evaluations should be performed every shift (± 2 hours) and with any change in neurologic functioning during the initial hospitalization for cell infusion.

Monitoring of CRP, ferritin, and LDH (only if LDH is elevated at baseline) levels may assist with the diagnosis and define the clinical course in regards to CRS/neurotoxicity. It is therefore, recommended that CRP and LDH (if elevated at baseline) be monitored when lab specimens are drawn starting at Day 0 and continuing through hospitalization. In addition, lactate can be monitored as clinically indicated.

Day 28 (± 4 days) will be the first disease evaluation post cell infusion. This is the primary efficacy endpoint for subjects with ALL, but subjects with lymphoma will also undergo disease evaluation at Day 28 (± 4 days). Evaluations will include general laboratory tests, full physical exam, neurologic exam, performance status, weight, vital signs, adverse event collection, concomitant medication monitoring, disease evaluation, and correlative sample collection. Quantitative immunoglobulins should be measured every 3 to 12 weeks post infusion until Month 3 (± 2 weeks) to monitor IgG levels. It is recommended to measure T and B cell lymphocyte subsets monthly starting at Day 28 to monitor for return of B cells, a possible indictor of loss of CAR T cell persistence.

The second disease evaluation will occur at the Month 3 visit (± 2 weeks). This is the primary efficacy endpoint for subjects with lymphoma. Evaluations will include general laboratory tests, full physical exam, neurologic exam, performance status, weight, vital signs, targeted adverse event and concomitant medication collection, disease evaluation, and correlative sample collection. Evaluations may be performed by local physician/medical provider in the event circumstances prevent the subject from returning to Stanford clinic, if all source documents are transmitted to the research team for inclusion in the medical record/research record.

5.5.3.1 Disease Evaluation Post Cell Infusion

Disease evaluation methods will be determined by the investigator based on subjects' location of disease; not all are required on all subjects, but methods should remain consistent while on study:

- Imaging Studies appropriate to sites relevant to subject's disease: subject with bulky disease will undergo PET/CT, other imaging studies (e.g. MRI of the brain) will be performed as determined by investigator
- Bone marrow aspirate: subjects with bone marrow involvement prior to therapy or if new abnormalities in the peripheral blood counts or blood smear cause suspicion of bone marrow involvement will undergo bone marrow aspirate, with biopsy if needed
- Lumbar puncture, for subjects with known or suspected CNS involvement only

- Lymph node biopsy in subjects with lymphoma only, if feasible, once between Day 7 and Day 28 (during peak CAR activity) for correlative studies

5.5.3.2 Small Volume Apheresis for Research

Subjects may undergo a small volume apheresis (approximately one to two blood volumes) to obtain peripheral lymphocytes for correlative studies. Every attempt will be made to use an indwelling catheter or peripheral lines for the small volume apheresis. If this is not feasible and an apheresis specimen cannot be collected, peripheral blood should be collected into the appropriate tubes for correlative samples.

A small volume apheresis for research will be considered at the following time points:

- Day 28 (± 7 days)
- Month 3 (± 1 month)
- Month 6 (± 1 month)
- Month 9 (± 1 month)
- Month 12 (± 2 months)

5.5.3.3 Targeted Adverse/Serious Adverse Event reporting

After Day 30 and for 24 months or until disease progression (whichever occurs first), targeted AE collection of neurological, hematological (Grade 3 or higher), infections, autoimmune disorders, and secondary malignancies will be performed if attributed as possibly, probably or definitely related to CD22-CAR. Targeted SAE collection will occur as described in **Section 7.3**.

5.5.3.4 Targeted Concomitant Medication Documentation

After Day 28 visit and for 24 months or until disease progression (whichever occurs first), targeted concomitant medication collection of gammaglobulins, immunosuppressive drugs, anti infectives, and vaccinations will be performed.

5.5.3.5 RCL Testing

The US Food and Drug Administration requires monitoring for replication competent lentivirus in all human subjects receiving lentiviral gene therapy. Even if a subject withdraws from other study evaluations, permission to continue RCL testing and annual gene therapy questionnaires will be requested. Blood collection for RCL testing will be performed at the following time points and sent to IU GTTL as described in **Section 13.4**

- Month 3 (± 1 month)
- Month 6 (± 1 month)
- Month 12 (± 2 months)

If all tested samples are negative for RCL through Month 12, blood samples will be collected and archived annually until Year 5 (± 2 months).

5.5.4 Evaluations Post Treatment Assessment Period (After Month 3)

Subjects who received CD22-CAR T cells will return to the clinic at the following intervals, unless there is no clinical response, documented disease progression or alternative disease therapy is

started, at which time the subject will proceed to long term gene therapy follow up (see **Section 5.5.5**). Correlative sample collection and post treatment assessments may be performed by the local medical care team or via TeleHealth if in the best interest of the patient, but results, progress notes and scans should be sent to the research team for inclusion in the medical record/research record.

5.5.4.1 Post Treatment Assessment Schedule

Subjects who received CD22-CAR T cells will be evaluated at the following timepoints (additional evaluations may be performed as clinically indicated):

- Month 6 (± 4 weeks)
- Month 9 (± 4 weeks)
- Month 12 (± 4 weeks)
- Every 6-12 months until 5 years post cell infusion
- Annually Year 6 to Year 15 (± 3 months)

5.5.4.2 Post Treatment Assessments:

Please refer to the **Section 9** Study Calendar for a list of all procedures at each visit.

Evaluations of disease status in subjects who have **not** progressed after CD22-CAR T cells will be conducted at Stanford for the first 24 months, when feasible. If not feasible, disease evaluations may be performed virtually or by referring physician or outside medical facility as long as documentation is forwarded to the study Principal Investigator/research team. If subject's disease has not progressed by Month 24, disease assessments will continue to be performed per standard of care.

Should a subject fail to return to the clinic for a scheduled protocol specific visit, sites will need to make 2 attempts by a combination of telephone 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.

Evaluations will include general laboratory tests (as clinically indicated), physical exam, neurologic exam, performance status, weight, vital signs, targeted adverse event and concomitant medication collection, disease evaluation, RCL testing, and correlative sample collection. A small volume apheresis for research will be considered at Month 6 (± 1 month), Month 9 (± 1 month), and Month 12 (± 2 months).

A PBMC sample (for CD22-CAR T cells, etc) should be collected at the time of progression or at time prior to starting any subsequent anticancer therapy without progression.

5.5.4.3 Gene Therapy Safety Monitoring (Month 6 to Year 5)

- Evaluations will be performed as per **Section 5.5.5.2** regardless of disease status or alternative therapies.

5.5.4.4 Annual Gene Therapy Questionnaire (Year 6 to 15)

- Evaluations will be performed as per **Section 5.5.5.2** regardless of disease status or alternative therapies.

After year 5, the subject will be contacted (in clinic or via phone or written questionnaire) to evaluate for development of delayed adverse events (See questionnaire in **Section 13.5,** Appendix E: Draft Letter and Questionnaire to Subjects for Long Term Follow-up for Delayed Adverse Events).

- If vector modified cells were detected in the blood during the previous visit, then blood for persistence of vector modified cells will be collected and tested until negative.
- Subjects are requested to inform the study research team of any changes to e-mail (if consented to use e-mail), phone, and address. E-mails will be sent to subjects via the Secure Email to ensure securely and confidentially over an SSL/encrypted connection.
- Distribution and collection of questionnaires
    - A draft letter to subject's primary doctor is provided in **Section 13.6,** Appendix F. This letter should be sent to the local health care provider via mail, fax or e-mail at the time the subject is referred back to the care of the local physician or with any change in primary doctors. E-mails will be sent to subjects via the Secure Email to ensure securely and confidentially over an SSL/encrypted connection.
    - Subjects will be sent a request for information (**Section 13.5,** Appendix E: Draft Letter and Questionnaire to Subjects for Long Term Follow-up for Delayed Adverse Events) and questionnaire annually.
        - If there is no response within 1 month, the study coordinator will follow up with a telephone call to request the information. The questionnaire may be completed with the subject responses over the phone at this time.
        - If there is no response to the telephone call, the letter and questionnaire (**Section 13.5**) should be sent to the subject via FedEx, signature required.
        - Each attempt to contact the subject and outcome must be documented in the medical record.

5.5.5 Long Term Gene Therapy Follow-up

At any time during the post treatment assessment period, the subject will proceed directly to the Long Term Gene Therapy Follow Up if he/she:

1. Did not respond to treatment (i.e., did not achieve a CR or PR), or
2. progresses following a response and is either not eligible for re-treatment or chooses not to pursue re-treatment, or
3. proceeds to alternative disease directed therapy,

The subject will subsequently be followed for survival, subsequent therapy, and long term gene therapy safety including RCL sampling. Correlative tests sampling may continue as per protocol at the discretion of the investigator. Long-term follow up laboratory evaluations and blood

collections may be completed by the outside medical facility/care provider.

Should a subject fail to return to the clinic or respond to contact for a scheduled protocol specific visit/contact, sites will need to make 2 attempts by a combination of telephone 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.

5.5.5.1 Long Term Gene Therapy Follow-up Schedule

- Month 6 (± 1 month), Month 12 (± 2 months) after CD22-CAR T cell infusion
- Every 12 months (± 2 months) for 5 years
- Beginning with year 6 (± 3 months), subjects will return to the clinic or be contacted by phone, e-mail or mail one time annually for up to 15 years after cell infusion.

5.5.5.2 Long Term Gene Therapy Follow-up Evaluations

The following procedures will be completed for all subjects who are enrolled and received CD22-CAR T cells at each Long Term Gene Therapy Follow Up visit:

- Physical exam, vital signs and performance status for year 1 through 5 (may be performed by the local physician)
- Gene Therapy Safety Monitoring to specifically document:
    - any new malignancies,
    - new incidence or exacerbation of a pre-existing neurologic disorder,
    - new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder,
    - new incidence of a hematologic disorder; and
    - other factors that may be relevant to the feasibility and scientific value of the long-term follow up observations for gene therapy toxicities (i.e. exposure to other cancer causing agents).
- RCL testing as described in **Section 13.4**
- Annual Gene Therapy Questionnaire (see **Section 13.5**) for year 6 through 15
- Laboratory evaluations as clinically indicated

5.5.6 Evaluations of Subjects Who Do NOT Receive CD22-CAR T cells

The following procedures/assessments will be completed for subjects who are enrolled but do not receive CD22-CAR T cells, at the time points outlined in the Section 9 Study Calendar until disease progression and/or toxicities attributable to the study participation resolve to ≤ Grade 1 or stabilize:

- Disease assessment per standard of care
- Adverse/Serious Adverse Event reporting and concomitant medication documentation until 30 days after last study treatment (e.g., leukapheresis, conditioning chemotherapy).

Should the subject fail to return to the clinic for a scheduled protocol specific visit, sites will need to make 2 attempts by a combination of telephone 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.

5.6 OPTION FOR ADDITIONAL DOSE(S) OF CD22-CAR T CELLS

On the day cells are infused, remaining cells that have been produced above and beyond the number of cells needed for a subject's dose level will be cryopreserved using standard techniques. Cells will be reserved for 1-2 additional doses with corresponding QA retention vials, and remaining cells will be released for research.

Subjects will have the option for additional infusions of CD22-CAR T cells (including preparative chemotherapy regimen, toxicity assessment and research blood sampling) if the following criteria are met:

5.6.1 Eligibility Criteria for subsequent cell infusions:

- ✓ Cell infusion of CD22-CAR T cells has been deemed safe. Dose escalation cohort for subjects with aggressive B-cell NHL has been completed without 2 subjects in a disease group experiencing DLTs. At least 3 subjects with ALL have completed the 28-day DLT observation period at the proposed dose level with DLT rate ≤30%.
- ✓ Response to previous infusion. Subjects who had a PR or SD with clinical benefit may elect to receive another infusion of cells. Subjects that initially had a CR may only receive a second dose if evaluable disease recurs. Clinical benefit is indicated by an improvement in the subject's health status (e.g., decreased transfusion requirement, improved cytopenias, decrease in number of blasts not sufficient to reach a PR, improved performance status or quality of life, etc.).
- ✓ At least 60 days have passed since the previous cell infusion.
- ✓ Circulating levels of CD22-CAR T cells must be < 5% by flow cytometry.
- ✓ Any toxicity (regardless of causality) after the previous CD22-CAR T cell infusion must resolve such that subjects meet all the initial eligibility criteria for major organ function and therapy washout as outlined in **Section 3.1**.
- ✓ An adequate number of cryopreserved CD22-CAR T cells and QA retention vials or an adequate number of apheresis product sufficient to generate an additional dose of CD22-CAR T cells must be available.

The cell dose (based on CAR transduced cells) for the second infusion shall not be greater than the current dose level completed or the safe dose if this has been determined.

As a rule, subjects who incurred DLT after receiving the first cell infusion will not be eligible to receive additional cell infusions unless IRB, APB and FDA approval is granted on a case by case basis.

5.6.2 Procedures for Additional Doses

Subjects may receive additional antineoplastic and lymphodepleting chemotherapy prior to the second infusion of CD22-CAR T cells.

Subjects must meet the eligibility criteria as described in **Section 5.4.2.1**. The subject's cell product will be tested for cell viability to confirm it still meets the IND product release criterion prior to the subject starting lymphodepletion chemotherapy. The lymphodepletion chemotherapy dosing used for lymphodepletion prior to additional doses may differ from the first infusion, at investigator's discretion. Cell infusion and evaluations after an additional dose of CD22-CAR will be the same as described above for the initial dose.

Any subject who receives subsequent doses of CD22-CAR T cells will NOT be evaluable for toxicity purposes of this study as they will be beyond the 28 day observation period for DLTs after their first cell infusion. However post-infusion monitoring will be the same as for the 1st infusion and all toxicities, including secondary reactions, will be recorded and reported. If two or more subjects develop Grade 4 toxicity at any time following the second CD22-CAR T cell infusion that is felt to be possibly, probably or likely related to the CD22-CAR T cells, then second infusions will be paused pending discussion with the FDA, IRB and APB regarding continuing second infusions as part of the experimental regimen.

5.6.2.1 Gene Therapy Follow Up After Additional Doses

If a subject receives more than one infusion of CD22-CAR T cells, the timing of gene therapy long term evaluations is restarted with each subsequent gene therapy administration. For example, a subject receives gene therapy and has undergone long term follow up for 1.5 years, develops progressive disease and is treated with a second gene therapy product. That subject will begin the blood sample collection at 3, 6 and 12 months (± 1 month) post cell administration and continue for a total of 15 years of follow up from the LAST gene therapy administration. The most recent RCL blood evaluation after the previous dose of CD22-CAR T cells will serve as the baseline RCL for the second dose; therefore, a second baseline RCL does not need to be repeated prior to additional doses of CD22-CAR T cells.

6 INVESTIGATIONAL AGENT AND COMMERCIAL DRUG INFORMATION

6.1 AUTOLOGOUS T CELLS TRANSDUCED WITH LENTIVIRAL VECTOR (CD22.BB.Z) CHIMERIC ANTIGEN RECEPTOR (CD22-CAR); FOLLOWING LYMPHODEPLETING CHEMOTHERAPY

6.1.1 Description

Cell therapy production will be conducted according to the appropriate manufacturing facility's SOPs and must meet the requirements for a GMP facility. The CD22-CAR replication incompetent lentiviral vector has been manufactured by Lentigen Technology Incorporated. All procedures will take place using good manufacturing process guidelines.

6.1.2 Stability

Stability testing is ongoing.

6.1.3 Administration

Guidance on administration of CD22-CAR T cells is specified in **section 5.4.2.**

6.1.4 Toxicities

In addition to standard transfusion types of reactions (chills, fever, rigors), the toxicities specific to CAR T cell therapy are described in detail in section **2.4**. Every effort will be made to mitigate the risk of these toxicities with the intensive monitoring plans outlined in section 5 and the supportive care measures outlined in section **4.2**. With growing experience with CAR therapy world-wide, the risk of severe neurotoxicity is increasingly better recognized—most notable with the recent occurrences of fatal neurotoxicity. With the incorporation of mandatory anti-seizure prophylaxis and prospective monitoring of neurologic function, we plan to rigorously monitor and evaluate for any neurotoxicity, with a plan to treat more severe neurotoxicity with steroids to mitigate the symptoms.

6.2 FLUDARABINE

(Refer to FDA-approved package insert for complete product information)

6.2.1 Description

Fludarabine phosphate is a synthetic purine nucleoside that differs from physiologic nucleosides in that the sugar moiety is arabinose instead of ribose or deoxyribose. Fludarabine is a purine antagonist antimetabolite.

6.2.2 How Supplied

Fludarabine will be obtained from commercially available sources by the Stanford Health Care Pharmacy Department. Drug is supplied in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.

6.2.3 Stability

Following reconstitution with 2 mL of sterile water for injection to a concentration of 25 mg/ml, the solution has a pH of 7.7. The fludarabine powder is stable for at least 18 months at 2-8^{0}C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Because no preservative is present, reconstituted fludarabine will typically be administered within 8 hours. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribonucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

6.2.4 Storage

Intact vials should be stored refrigerated (2-8°C).

6.2.5 Administration

Fludarabine is administered as an IV infusion in an appropriate solution over 30 minutes as described in Section **5.3.**

6.2.6 Toxicities

The primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in subjects treated with fludarabine. Hemolytic anemia has been reported after one or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fever, chills, fatigue, anorexia, nausea and vomiting, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL.

6.3 CYCLOPHOSPHAMIDE

(Refer to FDA-approved package insert for complete product information)

6.3.1 Description

Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkyating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after IV administration ranges from 3-12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

6.3.2 How Supplied

Cyclophosphamide will be obtained from commercially available sources by the Stanford Health Care Pharmacy Department.

6.3.3 Stability

Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8^{0}C.

6.3.4 Administration

It will be diluted in an appropriate solution and infused as described in Section **5.3.**

6.3.5 Toxicities

Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of subjects; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be

lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as a uroprotective agent in subjects receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all subjects. Subjects who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in subjects with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, subjects should be watched closely for cyclophosphamide toxicity (due to allopurinal induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophosphamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis, but is not a required premedication for this study.

6.4 CLOFARABINE INJECTION (CLOLAR ®)

(Refer to FDA-approved package insert for complete product information)

6.4.1 Description:

Clolar (clofarabine) injection is a purine nucleoside metabolic inhibitor indicated for the treatment of pediatric patients 1 to 21 years old with relapsed or refractory acute lymphoblastic leukemia after at least two prior regimens. The terminal half-life after IV administration was 5.2 hours.

6.4.2 How Supplied:

Clofarabine will be obtained from commercially available sources by the institution's Pharmacy Department. Clofarabine is supplied in single-use flint vials containing 20 mg of clofarabine in 20mL of solution.

6.4.3 Stability:

Vials containing undiluted clofarabine should be stored at 25°C (77°F); excursions permitted to 15-30°C (59-86°F). Following reconstitution as directed clofarabine should be infused with 24 hours of preparation.

6.4.4 Administration:

Clofarabine should be filtered through a sterile 0.2 micron syringe filter and then diluted with 5% Dextrose Injection, USP, or 0.9% Sodium Chloride Injection, USP, prior to IV infusion to a final concentration between 0.15 mg/mL and 0.4 mg/mL.

6.4.5 Toxicities

Clofarabine causes myelosuppression which may be severe and prolonged. Febrile neutropenia occurred in 55% and non-febrile neutropenia in an additional 10% of pediatric patients in clinical trials, increasing the risks of hemorrhage and infections. In addition hyperuricemia (tumor lysis), systemic inflammatory response syndrome (SIRS) and capillary leak syndrome, as well as venous occlusive disease of the liver (VOD) had been described. Additional toxicities are summarized in Section **2.3**.

6.5 BENDAMUSTINE HYDROCHLORIDE FOR INJECTION (BENDEKA ®)

(Refer to FDA-approved package insert for complete product information)

Bendamustine is contraindicated in patients with a known hypersensitivity (e.g., anaphylactic and anaphylactoid reactions) to bendamustine or mannitol.

6.5.1 Description:

Bendamustine is an alkylating drug which is FDA approved for treatment of patients with CLL and NHL that has progressed during or within six months of treatment with rituximab or rituximab-containing regimen. The mean terminal elimination $t_{1/2}$ of M3 and M4 are approximately 3 hours and 30 minutes respectively.

6.5.2 How Supplied:

Bendamustine will be obtained from commercially available sources by the institution's Pharmacy Department. Bendamustine for Injection is supplied in individual cartons of 5 mL clear multiple-dose vials containing 100 mg of Bendamustine hydrochloride as a clear, and colorless to yellow ready-to-dilute solution.

Dilute with either 0.9% Sodium Chloride Injection, USP, or 2.5% Dextrose/0.45% Sodium Chloride Injection, USP, or 5% Dextrose Injection, USP. The resulting final concentration of bendamustine hydrochloride in the infusion bag should be within 0.49 mg/mL to 5.6 mg/mL. After transferring, thoroughly mix the contents of the infusion bag. The admixture should be a clear, and colorless to yellow solution.

6.5.3 Stability:

Bendmustine Injection should be stored in the refrigerator 2°C – 8°C (36°F - 46°F). Retain in original package until time of use to protect from light.

BENDEKA is supplied in a multiple-dose vial. Although it does not contain any antimicrobial preservative, BENDEKA is bacteriostatic. The partially used vials are stable for up to 28 days when stored in its original carton under refrigeration (2°C to 8°C or 36°F to 46°F). Each vial is not recommended for more than a total of six (6) dose withdrawals. After first use, store the partially used vial in the refrigerator in the original carton at 2°C to 8°C or 36°F to 46°F and then discard after 28 days Administration:

6.5.4 Toxicities

Bendamustine causes myelosuppression which may be severe. In two NHL studies, 98% of

patients had Grade 3-4 myelosuppression; 3 patients died from myelosuppression-related adverse reactions- one each from neutropenic sepsis, diffuse alveolar hemorrhage with Grade 3 thrombocytopenia and pneumonia from an opportunistic infection (CMV). Infusion reactions occurred commonly and included fever, chills, pruritus and rash. Anaphylaxis occurred rarely, particularly in second or subsequent cycles of therapy. Tumor lysis syndrome and other skin reactions have also been reported. Additional toxicities are summarized in Section **2.3.**

6.6 PRE-MEDICATIONS

6.6.1 Acetaminophen (Tylenol)

Will be given as a pre-medication. This agent will be provided by the institution's pharmacy. Please refer to the package insert for complete pharmaceutical information on this product.

6.6.2 Diphenhydramine (Benadryl)

Will be given as a pre-medication IV or orally. This agent will be provided by the institution's Pharmacy Department. Please refer to the package insert for complete pharmaceutical information on this product.

6.6.3 Antimicrobial Prophylaxis

Subjects will receive appropriate antimicrobial prophylaxis (e.g., Bactrim for PCP and acyclovir for HSV and VZV prophylaxis) during lymphodepleting chemotherapy and following treatment as per institutional guidelines. This agent will be provided by the institution's Pharmacy Department. Please refer to the package insert for complete pharmaceutical information on this product.

6.6.4 Levetiracetam (Keppra)

Adult subjects will receive levetiracetam 500 mg orally twice a day (maximum dose of 750 mg/dose) or as per institutional guidelines at the discretion of the treating physician beginning the day before cell infusion (Day -1). This dose may be adjusted based on symptoms of neurotoxicity and/or with recommendations of neurologic consult. Levetiracetam should continue through Day 28 once neurotoxicity develops. This agent will be provided by the institution's Pharmacy Department. Please refer to the package insert for complete pharmaceutical information on this product.

6.6.5 Allopurinol

Subjects with high disease burden at risk for tumor lysis may be given allopurinol as per standard supportive care as per the investigator's discretion. Allopurinol will be administered at 300 mg orally once daily, starting at the time of initiation of the conditioning lymphodepletion chemotherapy regimen. This agent will be provided by the institution's pharmacy. Please refer to the package insert for complete pharmaceutical information on this product.

7 ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 POTENTIAL ADVERSE EVENTS

7.1.1 CD22-CAR T cells

Because these cells have been previously administered in only a small number of humans, there may be unanticipated adverse events.

Section 2.4 and **Section 6.1** discuss the potential risks of this investigational therapy based on previous studies with this or similar preparative regimens or cell products, including the risks of chemotherapy, risk of autoimmunity, risk of neurotoxicity, risk of the gene therapy component, and risk of cytokine storm. Transient, reversible mild effects have been observed with the administration of fresh cells, including chills, fever, rigors, diaphoresis, anorexia, nausea, diarrhea, headache and myalgias. General guidance for treatment of the most common toxicities are included in **Section 4.2**.

7.1.2 Risk of Apheresis:

Apheresis is a safe procedure that is routinely performed in healthy adults. Participants will be closely monitored and procedures to minimize risks and prevent side effects are incorporated into all aspects of the protocol. The institutions have broad expertise to adequately manage side effects. The potential risks of apheresis in this trial are as follows:

a. The most common side effects of apheresis are pain and bruising at IV sites. A central venous catheter may be required. Possible side effects include pain, bleeding, bruising, infection, thrombosis, vascular perforation.

b. During apheresis, mild side effects from citrate anticoagulant are common and include chills, numbness and tingling ("pins and needles"), anxiety, muscle cramps, and nausea. More serious side effects due to citrate-induced hypocalcemia are uncommon and include low blood pressure, seizures, weakness, and tetany. Citrate reactions rapidly resolve when the collection is slowed down or stopped. Prophylactic IV $CaCl_2$ and $MgSO_4$ infusions may be administered to subjects deemed to be at high risk of citrate toxicity. Risks of parenteral calcium and magnesium include extravasation necrosis and cardiovascular effects including bradycardia and blood pressure changes. However, side effects are unlikely given the low rate of infusion and use of large bore catheters for apheresis.

c. Transient mild thrombocytopenia is common after apheresis, but bleeding is unlikely.

d. Dilutional anemia occurs during apheresis, but this is unlikely to be clinically significant.

e. Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

7.2 ADVERSE EVENT DEFINITIONS

7.2.1 Adverse Event

An adverse event (AE) is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the subject, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All adverse events will be graded according to NCI CTCAE v5.0.

If a subject requires additional disease directed therapy after completing apheresis but before the start of lymphodepletion chemotherapy, these toxicities will not be considered study related adverse events and will NOT be collected or reported. Toxicities present at the initiation of lymphodepletion chemotherapy will be considered baseline conditions.

An abnormal laboratory value will be considered a reportable, recordable AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study therapy
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention, except for Grade 1 electrolyte changes or prophylactic therapies
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- Is a hematologic abnormality, including WBCs, hemoglobin, neutrophils, and platelets that constitutes a change in grade or attribution from baseline (e.g. worsens from baseline Grade 1 to Grade 2) during the first 28 days, or Grade 3 or 4 greater than 28 days post CD22-CAR T infusion.

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action(s) taken and about the subject's outcome.

7.2.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the investigational therapy caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the investigational therapy and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.2.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the protocol or informed consent documents or is not listed at the specificity or severity that has been observed; or is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the protocol or informed consent documents as

occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.2.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.2.5 Serious Adverse Event

An AE or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- In-patient hospitalization or prolongation of existing hospitalization
  - Note: Hospitalizations related to monitoring of expected CRS symptoms, scheduling issues (e.g. evening or weekend evaluations), and convenience considerations do not constitute a serious adverse event.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the subject or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Deaths occurring after the 30 day window due to disease progression or causes unrelated to study interventions will not be recorded as an SAE, but will be documented as a study outcome as per **Section 12.1.2**.

7.2.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.2.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the subject or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.2.8 Protocol Deviation

Any change, divergence, or departure from the IRB and APB-approved research protocol.

7.2.9 Non-compliance

The failure to comply with applicable IRB requirements, FDA or other regulatory requirements for the protection of human research subjects.

7.2.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document, or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.3 ADVERSE EVENT REPORTING

Both Serious and Non-Serious Adverse Events will be clearly noted in source documentation and listed on study specific AE logs. The Principal Investigator or designee will assess each AE to determine whether it is unexpected according to the Informed Consent, Protocol Document, or related to the investigation. All AEs will be tracked until resolution or until 30 days after the last study treatment, whichever is later.

All adverse events (as defined in **Section 7.2.1**) will be recorded and reported from the start of lymphodepletion chemotherapy and conclude 30 days after the last study treatment. Prior to the start of lymphodepletion chemotherapy, only unexpected serious adverse events related to study procedures will be collected. In the event that a subject requires disease directed therapy after undergoing leukapheresis but prior to commencing lymphodepletion chemotherapy, these adverse events and any adverse events unrelated to participation in this study will not be collected or reported.

Serious adverse events that occur more than 30 days after the last administration of study treatment and have an attribution of at least possibly related to the agent/intervention should be recorded and reported in the AE log.

In addition, any suspected adverse events that occur after 30 days, during the initial 5 years of follow up, will also be recorded and reported (section **5.5.5**). Suspected adverse events occurring in subjects who received cell therapy that are potentially related to the gene therapy nature of this study will be reported at the time of the investigator notification of their occurrence during 15 year follow up (Section **5.5.5.2**).

After Day 30 and for 24 months or until disease progression (whichever occurs first), targeted AE collection of neurological, hematological (Grade 3 or higher), infections, autoimmune disorders, and secondary malignancies will be performed if attributed as possibly, probably or definitely related to CD22-CAR.

7.3.1 Stanford Reporting

Serious Adverse Events (SAEs) (as defined in section **7.2.4**) Grade 3 and above, and all subsequent follow-up reports will be reported to the Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC), regardless of the event's relatedness to the investigation, for institutional

reporting purposes.

All SAEs Grade 3 and above attributed possibly, probably or definitely related to CD22-CAR T cells, and unexpected, will be reported to the APB using eProtocol within 10 working days of determination, or within 5 working days for deaths or life-threatening experiences. Any positive sterility results during manufacture, including prior to final product formulation, will be reported to the APB using eProtocol within 5 working days.

Events meeting the IRB definition of 'Unanticipated Problem' (as determined by the Principal Investigator) will be reported to the IRB using eProtocol within 10 working days of determination, or within 5 working days for deaths or life-threatening experiences.

APB and IRB will be notified via an expedited safety report if any stopping rules are triggered, as described in **Section 12.3**.

All adverse events will be reported to IRB and APB at annual continuing review.

7.3.2 IND Sponsor Reporting Criteria

The Principal Investigator must **immediately** (within 24 hours of knowledge of event) report to the IND Sponsor, using the study-specific form or MedWatch form, any serious adverse event, whether or not considered drug related or expected. The investigator must include an assessment of whether there is a reasonable possibility that the CD22-CAR T cells caused the event. The Principal Investigator is responsible for promptly providing the Sponsor with any information needed to determine whether the SAE requires submission of an IND Safety Report.

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study therapy and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

Non-serious adverse events must be recorded in a timely manner and reported to Sponsor at IRB continuing review and in IND Annual Reports.

Events will be submitted to Sponsor's Medical Representative, at:

David Miklos, M.D., PhD

Associate Professor of Medicine (Blood and Marrow Transplantation)
Clinical Director Cancer Cell Therapy
Medical Director of Stanford Cellular Therapeutics and Transplantation Laboratory
269 West Campus Dr, CCSR 2205, M/C 5623
Stanford, California, 94305-5623
650-452-8155

7.3.3 Reporting Pregnancy

- ✓ **Maternal exposure**

If a female subject becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor and the Stanford regulatory authorities (IRB, APB, DSMC). The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form

"Describe Event or Problem".

Pregnancy itself is not regarded as an AE unless there is a suspicion that the study treatment under study may have interfered with the effectiveness of a contraceptive medication. However, as subjects who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the Sponsor is requesting that pregnancy occurring while subject still has detectable CD22-CAR T cells in peripheral blood should be reported in an expedited manner as **Grade 3** "***Pregnancy, puerperium and perinatal conditions - Other (pregnancy)***" under the ***Pregnancy, puerperium and perinatal conditions***.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs within 4 months of last dose of lymphodepletion chemotherapy or while CD22-CAR T cells are detectable in peripheral blood, then the investigator should inform the Sponsor **immediately** (within 24 hours of knowledge of event).

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies. The same timelines apply when outcome information is available.

- ✓ **Paternal exposure**

Male subjects should refrain from fathering a child or donating sperm for 4 months after the last dose of lymphodepletion chemotherapy and while CD22-CAR T cells are detectable in peripheral blood.

Pregnancy of the subject's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring during the reporting period should, if possible, be followed up and documented.

7.4 FDA Reporting Criteria

7.4.1 IND Safety Reports to the FDA (Refer to 21 CFR 312.32)

The Sponsor will be responsible for reporting to the FDA any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information using the MedWatch Form 3500a.

7.4.2 FDA Annual Reports (Refer to 21 CFR 312.33)

The Sponsor will submit the Annual Report to the FDA according to 21CFR§312.33, and any associated FDA correspondences regarding the IND annual report.

7.4.3 Serious Adverse Event Reporting on Cell Therapy Products to the FDA

A sample from all products that are non-conforming or do not meet release specifications will be

used to conduct an out of specification investigation and the remainder either disposed of according to our facility biohazardous material disposal SOP or the FDA will be contacted by the manufacturing team to determine whether the product is suitable for infusion. The manufacturing facility will report all products manufactured including those that did not meet release criteria or were otherwise not infused in the annual IND report to the FDA.

All HCT/P deviations involving 351 cell products will be reported using MedWatch Form 3500a according to FDA publication "Guidance for Industry: MedWatch Form FDA 3500A: Mandatory Reporting of Adverse Reactions Related to Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) available at:
http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm074000.htm.

7.4.4 Action Plan for Positive Results on Cell Product Safety Testing:

In the unlikely event that a positive sterility test or mycoplasma test result is obtained after distribution of a cell product that has been administered, or after administration of the product to the subject, the following steps will be initiated IMMEDIATELY:

a. Clinical Laboratory personnel will notify Director of Manufacturing and Process Development of the test results.
b. Director of Manufacturing and Process Development will notify the Principal Investigator who will be updated with any substantive changes, including the final report on the identification and sensitivity from the positive sterility test. The manufacturer's QS Director or designee will determine the need for quality improvement based on the nature and extent of the incident.
c. If Director of Manufacturing is unable to reach Principal Investigator within 15 minutes, contact inpatient attending physician caring for the subject on the hospital service via hospital page with direct communication. NOTE: The Principal Investigator and/or designee will contact the attending physician, who will determine the extent of the work-up of a positive culture in consultation with appropriate infectious disease consultants, as well as determine an appropriate action treatment plan.
d. The Principal Investigator and/or attending physician will discuss the positive results with the subject, and specify the clinical therapy, antibiotic regimen and/or monitoring plan.
e. A contaminated sample of a product that has been administered to a subject will be handled in the same fashion as a Grade 4/5 toxicity. The Principal Investigator will be responsible for notifying the IRB and APB via an Unanticipated Problem (UP) report within 5 working days, and the Sponsor will notify the FDA via an expedited 7-day IND Safety Report.

In addition to the above, appropriate Safety reporting will be done as per the manufacturing facility's SOP for Sterility Testing . A sample of each product is retained by Quality Systems and will be sent to the Microbiology Laboratory for repeat testing and speciation. An Out-of-Specification (OOS) Investigation will be conducted by the Quality Systems staff of the manufacturing laboratory including root cause analysis, review of viable environmental monitoring results collected at the time of manufacturing on personnel, equipment and reagents. Whether or not attribution is established, a formal Corrective and Preventive Action plan will be issued by the manufacturing facility and appropriate remediation will be performed including

retraining of manufacturing personnel, elimination of any contaminated reagents and re-cleaning of the production facility followed by viable microbiological monitoring to establish effectiveness of cleaning.

8 CORRELATIVE/SPECIAL STUDIES

CAR T cell therapy targeting a single tumor associated antigen has mediated striking remissions in B cell leukemia and aggressive B-cell NHL; however, the complex interplay of tumor, T cell and intrinsic CAR properties that influence these outcomes are not well understood. The clinical experience to date has demonstrated variable patterns of tumor remission and CAR T cell efficacy and persistence. We aim to utilize this study as an opportunity to collect correlative data that will permit extensive study of both the B cell and T cell compartment prior to and following CAR T cell therapy. We aim to integrate multi-dimensional technologies to permit complex analyses of the apheresis product, the CAR T cell product pre-infusion and *in vivo* expanded CAR T cells following antigen encounter. We additionally aim to investigate properties of B cell tumors that render them susceptible to CAR T cell cytotoxicity and study physiologic and malignant B cell remodeling under the pressure of multi-targeted CAR therapy.

Overall goal of study correlatives:

- Explore the T cell and tumor properties that influence CAR T cell efficacy and persistence and permit disease relapse

Specific Aims:

- Assess the impact of T cell subset composition as delineated using flow cytometry, mass cytometry and ATAC-Seq on CAR T cell expansion and persistence
- Where possible, use TCR sequencing to fate map cells contained in the apheresis of manufactured product to persistent CD22-CAR T cells as an exploratory aim to identify subsets with a greater likelihood of T cell persistence in the setting of adoptive cell therapy.
- Characterize antigen expression on relapsed B-ALL or aggressive B-cell NHL following CAR-mediated targeting
- Utilize the CAR-mediated arrest of B cell maturation to gain insight on physiologic and pathophysiologic B cell development, maturation arrest and alternative survival pathways in normal and malignant B cells.
- Explore the level CD22 surface expression and CD22 site density on leukemic blasts, and malignant lymphoma cells when feasible and correlate with clinical response to CAR T cells.
- To establish the utility of chromatin structure and epigenomic technology to characterize CAR T cell therapies.

8.1 CORRELATIVE STUDIES

8.1.1 Sample Collection Schedule

The samples to be collected and schedule for sample collection is detailed in **Section 13.7** Appendix G: Correlative Sample Schedule . Every effort will be made to collect correlative samples as described in Appendix G, but changes in tests, schedules, sample volume limitations and/or conduct of apheresis, and conflicts with schedules or patient conditions may result in missed or altered sample collection. These variations will be noted but will NOT constitute a protocol

deviation given the exploratory nature of correlative testing. At the discretion of the principal investigator, correlative samples may be omitted if not applicable to the subject's disease or not in the best interest of the subject. Omitted correlative samples for these reasons should not be considered a protocol deviation.

8.1.2 Blood Drawing Limits for Research Purposes

The maximum amount of blood that may be drawn per day from adult subjects is 2.5% of total blood volume (192-200 mL). The volume to be drawn per day for research purposes will comply with institutional policy. Subjects may undergo a small volume apheresis (approx. one to two blood volumes) in lieu of standard blood draw to obtain peripheral blood lymphocytes for correlative studies including Day 28 (± 1 week), and Month 3 (± 2 weeks), 6 (± 1 month), 9 (± 1 month), and 12 (± 2 months).

8.2 MONITORING GENE THERAPY TRIALS: PERSISTENCE, AND RCL, AND TOXICITY

8.2.1 Monitoring Persistence

Due to the nature of these studies, it is possible that expansion of specific T-cell clones will be observed as tumor reactive T-cells proliferate in response to tumor antigens. Therefore, care will be taken to track T-cell persistence both immunologically and molecularly by flow cytometry using anti-CD22 anti-idiotype antibody at the time points listed in **Section 13.7**.

8.2.1.1 Persistence of genetically engineered transduced cells

If any subject has more than 5% persistence of gene transduced cells at Month 6, samples will be subjected to flow cytometry using anti-CD22 anti-idiotype antibody that would allow the identification of clonality of persisting gene transduced cells. Such techniques may include analysis of BV chain expression, T cell cloning or LAM-PCR. If a predominant or monoclonal T cell clone derived from gene transduced cells is identified during the follow-up, the integration site and sequence will be identified and subsequently analyzed against human genome database to determine whether the sequences are associated with any known human cancers. If a predominant integration site is observed, the T cell cloning or LAM-PCR test will be used at an interval of no more than 3 months after the first observation to see if the clone persists or is transient. In all instances where monoclonality is persistent and particularly in instances where there is expansion of the clone, regardless of whether or not the sequence is known to be associated with a known human cancer, the subject should be monitored closely for signs of malignancy, so that treatment, if available, may be initiated early.

8.2.2 Safety Assessment Testing for Replication Competent Lentivirus in Lentiviral Vector Based Gene Therapy Products during Subject Follow up

In compliance with OSP and FDA's ***Guidance for Industry: Gene Therapy Clinical Trials – Observing Participants for Delayed Adverse Events (2006)***, subjects who have received at least one dose of a genetically engineered cellular therapy will be evaluated for long term safety and occurrence of adverse events according to the requirements established by FDA guidance and the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines) (2016).

8.2.2.1 Replication-competent Lentivirus Evaluations

Blood samples will undergo analysis at Indiana University Gene Therapy Testing Laboratory (IU GTTL) for detection of RCL by detection of p24 antigen and reverse transcriptase activity (Product Enhanced Reverse Transcriptase [PERT]) at the following time points:

- prior to cell infusion (at baseline)
- at Month 3 (±1 month),
- at Month 6 (± 1 month), and
- at Month 12 (± 2 months) post cell administration.

Samples sent to IU GTTL MUST be drawn on Monday, Tuesday, Wednesday or Thursday, and shipped on ice priority overnight. The lab cannot accept samples on the weekends (see **Section 13.4**). If all samples in the first year are negative, annual blood samples will be archived for the next 4 years (± 2 months) (for a total of 5 years after cellular therapy) with a brief clinical history conducted annually. If any post-treatment samples are positive, further analysis of the RCL and more extensive subject follow-up will be undertaken, in consultation with the FDA.

If a subject dies or develops neoplasms during this follow up, efforts will be made to assay a biopsy sample for RCL. Subjects who receive additional CD22-CAR T cell infusions will re-start RCL testing based on the new infusion date.

9 STUDY CALENDAR

			Preparative Regimen and Cell Infusion			Post Treatment Assessment				Long Term Follow-Up[l]		
Procedure	**Screening[a] (≤ 28 days of enrollment, unless specified)**	**Apheresis[d]**	**Day -5, Day -4, Day -3**	**Prior to cell infusion (< 24 h)**	**Day 0**	**Day 1 to Day 14[i] (± 2 d)**	**Twice weekly, Day 15 to Day 27 (± 4 d)**	**Day 28 (± 4 d)**	**Monthly, Month 2-3 (±2 weeks)**	**Month 6 (±1 mo) 9, (±1 mo) 12 (±2 mo) q6-12 months to 5 years**	**Annually, Year 6 to 15 (±3 mo)**	**At Disease Progression**
Medical History	X											
Physical Exam	X			X	X	X[i]	X[i]	X	X	X		
Vital signs	X	X	X	X	X[f]	X[f]	X[f]	X	X	X		
Neurologic exam				X	X[h]	X[h]	X[h]	X[h]	X[h]	X[h]		
Performance Status	X							X	X	X		
Height	X		X[r]									
Weight	X	X	X	X				X	X			
Labs[c]												
• CBC with diff	X	X[d]	X	X	X	X[i]	X[i]	X	X	X		
• PT/PTT	X											
• Chemistries[b]	X	X[d]	X	X	X	X[i]	X[i]	X	X			
• Phosphorus	X	X[d]	X[r]		X	X[t]	X[t]					
• Magnesium	X	X[d]	X[r]	X	X	X[t]	X[t]					
• CRP	X	X[d]	X[r]	X	X	X[t]	X[t]	X				
• Ferritin	X		X[r]			X[t]	X[t]	X				
• Uric acid and LDH			X[r]		X[t]	X[t]	X[t]					
• CD22 staining by IHC or flow cytometry	X[p]											
• Quantitative IgG immunoglobulins								X[m]	X[m]			
• Urine analysis	X											
• β-HCG pregnancy test on all females of child-bearing potential	X	X[c]	X[c]									
• RCL blood sample				X[n]					X[n]	X[n]		
ECG	X											
ECHO, MUGA or cardiac MRI	X[q]											
Correlative Research Studies	Correlative samples outlined in Section 13.7, Appendix G											
• Leukapheresis		X						X[j]	X[j]	X[j]		

			Preparative Regimen and Cell Infusion			Post Treatment Assessment				Long Term Follow-Up[l]		
Procedure	**Screening[a] (≤ 28 days of enrollment, unless specified)**	**Apheresis[d]**	**Day -5, Day -4, Day -3**	**Prior to cell infusion (< 24 h)**	**Day 0**	**Day 1 to Day 14[i] (± 2 d)**	**Twice weekly, Day 15 to Day 27 (± 4 d)**	**Day 28 (± 4 d)**	**Monthly, Month 2-3 (±2 weeks)**	**Month 6 (±1 mo) 9, (±1 mo) 12 (±2 mo) q6-12 months to 5 years**	**Annually, Year 6 to 15 (±3 mo)**	**At Disease Progression**
Disease Evaluation												
• PET/CT chest/abdomen/pelvis, brain MRI (or other appropriate imaging)	X[k]		X[k]					X[k]	X[k]	X[k]		
• Lumbar puncture	X[k]							X[k]	X[k]	X[k]		
• Bone marrow aspirate	X[k]		X[k]					X[k]	X[k]	X[k]		X
• MRD blood sample (Subjects with ALL only)			X[k]					X[k]	X[k]	X[k]		
• Lymph node biopsy	X[s]					X[s]	→	X[s]				X[s]
Treatment Regimen												
•												
• Lymphodepleting chemotherapy			X[e]									
• CD22-CAR T cells infusion					X[g]							
Response Evaluation[k,l]								X	X	X	X	X
Adverse Events[v]		X	→	→	→	→	→	X	X[o]	X[o]		X[o]
Concomitant Medications	X	→	→	→	→	→	→	X	X[o]	X[o]		X[o]
Long term follow up questionnaires											X[u]	

a. Within 28 days prior to enrollment, unless otherwise specified.
b. Laboratory evaluation to include; Chemistries: Sodium (Na), Potassium (K), Chloride (Cl), Total CO_2 (bicarbonate), Urea nitrogen (BUN), Creatinine, Glucose, Calcium (Ca) total, ALT/GPT, AST/GOT, Alkaline Phosphatase, Bilirubin, Albumin, Total Protein); creatinine clearance may be performed if the serum creatinine is elevated. The PI may order additional tests in some subjects if needed to fully assess clinical status and obtain baseline results. Examples of such tests include: haptoglobin, soluble IL2R, immunoglobulin levels, and viral serology or PCR for cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV), and Epstein-Barr virus (EBV).
c. Within 7 days prior to leukapheresis procedure and Day -5.
d. For cell acquisition for product development. Visit may be skipped if subject has cryopreserved cells that meet IND criteria. Labs required within 7 days prior to leukapheresis.
e. Subjects will receive hydration and prophylactic medications according to institutional standard practice prior to, during and after lymphodepletion chemotherapy.
f. Vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry, temperature) collected on Day 0: prior to infusion, at the conclusion of the infusion (±10 min), 60 (±10 min) minutes after infusion, and as clinically indicated; then Day 1-28: every shift (± 2 hrs) during hospitalization, and with each visit after discharge.
g. Premedications will be provided as described in protocol **Section 6.6**. Subject may be offered additional CAR T cell treatments if enough cells were manufactured from the initial preparation, the subject experienced at least partial response to the first infusion, and the subject meets all eligibility criteria. Subsequent infusions will follow the same procedures as the first.
h. Neurologic exam daily and ICE score every shift (± 2 hrs) during hospitalization, then neurologic exam with each visit after discharge. After Day 28, continue until end of neurotoxicity or at investigator discretion.
i. Five (5) times per week from Day 1 to Day 14 (± 2 days) (unless otherwise indicated) with visits no more than 48 hours apart, and then twice weekly until Day 28 (± 4 days).
j. Subjects may undergo a small volume apheresis (approx. one to two blood volumes) in lieu of standard blood draw to obtain peripheral blood lymphocytes for correlative studies including Day 28 (±7 days), and 3 (± 2 weeks) , 6 (± 1 month) , 9 (± 1 month) , and 12 (± 2 months) months.
k. Subjects with leukemia will undergo bone marrow biopsy and patients with aggressive B-cell NHL will undergo PET/CT (or PET at investigator discretion) at screening, Day 28 (±4 days) and Month 3 (± 1 month), 6 (± 1 month), then per standard of care frequency. Disease evaluations may be repeated if >28 days passes between enrollment and start of lymphodepletion chemotherapy or if bridging anti-neoplastic therapy is used post-enrollment (per **Section 4.2.5**). Other methodologies including lumbar puncture, x-rays, MRI, MRD evaluation, lymphocyte subsets, etc determined by investigator based on sites and type of disease may be used; consistency throughout protocol evaluations is necessary. Day 28 will be considered the response evaluation for subjects with ALL. Month 3 will be considered the response evaluation for subjects with aggressive B-cell NHL.
l. Subject will be monitored by physical exams, disease evaluations, vitals, CBC with diff, Chemistries, and IgG levels until relapse or subject proceeds to other therapies at investigator's discretion; at which time Long-Term Gene Therapy follow-up will proceed as per **Section 5.5.5**
m. Every 3-12 weeks (±2 weeks) post infusion until Month 3.
n. RCL samples collected and sent to IU GTTL: prior to cell infusion; and at 3 (± 1 month), 6 (± 1 month), and 12 months (± 2 months) post cell infusion. Subsequent RCL blood samples may be stored annually for 4 additional years if all RCL in first year negative.
o. Targeted adverse/serious adverse event reporting and concomitant medication collection until Month 24, disease progression, or start of alternate therapy (whichever occurs first).
p. <u>For R/R ALL</u>: CD22 expression can be any time since diagnosis, but must be re-demonstrated if patient has received targeted anti-CD22 therapy. <u>For R/R aggressive B-cell NHL</u>: CD22 expression is not required. Subjects must have archival tissue available or must be willing to undergo a biopsy of easily accessible disease.
q. Testing performed within 180 days or after most recent anthracycline based treatment or mediastinal radiation therapy may be used for confirmation of eligibility
r. Height within 30 days prior to Day -5. Others collected once, Day-5 or Day -6 only.
s. If feasible and easily accessible, lymph node biopsy will be obtained from subjects with lymphoma at baseline, and once between Day 7 and Day 28. A PBMC sample (for CAR T cells, etc) and lymph node biopsy may be collected at the time of progression, prior to starting any subsequent anticancer therapy, if clinically feasible.
t. LDH testing only required if elevated at baseline (prior to lymphodepletion chemotherapy). Uric acid only at baseline (Day -5 or -6). Phosphorus, magnesium, CRP, Ferritin, and LDH daily while hospitalized during initial hospitalization, then Day 14 (± 3 days) and Day 21 (± 3 days).
u. After 5 years, health status data will be obtained from surviving subjects via telephone contact or mailed questionnaires. The long term follow up period for lentiviral vectors is 15 years.
v. All adverse events will be recorded and reported from the start of lymphodepletion chemotherapy and conclude 30 days after the last study treatment. Prior to the start of lymphodepletion chemotherapy, only unexpected serious adverse events related to study procedures will be collected (see **Section 7.3.1**).

10 MEASUREMENTS AND MONITORING

10.1 PRIMARY AND SECONDARY ENDPOINTS

10.1.1 Primary Endpoints:

The primary endpoint for feasibility is defined by the rate of successful manufacture of the CD22-CAR T cells produced with the Miltenyi CliniMACS Prodigy® system to satisfy the targeted dose level and meet the required release specifications within 7 or 10 days of manufacturing initiation.

In patients with relapsed/refractory aggressive B-cell NHL, a primary endpoint for safety is the maximum tolerated dose (MTD). The MTD is defined as the dose level with 0 DLTs out of 3 feasible patients or 1 DLT out of 6 feasible patients; in this study, the recommended phase 2 dose (RP2D) may not be the MTD as defined above. If the MTD is not found to be feasible, the dose below that is found to be safe and feasible will be the RP2D.

For both disease populations, primary endpoints for safety of CD22-CAR T cells as evidenced by the incidence and severity of dose limiting toxicities (DLT), adverse events, serious adverse events, laboratory abnormalities, changes in vital signs, and changes in physical examination following infusion of CD22-CAR T cells, recorded and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.

10.1.2 Secondary Endpoints

The secondary endpoints are the overall response rates per response criteria in adults with relapsed/refractory CD22 expressing B-cell ALL and adults with relapsed/refractory aggressive B-cell NHL (see below for response criteria for each disease population).

10.1.2.1 Response Criteria

- The response rate as measured by Response Criteria for ALL (Refer to **Section 13.3.1** Response Criteria for ALL)(*Modified from: Cheson BD, et al.)
  - Complete Response (CR)
  - Partial Response (PR)
  – - Hematological Activity
  - Stable Disease (SD)
  -Progressive Disease (PD)

- The response rate as measured by Response Criteria for Lymphoma (Refer to **Section 13.3.2** Response Criteria for Lymphoma):
  - Complete Response (CR)
  - Partial Response (PR)
  - No Response or Stable Disease (SD)
  - Progressive Disease (PD)

10.2 INSTITUTIONAL REVIEW OF PROTOCOL

The protocol, the proposed informed consent and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB, APB and Stanford Cancer Institute Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB and APB prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators.

10.3 DATA AND SAFETY MONITORING PLAN

10.3.1 Clinical Team

The clinical research team will meet on a regular basis during dose escalation and when subjects are being actively treated on the trial to discuss cell manufacturing, toxicities, eligibility questions, trial accrual, and treatment needs. Decisions about dose level enrollment and dose de-escalation if applicable will be made based on the toxicity data from prior subjects in each disease group. Members from cell processing facilities will join as needed.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB, DSMC, APB and to the Sponsor.

The principal investigator will review adverse event and response data on each subject to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

10.3.2 Data Safety Monitoring Committee

The Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) will be the monitoring entity for the clinical study. The DSMC members include at least four physicians, one oncology nurse, one oncology data manager and one biostatistician, and are selected in consultation with Stanford Cancer Institute leadership to cover expertise in areas such as medical oncology, radiation oncology, hematology, blood and marrow transplantation, and pediatric oncology. Members with additional expertise may be appointed on an ad hoc basis. The DSMC will audit study-related activities to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). The DSMC will review monthly any serious adverse events (Grade $\geq$3) and protocol deviations, and annually audit regulatory binders, patient eligibility, data entry, IRB compliance and all serious adverse events and adverse events associated with the research to ensure the protection of human subjects. Results of the DSMC audit will be communicated to the IRB and APB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

10.3.3 Safety Monitoring Committee

The clinical team has assembled an independent SMC consisting of Stanford and external academic investigators who are independent of the clinical trials under review and who are

knowledgeable in cellular therapies to review all serious adverse events (SAEs), subject deviations, and internal and external audit/monitoring reports on a more frequent basis. The SMC will confirm dose escalation decisions based on available AEs and dose limiting toxicity (DLT) determinations for each dose cohort, and determine whether the study needs to be halted based on protocol specified safety rules (**Section 12.3**).

10.4 DATA MANAGEMENT PLAN

Study data will be collected on paper or electronic case report forms (CRFs). This study will utilize a Medrio electronic clinical database for eCRFs. If there is a gap between study open to enrollment and Medrio database activation, paper CRFs will be utilized until the Medrio eCRFs are available. CRFs should summarize the clinical findings and observations necessary to ensure safety of participants on the study, and to document the study outcomes. All data will be kept secure. Paper study files will be kept in a locked, secure location. Electronic study data will be stored in encrypted, backed-up, password protected computers. Personal identifiers will not be used when collecting and storing data. An enrollment log will be maintained in the regulatory binder/file which is the only location of personal identifiers with unique subject identification number.

11 COLLABORATIVE AGREEMENTS

In order to track cell product throughout manufacture and back to the bedside for infusion, the cell manufacturer will be provided selected protected health information. This collaborative agreement is disclosed in the informed consent document.

A collaborative research agreement exists with Adaptive Biosciences, Inc. to exchange data and biospecimens for correlative studies on circulating tumor DNA.

Additionally, a collaboration with Syncopation Life Sciences will share clinical and manufacturing data as well as biospecimens.

12 STATISTICAL CONSIDERATIONS

The aims of this clinical trial are three-fold:

1. To evaluate the feasibility of manufacturing CD22-CAR T cellular products in the Miltenyi CliniMACS Prodigy® system across both diseases, ALL and aggressive B-cell non-Hodgkin lymphoma. The CliniMACS Prodigy® represents the next generation in automated cell processing, combining and streamlining cell processing workflows into one closed system.
2. To establish safety in two adult disease populations. CD22-CAR T cells have not been previously administered to subjects with aggressive B-cell non-Hodgkin lymphoma, and we will establish the maximum tolerated dose (MTD) or recommended Phase 2 dose (RP2D) of CD22-CAR T cells in this patient population. Simultaneously, we will seek to expand the prior pediatric and young adult experience with this CD22-CAR T cell agent in adults with CD22+ expressing B-cell ALL at the established dose of $1x10^6$ transduced T cells/kg. Once MTD/RP2D is determined in subjects with aggressive B-cell non-Hodgkin lymphoma, this disease population will undergo the same safety evaluation.

3. To evaluate in a preliminary fashion efficacy of CD22-CAR T cells in two disease populations, subjects with relapsed/refractory ALL and subjects with relapsed/refractory aggressive B-cell non-Hodgkin lymphoma.

The precedent for conducting clinical trials of this scope was established by the ongoing clinical trials CCT5001/IRB-41382 and CCT5007/IRB-41383 conducted by Stanford Center for Cancer Cell Therapy.

A formal statistical analysis plan (SAP) will be prepared and finalized before database lock for the final analysis for the study report. The SAP will provide details regarding the definition of analysis subjects (populations), analysis variables, and analysis methodology to meet all study objectives.

The principle and key elements of the SAP are provided as follows:

- In general, safety and efficacy data will be summarized for each disease group with descriptive statistics, including means, standard deviations, medians, minimums and maximums for continuous variables, the number of subjects and percent in each category for categorical variables.
- Data from each individual will be tabulated as appropriate. Efficacy and safety endpoints will be tabulated by dose cohort and time point.

12.1 PRIMARY OUTCOMES:

- Feasibility rate of successful manufacturing of CD22-CAR T cells for the designated dosage in each disease population, using the Miltenyi CliniMACS Prodigy® system.
- DLT rate and maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D) of CD22-CAR T cells in adults with relapsed/refractory aggressive B-cell NHL.
- DLT rate of an established dose of CD22-CAR T cells in adults with relapsed/refractory CD22 expressing B-cell ALL

12.1.1 Feasibility

Feasibility will be defined as the successful manufacturing of CD22-CAR T cells that meet established release criteria to satisfy the targeted dose level. Although we anticipate reaching the targeted cell dose during manufacture, feasibility of manufacturing cells remains a primary objective in this subject population.

The study will have one interim evaluation for feasibility in each disease population. Feasibility for patients with R/R aggressive B-cell NHL will be evaluated after the first 6 subjects' cell production. Feasibility for patients with R/R ALL will be evaluated after the first 10 subjects' cell production. If the targeted number of cells cannot be produced in at least 3 of the first 6 patients with aggressive B-cell NHL or at least 5 of the first 10 patients with ALL on this trial that meet the established release criteria, further enrollment in that disease population will be paused pending evaluation of the manufacturing process. The Protocol Director, in collaboration with the Cancer Cell Therapy (CCT) Manufacturing team, the CCT Clinical Research Operations & Procedures team, representatives from the manufacturing facilities, and IND Sponsor's authorized representatives, would work to modify the manufacturing process to improve rate of successful manufacture. Any changes to the protocol or IND will be submitted to FDA, and APB and IRB approval will be obtained prior to continuing enrollment. Based on previous experience, the probability of successful manufacturing of cells is 95%. Thus, the probability of suspending the

trial for lack of feasibility is negligible ($p < 0.001$).

If cell growth limitations preclude administration of the targeted cohort cell dose, the subject will receive as many cells as possible, and be considered evaluable for feasibility, but not evaluable for toxicity. If a minimum of 1.0×10^5 CD22-CAR-transduced T cells per kg cannot be obtained for infusion, the subject may be treated but will not be evaluable for toxicity or response, but will be considered a feasibility failure. The study has a goal of 80% feasibility to warrant further investigation of the experimental therapy. Subsequently, contingent upon meeting the first feasibility rule after 6 R/R aggressive B-cell NHL subjects, the study will allow for a total of 10 R/R aggressive B-cell NHL subjects (maximum of 4 subjects to be replaced during dose-escalation) to be replaced for safety and efficacy evaluations due to inability to achieve the target cell product. Similarly, contingent upon meeting the first feasibility rule after 10 R/R ALL subjects, the study will allow for a total of 6 R/R ALL subjects to be replaced for safety and efficacy evaluations.

12.1.2 Safety

The safety and tolerability of CD22-CAR T cell regimen will be assessed in each disease group by:

- Suspected adverse events, and
- Suspected serious adverse events

As evidenced by:

  - Changes in clinical laboratory tests (clinical chemistry, hematology, etc).
  - Changes in vital signs (blood pressure, pulse, respiratory rate and body temperature).
  - Changes in physical exams. Signs and symptoms assessed may require additional testing as clinically indicated such as ECG, PFT, radiographic studies, etc.
  - Subject reported signs and symptoms

Safety data will be analyzed per standard methods and interpreted descriptively for each disease group (ALL and aggressive B-cell NHL). Safety data will be summarized for each disease group separately and for the dose cohorts of subjects with aggressive B-cell NHL, if needed. Adverse events will be assessed using the CTCAE version 5.0 for type and severity of event. Serious Adverse Events will be summarized for the targeted dose level of a disease group. Reasons for discontinuation of study therapy will be tabulated.

Laboratory testing includes hematology, serology, serum chemistry, and urinalysis. Baseline laboratory testing will be those results obtained prior to initiating the conditioning lymphodepletion chemotherapy regimen. The study will utilize local lab for all clinical laboratory testing. Laboratory data will be tabulated based on the following result class.

- Normal: result is within the local lab normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance and investigator determination noted in source documents.

Vital signs collected immediately prior to receiving study drug will be the baseline vital signs.

Observed vital sign values and change from baseline in vital signs at each visit will be summarized without formal statistical testing.

Vital sign result may also be tabulated based on the following result class.

- Normal: result is within the normal range
- Abnormal: result is either higher or lower than the normal range

All abnormal values will be assessed for clinical significance; clinical significance will be captured in the case report form. Number and percent of subjects within each result class will be tabulated by time point for each vital sign.

Findings of physical examinations will be tabulated by dose cohorts without formal statistical analysis.

12.1.2.1 Safety Evaluation for Subjects with Aggressive B-cell NHL

To evaluate the safety of administering escalating doses of autologous CD22-CAR engineered T cells that can be feasibly produced to meet established release specifications in adults with aggressive B-cell NHL, defined as DLBCL, PMBCL, and tDLBCL, following a lymphodepleting conditioning regimen, the following dose levels will be used:

- Dose Level 1: 1×10^6 transduced T cells/kg (± 20%)
- Dose Level 2: 3×10^6 transduced T cells/kg (± 20%)
- Dose Level 3: 1×10^7 transduced T cells/kg (± 20%)

A dose limiting toxicity (DLT) as defined in **Section 5.4.7.1**. Based on preliminary data, the probability of a DLT is less than 20%. In this study, the maximum acceptable DLT rate is 30%.

The MTD/RP2D for patients with R/R aggressive B-cell NHL will be determined using a '3+3' dose escalation design. The first cohort of 3 patients will be treated at dose level 1 and escalation will proceed based on the number of DLTs in each cohort and constrained by the feasibility of producing the current dose level. The MTD/RP2D is defined as the feasible dose level achieving 0 DLT out of 3 evaluable subjects or 1 (or fewer due to feasibility) DLT out of 6 evaluable subjects. The maximum number of subjects to be enrolled in the '3+3' dose-escalation is 19 R/R aggressive B-cell NHL subjects (max 15 subjects required by '3+3' plus max 4 subjects replaced for feasibility). Ignoring feasibility, basic operating characteristics of the dose-escalation design are presented in the table below.

True MTD	True DLT probability at each dose			Average* Number of patients enrolled	Average* Number of DLTs	Probability* correct dose declared MTD	Probability* all doses found toxic
Dose level 1	30%	40%	50%	8	3	34%	50%
Dose level 2	15%	30%	50%	10	3	34%	17%
Dose level 3	10%	20%	30%	11	2	35%	9%

*From 1000 simulations using *ssim3p3( )* function in R package: 'UBCRM'

After the MTD/RP2D is established, a dose-expansion cohort will enroll up to 33 evaluable

subjects (for a total of 36 evaluable subjects treated at the final MTD/RP2D) to monitor safety and indicate efficacy. The sample size for the aggressive B-cell NHL dose-expansion cohort is determined to indicate efficacy (see **Section 12.2.1** for more details).

The trial will continue to evaluate safety after the first 11 (or 14) subjects in the dose-expansion cohort, corresponding to a total of 17 evaluable subjects treated at the MTD/RP2D with either 6 (or 3) subjects from the dose-escalation cohort(s), and every 5 subjects thereafter. Safety boundaries will be used to monitor a DLT rate of 30%; however, given the expected toxicity rate we do not anticipate crossing these boundaries. If the number of DLTs reported in the table below is met, the MTD/RP2D dose level will be de-escalated by one dose level and the dose expansion cohort will complete enrollment with the updated RP2D dose level, to further assess safety at this dose level and gather preliminary evidence for efficacy. If de-escalation occurs, new safety boundaries will be generated based on the number of currently enrolled subjects and will take place after every 5 additional subjects. If Dose Level -1 is found to be unsafe, the trial will stop enrollment for this disease population.

Table 7. Maximum DLTs during Dose Expansion in Subjects with Aggressive B-cell NHL

Total number of evaluable patients treated at MTD/RP2D	Stop and De-escalate if Number of DLTs reached
17	8+
23	9+
28	11+

The toxicity boundaries correspond to Pocock boundaries with a 10% significance level used for all looks and are calculated using the function *toxbdry( )* in the R package 'clinfun' assuming a DLT rate of 25% is acceptable.

12.1.2.2 Safety Evaluation in Subjects with ALL

Safety evaluations will occur after the first 10 R/R CD22-positive ALL subjects are evaluable for safety assessment and every 5 subjects thereafter. After 15 evaluable subjects, both safety and futility evaluations will be implemented simultaneously. If the tabulated threshold of DLTs in the table below is met, the established CD22-CAR T-cell dose will be decreased by one dose level (as defined in **Section 5.4.6**) for subjects with R/R ALL. If de-escalation occurs, new safety boundaries will be generated based on the number of currently enrolled patients. The cohort will complete enrollment up to a maximum of 28 evaluable patients with safety assessments after every 5 patients treated at the updated dose level. If dose level -1 is found to be unsafe, the trial will stop enrollment for this disease population.

Table 8. Maximum DLTs during Dose Expansion in Subjects with ALL

Total number of evaluable patients treated at MTD/RP2D	Stop and De-escalate if Number of DLTs reached
10	5+
15	7+

20	9+

The toxicity boundaries correspond to Pocock boundaries with a 10% significance level used for all looks and are calculated using the function *toxbdry( )* in the R package 'clinfun' assuming a DLT rate of 25% is acceptable.

12.2 SECONDARY OUTCOMES

Although a secondary outcome, efficacy as measured by overall response rate will be important in determining if a Phase 2 study is warranted in either disease group. The clinical responses in each disease group will be evaluated at Day 28, 3 months, and every 3 months up to 1 year, as per **Section 9**, until disease progression at which time, subjects will be followed as per **Section 5.5** until off-study criteria are met.

Overall survival (OS) and progression-free survival (PFS) will be assessed in each disease group. Progression-free survival (PFS) will be measured from the start of the lymphodepleting chemotherapy preparative regimen until the documentation of disease progression or death due to any cause, whichever occurs first. Overall survival (OS) will be determined as the time from the start of the lymphodepleting chemotherapy preparative regimen until death.

12.2.1 Efficacy Evaluation for Subjects with Aggressive B-cell NHL

An overall response rate (CR or PR) of 25% at 3 months is determined to be sufficient activity worthy of further investigation. A futility analysis will be conducted when 17 *evaluable* subjects have been treated at the MTD/RP2D [with 3 (or 6) from the dose-escalation cohort(s) and 14 (or 11) from the dose-expansion cohort] and have reached the Month 3 PET imaging assessment. If 4 or fewer out of 17 evaluable R/R aggressive B-cell NHL subjects have a CR or PR at 3 months, further enrollment to this disease population will stop and the therapy at the MTD/RP2D will be declared inactive.

Assuming no futility stopping, dose level -1 is not found unsafe, and no failure of manufacturing the required doses, the study will enroll for a maximum sample size of 36 *evaluable* aggressive B-cell NHL subjects treated at the MTD/RP2D. This corresponds to a dose-expansion cohort size of 33 (or 30) *evaluable* subjects depending on how many subjects were used to establish the MTD/RP2D in the dose-escalation. With 36 subjects, the Minimax Simon two-stage design has 80% power at a 5% significance level to test the null hypothesis that the ORR ≤ 25%, assuming a target ORR of 45%. If 14 or more respond at 3 months out of the total of up to 36 evaluable R/R aggressive B-cell NHL subjects treated at the MTD/RP2D, the therapy will be declared active and worthy of further investigation.

12.2.2 Efficacy Evaluation for Subjects with ALL

A futility analysis will be conducted when 15 *evaluable* subjects have been treated at the established dose and have reached Day 28 bone marrow assessment. If 7 or fewer out of 15 evaluable R/R ALL subjects have a CR, further enrollment to the cohort at this dose level will stop for futility; however, upon review by principal investigators, further enrollment to the cohort may continue at dose level 2 if DLT rate at dose level 1 is sufficiently safe. Assuming no futility stopping, dose level -1 is not found unsafe, and no failure of manufacturing the required doses, the study will enroll a maximum sample size of 28 *evaluable* ALL subjects treated at the established

dose. If 18 or more respond at Day 28 out of the total 28 evaluable subjects, the therapy will be declared sufficiently active for efficacy and worthy of further investigation. With 28 evaluable patients, the Minimax Simon two-stage design has 80% power at a 10% significance level to test the null hypothesis that the ORR ≤ 50%, assuming a target ORR of 70%.

12.3 PROTOCOL STOPPING RULES

To summarize, the study will be halted pending discussions with the FDA, IRB and APB if any of the following conditions are met:

- DLT stopping rule for a maximum acceptable DLT rate of 30% triggered at Dose Level -1 for either disease group cohort (ALL or aggressive B-cell NHL).
- Development of EBV lymphoma or polyclonal lymphoproliferative disease (PLPD) in any subject.
- Any Grade 5 event at least possibly related to the research regimen.
- If the targeted number of cells cannot be produced in at least 3 of the first 6 subjects with aggressive B-cell NHL or at least 5 of the first 10 patients with ALL on this trial, further enrollment will be paused pending evaluation of the manufacturing process regardless of the safety evaluation.
- If more than 10 aggressive B-cell NHL subjects (max 4 during dose-escalation) or more than 6 ALL subjects need to be replaced for the safety evaluation due to inability to achieve the target cell product, further enrollment to that disease population will stop.
- If 7 or fewer subjects out of the first 15 evaluable ALL subjects have a response (CR) at Day 28, accrual will stop after evaluation of the safety objectives. Similarly if 4 or fewer subjects out of the first 17 evaluable aggressive B-cell NHL subjects treated at MTD/RP2D have a response (CR or PR) at Month 3, accrual will stop after evaluation of the safety objectives.

12.4 SAMPLE SIZE

12.4.1 Accrual estimates

Given the number of subjects seeking out adoptive cell therapy trials the rate limiting factor at this time is the time and labor intensive nature of manufacturing the CD22-CAR T cells. We anticipate enrollment of 1-2 subjects per month per each disease group, given the number of subjects with B-ALL and aggressive B-cell NHL treated in Stanford Cancer Therapy Clinics. The recruitment period for this study is expected to be 2-3.5 years. Subjects will be followed for a total of 15 years post treatment. The total duration of this study is expected to be 7-8.5 years of active treatment and short term follow up, and a total of 15 years of long term follow up after the last subject completes study therapy.

12.4.2 Sample size justification

The primary objectives of this study are safety and feasibility. The '3+3' design will be used for dose-escalation in R/R aggressive B-cell NHL subjects across three possible dose levels of CD22-CAR T-cells under investigation. We will perform an interim analysis for feasibility after the 6 subjects and will continue to enroll if 3 or more subjects are successful in feasibility of manufacturing cells. A minimum of 9 subjects and maximum of 22 subjects (max of 18 *evaluable*

from dose-escalation and max of 4 additional from feasibility) may be required to establish the MTD/RP2D for R/R aggressive B-cell NHL subjects. After the MTD/RP2D is established in this disease population, the trial will expand to enroll a dose-expansion cohort at the established dose to monitor safety and indicate efficacy. The dose-expansion cohort will have three interim evaluation time points for safety and one interim evaluation for futility. The first interim analysis will evaluate both safety and futility after 17 *evaluable* subjects have been treated at the established MTD/RP2D (including subjects from both dose-escalation and dose-expansion). If 5 or more subjects respond (CR or PR) at 3 months and 8 or fewer subjects experience a DLT, the trial will continue to enroll and evaluate safety after 23 and 28 *evaluable* subjects have been treated at the established dose level. To indicate efficacy, a Minimax Simon two-stage design was used to motivate the maximum sample size for the dose-expansion cohort. With a total of 36 *evaluable* subjects (using both dose-escalation and dose-expansion cohorts treated at established dose), the Minimax Simon two-stage design has 80% power at a 5% significance level to test the null hypothesis that the ORR ≤ 25%, assuming a target ORR of 45%, where ORR is measured by CR or PR. The study has a feasibility goal of 80% and will allow for an additional 10 subjects to be replaced for safety and efficacy evaluations due to inability to achieve the target cell product (max of 4 subjects during dose-escalation). **For relapsed/refractory aggressive B-cell NHL subjects, a maximum of 18+10+33 = 61 patients may be required to establish safety and feasibility, and indicate sufficient efficacy.**

Simultaneously, a minimum of 10 patients with relapse/refractory ALL may be enrolled at the established dose (1 x 10^6 transduced T cells/kg ± 20%). We will perform an interim analysis for feasibility after first 10 patients and will continue to enroll if 5 or more patients are successful in feasibility of manufacturing cells. We will perform a safety evaluation for possible dose de-escalation after 10 evaluable patients have been observed for the 28 day DLT assessment period, and additional safety evaluations every 5 patients thereafter. After 15 evaluable patients have been observed, we will perform safety and futility evaluations. If 8 or more patients respond (CR only) and 7 or fewer patients experience a DLT at 28 days, the trial will continue at the established dose. With a total of 28 *evaluable* patients, the Minimax Simon two-stage design has 80% power at a 10% significance level to test the null hypothesis that the ORR ≤ 50%, assuming a target ORR of 70%, where ORR is measured by CR only. The study has a feasibility goal of 80% and will allow for an additional 6 relapse/refractory ALL patients to be replaced for safety and efficacy evaluations due to inability to achieve the target cell product. **For relapse/refractory ALL patients, a maximum of 28+6= 34 patients may be required to establish safety and feasibility, and indicate sufficient efficacy.**

Table 9. Enrollment Table by Objective

Objective	**Subjects with Aggressive B-cell NHL**	**Subjects with ALL**
Manufacturing Feasibility	Evaluated in first 6 subjects	Evaluated in first 10 subjects
Safety – Preliminary (MTD)	**Up to 18 (6 + 6 +6) subjects**	**10 evaluable subjects**
Safety – Secondary (Dose Expansion)	After 17 evaluable subjects at MTD/RP2D, then every 5 subjects	After 15 evaluable subjects at target dose, then every 5 subjects
Efficacy – Preliminary Futility	17 evaluable subjects at MTD/RP2D	15 evaluable subjects at target dose
Efficacy – Full Analysis	**Up to 36 evaluable subjects**	**Up to 28 evaluable subjects**

	at MTD/RP2D	**at target dose**
Number of Replacements allowed	**Up to 10**	**Up to 6**
Total number of subjects	**18 + 33 + 10 = 61**	**10 + 18+ 6 = 34**

13 APPENDICES

13.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

PERFORMANCE STATUS CRITERIA *Karnofsky and Lansky performance scores are intended to be multiples of 10.*					
ECOG (Zubrod)		**Karnofsky**		**Lansky**	
Score	**Description**	**Score**	**Description**	**Score**	**Description**
0	Fully active, able to carry on all pre-disease performance without restriction.	100%	Normal, no complaints, no evidence of disease.	100%	Fully active, normal.
		90%	Able to carry on normal activity; minor sighs of symptoms of disease.	90%	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory, able to carry out light or sedentary work, e.g., light housework, office work.	80%	Able to carry on normal activity with effort; some signs or symptoms of disease.	80%	Active, but tires more quickly.
		70%	Cares for self, unable to carry on normal activity or do active work.	70%	Both greater restriction of, and less time spent in, play activities.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.	60%	Requires occasional assistance but is able to care for most of own needs.	60%	Up and around, but minimal active play; keeps busy with quieter activities.
		50%	Requires considerable assistance and frequent medical care.	50%	Gets dressed, but lies around much of the day; no active play; able to participate in quiet play and activities.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	40%	Disabled; requires special care and assistance.	40%	Mostly in bed; participates in quiet activities.
		30%	Severely disabled; hospitalization indicated, although death not imminent.	30%	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any self-care. Totally confined to a bed or chair	20%	Very ill; hospitalization necessary; active supportive treatment required.	20%	Often sleeping; play entirely limited to very passive activities.
		10%	Moribund, fatal process progressing rapidly	10%	No play; does not get out of bed
5	Dead	0%	Patient expired	0%	Unresponsive; Dead

13.2 APPENDIX B: GUIDELINES TOXICITY ASSESSMENT AND MANAGEMENT

13.2.1 Guidelines for Grading Suspected Cytokine Release Syndrome according to ASBMT CRS Consensus Grading#

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever[†]	Temperature ≥ 38° C	Temperature ≥ 38° C	Temperature ≥ 38° C	Temperature ≥ 38° C
With either:				
Hypotension	None	Not requiring vasopressors	Requiring one vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
And/or[§]**:**				
Hypoxia	None	Requiring low-flow nasal cannula^ or blow-by	Requiring high-flow nasal cannula, facemask, non-rebreather mask, or Venturi mask	Requiring positive pressure (e.g.: CPAP, BiPAP, intubation and mechanical ventilation

CPAP: Continuous positive airway pressure; BiPAP: Bilevel positive airway pressure

Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading.

[†] Fever is defined as temperature ≥ 38° C not attributable to any other cause. In patients who have CRS then receive anti-pyretics or anti-cytokine therapy such as tocilizumab, steroids or anakinra, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

[§] CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with a temperature of 39.5 ° C, hypotension requiring one vasopressor and hypoxia requiring low-flow nasal cannula is classified as having Grade 3 CRS.

^ Low-flow nasal cannula is defined as oxygen delivered at ≤ 6 liters/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at > 6 liters/minute.

13.2.2 Guidelines for Managing Suspected Cytokine Release Syndrome

Grade 1 Temperature > 38°C WITH no hypotension AND/OR no hypoxia	*Symptoms are not life threatening and require symptomatic treatment only* Treatment: ➢ Supportive Care ➢ VS Q4-6 hr (incl orthostatic BP) and prn, monitor for progression ➢ Assess for infection, pan-culture with CSR, treat febrile neutropenia if present ➢ Acetaminophen, NSAIDS and cooling blanket PRN
Grade 2 Temperature > 38°C WITH hypotension not requiring pressors AND/OR requires nasal cannula oxygen to maintain SpO2 > 92%	*Symptoms require and respond to moderate intervention* Treatment: Echocardiogram as needed ➢ Notify ICU if not improving **First ONSET only :** ➢ Tocilizumab 8 mg/kg IV X 1 ➢ Dexamethasone 10 mg IV X 1 ➢ If orthostatic, give bolus and reassess. ➢ If ongoing orthostatic or re-occurs > 24 hrs, treat as grade 2 **Grade 2 recurrent** (recurs after > 24 hrs of toci) **or refractory /ongoing** (not responding > 24 hrs) **:** ✓ Anakinra 100 mg SQ q6h X 3 days ✓ Dexamethasone 10 mg IV q6h ✓ Taper dex when ≤ gr 1. ✓ Monitor organ function closely (incl. cardiac, respiratory, renal, neurologic, liver) : VS q2-4h and prn, telemetry and continuous O2, anticipate transfer if worsening symptoms. ➢ Be vigilant for HLH, trend DIC screen and ferritin
Grade 3 Temperature > 38°C WITH hypotension requiring 1 pressor +/- vasopressin AND/OR requires facemask or high-flow oxygen to maintain SpO2 > 92%	*Symptoms require and respond to aggressive intervention* Treatment: ➢ Transfer to ICU ➢ Tocilizumab 8 mg/kg IV X 1 (even if given previously) ➢ Anakinra 100 mg SQ q6h X 3 days ➢ Dexamethasone 10 mg/IV q6h ➢ Manage hypotension and hypoxia with ICU support ➢ Be vigilant for HLH, trend DIC screen and ferritin
Grade 4 Temperature > 38°C WITH hypotension requiring > 1 pressor (not including vasopressin) AND/OR requires intubation or NIPPV	*Life-threatening symptoms* Treatment: ABC's, stabilize, transfer or treat in ICU ➢ Tocilizumab 8 mg/kg IV X 1 (even if given previously) ➢ Anakinra 100 mg SQ q6h X 3 days ➢ Solumedrol X 3 days then taper ➢ Manage hypotension and hypoxia with ICU support ➢ Be vigilant for HLH, trend DIC screen and ferritin

13.2.3 Encephalopathy assessment tools for grading Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS)

13.2.3.1 Immune Effector Cell-associated Encephalopathy (ICE) Assessment

Directions: Answer whether each task was performed correctly (Not Done/Yes/No). If you answer YES put 1 in the Score column; If you answer NO (or Not Done) put 0 in the Score column.

Tasks	Performed correctly?	Score
Orientation		
1. What is the current year?		**0**
2. What is the current month?		**0**
3. What is the current city?		**0**
4. What hospital are you in?		**0**
Naming		
5. Name this object (*point to an object in the room)*		**0**
6. Name this object (*point to an object in the room)*		**0**
7. Name this object (*point to an object in the room)*		**0**
Following commands		
8. Show me (insert object, e.g. 2 fingers) or Close your eyes and stick out your tongue.		**0**
Writing		
9. Write a simple sentence (*provide paper and pencil)*		**0**
Attention		
10. Count backwards from 100 in 10's.		**0**
Total Score		

Scoring ICE

No impairment: Score 10

Grade 1 ICANS: Score 7-9

Grade 2 ICANS: Score 3-6

Grade 3 ICANS: Score 0-2

Grade 4 ICANS: Score 0 due to subject unarousable and unable to perform ICE assessment.

13.2.3.2 ASBMT Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS) Consensus Grading for Adults

Neurotoxicity Domain	Grade 1 (Some or all of the following: spontaneous awakening, mild confusion, word-finding difficulty, +/- new tremor, no clinical seizures)	Grade 2 (Some or all of the following: awakening to voice, moderate confusion, expressive aphasia, perseveration, no clinical seizures)	Grade 3 (Some or all of the following: awakening to tactile stimulus, global aphasia, myoclonus, clinical seizure, brief electrographic seizures)	Grade 4 (Some or all of the following: coma/posturing, focal weakness, status epilepticus, pupillary/CN abnormalities, diffuse cerebral edema)
ICE Score^	7-9	3-6	0-2	0 (unarousable, unable to perform ICE)
Depressed level of consciousness‡	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma
Seizure	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly; or Non-convulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (> 5 min); or Repetitive clinical or electrical seizures without return to baseline in between.
Motor findings§	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis
Raised ICP / Cerebral edema	N/A	N/A	Focal/local edema on neuroimaging#	Diffuse cerebral edema on neuroimaging; Decerebrate or decorticate posturing; or Cranial nerve VI palsy; or Papilledema; or Cushing's triad

ICANS grade is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause. For example, a patient with and ICE score of 3 who has a generalized seizure is classified as having a Grade 3 ICANS.

^A patient with an ICE score of 0 may be classified as having Grade 3 ICANS if the patient is awake with global aphasia. But a patient with an ICE score of 0 may be classified as having a Grade 4 ICANS if the patient is unarousable.

‡Depressed level of consciousness should be attributable to no other cause (e.g. no sedating medication).

§Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0 but they do not influence ICANS grading.

#Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v5.0.

ICE: Immune effector Cell-associated Encephalopathy; **ICP:** Intracranial pressure; **EEG:** electroencephalogram

13.2.3.3 Neurologic Recommended Treatments by Grade

Neurotoxicity Grade	Grade 1 **(Some or all of the following:** spontaneous awakening, mild confusion, word-finding difficulty, +/- new tremor, no clinical seizures)	Grade 2 **(Some or all of the following:** awakening to voice, moderate confusion, expressive aphasia, perseveration, no clinical seizures)	Grade 3 **(Some or all of the following:** awakening to tactile stimulus, global aphasia, myoclonus, clinical seizure, brief electrographic seizures)	Grade 4 **(Some or all of the following:** coma/posturing, focal weakness, status epilepticus, pupillary/CN abnormalities, diffuse cerebral edema)
Recommended Interventions by Grade	Neuro chks q4h, with ICE q12 or as clinically indicated. Cont Keppra 500 mg BID, reconcile meds to identify contributors	Consult Neurology, neuro chks q2h X 6 hrs, then q4h if stable, telemetry and continuous O2, CT of the head, consider EEG, Consider incr Keppra to 1000 mg BID, tocilizumab 8 mg/kg if concurrent CRS and no prior toci.	Notify Neuro consult, discuss imaging with neurology, neuro chks q2h X 12-24 hours, Discuss with MICU. ICE score q 4 X 12-24 hrs. telemetry and continuous O2 consider empiric increase in AED, Consider possibility of diffuse cerebral edema, continue Keppra 1000 mg BID, add Vimpat 100 mg BID, Dexamethasone 10 mg IV q6h, Tocilizumab per CRS pathway, not to exceed 3 doses in 24 hrs.	ABC's, stabilize patient, transfer to MICU, emergent CT head when stabilized, neuro chks q1h with ICE scores and pupillometry, Adjust antiepileptic drugs based on EEG and discussion with NCC, Methylprednisolone 1g IV daily; reassess daily, taper when able • Tocilizumab per CRS pathway, not to exceed 3 doses in 24 hours, Consider Anakinra 100mg SQ every 6 hrs x 3 days

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13.3 APPENDIX C: RESPONSE CRITERIA

13.3.1 Response Criteria for ALL

13.3.1.1 Introduction

This document provides the working definitions and specifications for a consistent and efficient analysis of efficacy, assessing clinical activity in acute lymphoblastic leukemia (ALL). The current document is written primarily for the relapse and refractory disease setting. Modifications may be indicated for earlier disease settings. This document is based on the standardized response criteria defined by National Comprehensive Cancer Network (NCCN) Guidelines (NCCN 2013 v1) and further supported by the workshop report on acute leukemia from American Society of Hematology (ASH)[122] (Appelbaum et al 2007) and the International Working Group (IWG) guideline for acute myeloid leukemia (AML)[123] (Cheson et al 2003). The Cheson IWG guideline and Appelbaum ASH report were used in recent drug approvals (e.g. Marqibo) in ALL, prior to the NCCN guideline availability. The NCCN guidance is a more recently published United States based guideline for ALL.

The objectives of this document are to:

- Ensure that the definitions of responses in the clinical study protocol correctly reflect the above mentioned guidelines.

- Provide guidance for the response assessment and clinical monitoring to ensure consistency in applying the guidelines.

13.3.1.2 Types of Efficacy Assessments

Disease characterization at baseline and evaluation of response rely on the following:

- Bone marrow assessment
- Peripheral blood assessment
- Extramedullary disease assessment, including
  - CNS disease
  - Other extramedullary sites
- Minimal residual disease (MRD) assessment of bone marrow

Response Criteria

Response Category	Definition
Complete remission (CR)	All the following criteria are met: **Bone Marrow** • < 5% blasts **Peripheral Blood** • Neutrophils > 1.0 X 10^6/L, and • Platelets > 100 X 10^6/L, and • Circulating blasts < 1% **Extramedullary disease** • No clinical evidence of extramedullary disease (by physical exam and CNS symptom assessment), and • If additional assessments (e.g. CSF assessment by LP, CNS imaging, bipsy, etc.) are

	performed, results must show remission status. **Transfusion independency** • No platelet and/or neutrophil transfusions less than or equal to 7 days before the date of the peripheral blood sample for disease assessment.
Complete remission with incomplete blood count recovery (CRi)	All criteria for CR as defined above are met, except that the following exist: • Neutrophils > 1.0 X 10^6/L, and/or • Platelets > 100 X 10^6/L, and/or • Platelet and/or neutrophil transfusions less than or equal to 7 days before the date of the peripheral blood sample for disease assessment.
No response	Failure to attain the criteria needed for any response categories or relapse
Relapsed Disease	Only in patients who achieved a CR or Cri and who have: • Reappearance of blasts in the blood (> 1%), or • Reappearance of blasts in the bone marrow (> 5%), or • (Re-) appearance of any extramedullary disease after CR or CRi
Unknown	"Unknown" is assigned in case the baseline assessment or the response assessment is not done, incomplete, indeterminate, or not performed within the respective time frame. If there is evidence of relapse, the overall response will be assessed as "relapsed disease" with the relapsed component alone.

In order for the best overall disease response to be categorized as CR or CRi, there must be no clinical evidence of relapse as assessed by peripheral blood and extramedullary disease assessment (physical exam and CNS symptom assessment) at a minimum of 4 weeks (28 days) after the initial achievement of CR or CRi. Please note, if additional assessments (e.g. bone marrow, CSF assessment by LP, CNS imaging, biopsy, etc.) are performed in the same evaluation for disease response evaluation purposes, they will also need to show remission status.

13.3.2 Response Criteria for Lymphoma

Response Criteria Lymphoma (From: Cheson BD, et al : Recommendations for Initial Evaluation, Staging, and Response Assessment of Hodgkin and Non-Hodgkin Lymphoma: The Lugano Classification. J Clin Oncol 2014 ; 32 :3059-3067)

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B-cell Lymphomas

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LUGANO RESPONSE CRITERIA FOR NON-HODGKIN'S LYMPHOMA

PET should be done with contrast-enhanced diagnostic CT and can be done simultaneously or at separate procedures.

Response	Site	PET-CT (Metabolic response)	CT (Radiologic response)[d]
Complete response	Lymph nodes and extralymphatic sites	Score 1, 2, or 3[a] with or without a residual mass on 5 point scale (5-PS)[b,c]	All of the following: Target nodes/nodal masses must regress to ≤1.5 cm in longest transverse diameter of a lesion (LDi) No extralymphatic sites of disease
	Non-measured lesion	Not applicable	Absent
	Organ enlargement	Not applicable	Regress to normal
	New Lesions	None	None
	Bone Marrow	No evidence of FDG-avid disease in marrow	Normal by morphology; if indeterminate, and flow cytometry IHC negative
Partial response	Lymph nodes and extralymphatic sites	Score 4 or 5[b] with reduced uptake compared with baseline. No new or progressive lesions. At interim these findings suggest responding disease. At end of treatment these findings may indicate residual disease.	All of the following: ≥50% decrease in SPD of up to 6 target measurable nodes and extranodal sites When a lesion is too small to measure on CT, assign 5mm x 5mm as the default value. When no longer visible, 0x0 mm For a node >5mm x 5mm, but smaller than normal, use actual measurement for calculation
	Non-measured lesion	Not applicable	Absent/normal, regressed, but no increase
	Organ enlargement	Not applicable	Spleen must have regressed by >50% in length beyond normal
	New Lesions	None	None
	Bone Marrow	Residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). If there are persistent focal changes in the marrow in the context of a nodal response, consider further evaluation with biopsy, or an interval scan.	Not applicable

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Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

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LUGANO RESPONSE CRITERIA FOR NON-HODGKIN'S LYMPHOMA

PET should be done with contrast-enhanced diagnostic CT and can be done simultaneously or at separate procedures.

Response	Site	PET-CT (Metabolic response)	CT (Radiologic response) [d]
No response or stable disease	Target nodes/nodal masses, extranodal lesions	Score 4 or 5[b] with no significant change in FDG uptake from baseline at interim or end of treatment. No new or progressive lesions	<50% decrease from baseline in SPD of up to 6 dominant, measurable nodes and extranodal sites; no criteria for progressive disease are met
	Non-measured lesion	Not applicable	No increase consistent with progression
	Organ enlargement	Not applicable	No increase consistent with progression
	New Lesions	None	None
	Bone Marrow	No change from baseline	Not applicable
Progressive disease	Individual target nodes/nodal masses Extranodal lesions	Score 4 or 5 [b] with an increase in intensity of uptake from baseline and/or New FDG-avid foci consistent with lymphoma at interim or end-of-treatment assessment[e]	Requires at least one of the following PPD progression: An individual node/lesion must be abnormal with: LDi >1.5 cm and Increase by ≥50% from PPD nadir and An increase in LDi or SDi from nadir 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. If no prior splenomegaly, must increase by at least 2 cm from baseline New or recurrent splenomegaly
	Non-measured lesion	None	New or clear progression of preexisting nonmeasured lesions
	New Lesions	New FDG-avid foci consistent with lymphoma rather than another etiology (eg, infection, inflammation). If uncertain regarding etiology of new lesions, biopsy or interval scan may be considered[e]	Regrowth of previously resolved lesions A new node >1.5 cm in any axis A new extranodal site >1.0 cm in any axis; if <1.0 cm in any axis, its presence must be unequivocal and must be attributable to lymphoma Assessable disease of any size unequivocally attributable to lymphoma
	Bone Marrow	New or recurrent FDG-avid foci	New or recurrent involvement

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Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

LUGANO RESPONSE CRITERIA FOR NON-HODGKIN'S LYMPHOMA

Footnotes

[a] Score 3 in many patients indicates a good prognosis with standard treatment, especially if at the time of an interim scan. However, in trials involving PET where de-escalation is investigated, it may be preferable to consider score 3 as an inadequate response (to avoid under-treatment).
[b] See PET Five Point Scale (5-PS).
[c] It is recognized that in Waldeyer's ring or extranodal sites with high physiological uptake or with activation within spleen or marrow, e.g. with chemotherapy or myeloid colony stimulating factors, uptake may be greater than normal mediastinum and/or liver. In this circumstance, CMR may be inferred if uptake at sites of initial involvement is no greater than surrounding normal tissue even if the tissue has high physiological uptake.
[d] FDG-avid lymphomas should have response assessed by PET-CT. Diseases that can typically be followed with CT alone include CLL/SLL and marginal zone lymphomas.
[e] False-positive PET scans may be observed related to infectious or inflammatory conditions. Biopsy of affected sites remains the gold standard for confirming new or persistent disease at end of therapy.

PET Five Point Scale (5-PS)

1	No uptake above background
2	Uptake ≤ mediastinum
3	Uptake > mediastinum but ≤ liver
4	Uptake moderately > liver
5	Uptake markedly higher than liver and/or new lesions
X	New areas of uptake unlikely to be related to lymphoma

SPD – sum of the product of the perpendicular diameters for multiple lesions
LDi – Longest transverse diameter of a lesion
SDi – Shortest axis perpendicular to the LDi
PPD – Cross product of the LDi and perpendicular diameter

Measured dominant lesions – Up to 6 of the largest dominant nodes, nodal masses and extranodal lesions selected to be clearly measurable in 2 diameters. Nodes should preferably be from disparate regions of the body, and should include, where applicable, mediastinal and retroperitoneal areas. Non-nodal lesions include those in solid organs, e.g., liver, spleen, kidneys, lungs, etc, gastrointestinal involvement, cutaneous lesions of those noted on palpation.
Non-measured lesions – Any disease not selected as measured, dominant disease and truly assessable disease should be considered not measured. These sites include any nodes, nodal masses, and extranodal sites not selected as dominant, measurable or which do not meet the requirements for measurability, but are still considered abnormal. As well as truly assessable disease which is any site of suspected disease that would be difficult to follow quantitiatively with measurement, including pleural effusions, ascites, bone lesions, leptomeningeal disease, abdominal masses and other lesions that cannot be confirmed and followed by imaging.

Reprinted with permission. © 2014 American Society of Clinical Oncology. All rights reserved. Cheson B, Fisher R, Barrington S, et al. Recommendations for Initial Evaluation, Staging and Response Assessment of Hodgkin and Non-Hodgkin Lymphoma – the Lugano Classification. J Clin Oncol 2014;32:3059-3067.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

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13.4 APPENDIX D: MONITORING GENE THERAPY TRIALS: REPLICATION COMPETENT LENTIVIRUS (RCL)

Subject Testing for RCL PCR

Subject blood samples will be obtained at the following time points:

1. Prior to cell infusion
2. 3 months(± 1 month)
3. 6 months (± 1 month)
4. 12 months (±2 months)

All Samples are scheduled from the most recent cell infusion, and should proceed even after disease progression. If any post-treatment samples are positive, further RCL testing and subject work-up should be undertaken.

Procedures:

Samples will be collected from subjects returning to clinic or, at investigator discretion, may be collected by a local medical provider. Samples will be shipped directly to Indiana University GTTL, as described in Stanford Center for Cancer Cell Therapy SOP 'Remote RCR/RCL Collection'. Procedures in brief include:

1. 4 mL blood sample collected in EDTA tube (lavender/purple top). If necessary, sample may be collected as 2 x 2 mL EDTA tubes.
2. Sample will be packaged according to biohazardous shipping regulation (UN 3373, 'Diagnostic Specimens') and shipped on ice priority overnight to Indiana University GTTL.

   Attn: Lisa Duffy
   IU GTTL
   980 West Walnut St, R3-C668
   Indianapolis, IN 46202-5188
   Phone: 317-274-0323

   a. 'NGVB Sample Submission Form' and 'Sample Submission Form Master Agreements' must accompany sample for proper processing
   b. Sample shipment must occur Monday-Thursday, as IU GTTL is unable to accept samples over the weekend or holidays
3. Notify IU GTTL representatives by email when sample delivery is expected.

Document Retention

1. RCL Reports from Indiana University will be sent to the Sponsor Investigator or designated representative; who will distribute the results. Groups requesting RCL results notification include:
   a. Principal Investigator and co-investigators
   b. IND Sponsor, or delegated representative
   c. CCT-Clinical Research Operations & Procedures (CCT-CROP) [regulatory support]
   d. CCT Manufacturing team
2. Hard copies will be retained in the subject's research record or other suitable study file in a locked file cabinet.

13.5 APPENDIX E: DRAFT LETTER AND QUESTIONNAIRE TO SUBJECTS FOR LONG TERM FOLLOW-UP FOR DELAYED ADVERSE EVENTS

[date]

[name and address]

Dear [subject name],

You have participated in a clinical research study that requires that the study doctors and nurses monitor your health for 15 years. In addition to the annual visits you will be attending, **we would like for you to report certain events listed below to your study doctor or nurse if they occur**:

1. Your doctor tells you that you have been diagnosed with any new type of cancer, including blood disorders such as leukemia or lymphoma (this would be separate from your cancer diagnosis).
2. You develop loss of feeling in any part of your body, especially hands and feet; you develop a loss of control of any body part (arms, legs…); you have a seizure; you experience memory loss. In addition, if you experience a worsening of any of the symptoms listed, please contact your study nurse or doctor. These types of symptoms are called neurological disorders. If your primary doctor or specialist tells you that you have developed neurological symptoms, contact your study doctor or nurse.
3. You develop arthritis or autoimmune disease, or worsening of any previously experienced arthritis or autoimmune disease which you were experiencing prior to participation in the study. If you are experiencing symptoms of arthritis or have been told by your doctor that you have an autoimmune disease, contact your study doctor or nurse.

Please complete the attached questionnaire and return it in the Fed-Ex envelope to the study coordinator.

If you experience any of the events listed above during the upcoming year, please contact your study physician or the study nurse listed below as soon as you can. They may ask you questions about your health and will record your symptoms/disease and then monitor your health if they decide that it is necessary. When you call, please mention that you participated in a gene therapy clinical trial at the < put your institution here> . Your subject identification number under this protocol is (#XXX).

Study Coordinator:
Name
Address
Phone
Email

If you have any questions about this letter or the follow up procedures for the study itself, please do not hesitate to contact the above study nurse.

Thank you for your continued participation in our clinical research study. Best regards,

[study coordinator]

Questionnaire to Subjects for Long Term Follow-up for Delayed Adverse Events

Subject Identification [put subject study number here]

Within the past year, have you:

1. **Had any problems with your health?** ☐ YES ☐ NO

If Yes, please explain: _____

_____

2. **Required any hospitalizations?** ☐ YES ☐ NO

If Yes, please describe when and the reason: _____

_____

_____

3. **Seen any healthcare provider?** ☐ YES ☐ NO

If Yes, please describe when and the reason: _____

_____

_____

4. **Started on any new medications?** ☐ YES ☐ NO

If Yes, please list: _____

_____

5. **Developed any new conditions or illnesses?** ☐ YES ☐ NO

If Yes, please describe: _____

_____

Please share any other new health concerns or problems:

_____

_____

When you have completed this questionnaire, please return it to:

Study Coordinator:
Name
Address
Phone
Fax
Email

A pre-addressed stamped envelope has been enclosed for your convenience, if you choose to mail this questionnaire. We will also accept faxed or e-mailed completed questionnaires as well.

Thank you very much for your participation.

13.6 APPENDIX F: PHYSICIAN (LOCAL MEDICAL PROVIDER) LETTER

[date]

[name and address]

Dear [physician name],

Your subject [subject name] has participated in a clinical research study that requires 15 year monitoring for adverse events. To aid in reporting adverse events that are possible related to the clinical research study, we are asking the subjects on our research study to designate a primary care or infectious disease physician that may help in the monitoring and reporting of adverse events. Your subject has designated you. **If upon any of your visits with your subject, any of the following events are reported or discovered, please contact the study nurse or physician as soon as possible**:

1. New malignancies
2. New incidence of exacerbation of a pre-existing neurologic disorder
3. New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder
4. New incidence of a hematologic disorder.

If your subject experiences any of these events, please contact the study coordinator below as soon as you can so that they can record the event and then monitor your subject's health if necessary. When you call, please mention that the subject has participated in a gene therapy clinical trial in the < designate location, institution and sponsor investigator> .

Study Coordinator
Name
Address
Phone
Email

If you have any questions about this letter or the study itself, please do not hesitate to contact the above study nurse.

Thank you for your support in helping us to monitor for delayed adverse events. Best regards,

13.7 APPENDIX G: CORRELATIVE SAMPLE SCHEDULE

Correlative Sample Collection Matrix

mL	type	purpose	Apheresis[6]	Product[6]	Prior to LD chemo (± 7 d)	D0	D3 (± 2 d)	D7 (± 2 d)	D10 (± 2 d)	D14 (± 2 d)	D21 (± 4 d)	D28 (± 7 d)	M2 (± 2 w)	M3 (± 2 w)	M4 (± 2 w)	M5 (± 2 w)	M6 (± 1 m)	M9 (± 1 m)	M12 (± 2 m)	At Progression
Apheresis Product																				
	collected by manufacturer		X																	
	collected by Stanford											X[1]		X[1]			X[1]	X[1]	X[1]	
CAR T cell Product																				
	collected by manufacturer	baseline measurements for correlatives				X														
PBMCs																				
10	Green top (Heparin)	Flow panel (CAR-FACS)[4]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
10	Green top (Heparin)	CyTOF[4]	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
10	Lavender top (EDTA)	CAR qRT-PCR[4]				X	X	X[2]	X	X	X	X	X	X[2]	X	X	X[2]	X	X[2]	X[2]
10	Lavender top (EDTA)	TCR sequencing/ CAR-T cell fate	X	X				X		X		X		X			X		X	X
5	Lavender top (EDTA)	ATAC-Seq	X	X						X		X		X			X		X	X
10	Green top (Heparin)	Sample banking				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Plasma																				
4	Lavender top (EDTA)	Cytokines - Luminex (HIMC)[4]				X	X	X	X	X	X	X								
8	Lavender top (EDTA)	MRD - DLBCL	X		X	X		X		X	X	X	X	X			X	X	X	
Bone Marrow Aspirate																				
2	Green top (Heparin)	Flow panel (CAR-FACS)[4]			X							X		X			X			X
5	Green top (Heparin)	CyTOF[4]			X							X		X			X			X
5	Lavender top (EDTA)	TCR sequencing/ CAR-T cell fate			X							X		X			X			X
5	Lavender top (EDTA)	ATAC-Seq			X							X		X			X			X
3	Green top (Heparin)	Sample banking			X							X		X			X			X
Lymph Node Biopsy																				
	CT guided: 4 cores collected in RPMI				X[3]							X[3]								X[3]
	CT guided: 4 cores collected in hydrothermacil				X[3]							X[3]								X[3]
	CT guided: 4 tubes FNA product				X[3]							X[3]								X[3]
	Not CT guided: 6 tubes FNA product				X[3]							X[3]								X[3]
Lumbar Puncture/CSF																				
1	Plastic Falcon tube				X							X[3]								X
Total Blood Volume per visit					18	42	44	52	44	67	52	67	48	53	40	40	53	48	53	45

Notes

1 A small volume apheresis (1-2 blood volumes) may be collected in lieu of PBMC blood tubes listed below
2 Sample collected used for both CAR qRT-PCR and TCR sequencing
3 Either CT guided or not CT guided will be collected. One sample between Day 7 to 28 at peak CAR activity
4 Stop further collection if CAR is undetectable
5 Collect at any time of neurotoxicity and at Day 28 (for subjects with CNS involvement at baseline), at discretion of investigator
6 Collected from the apheresis sample or product (NOT FROM PATIENT directly)

13.8 APPENDIX H: ENROLLMENT USING TWO STAGES: 1) LEUKAPHERESIS AND 2) LYMPHODEPLETION CHEMOTHERAPY AND CELL INFUSION

If patient or manufacturing scheduling requires, consenting and screening may be completed in two stages: 1) for leukapheresis, and 2) for lymphodepleting chemotherapy and CD22-CAR T cell infusion, as outlined with the following checklists. In addition the screening evaluations and Study Calendar are outlined using this two stage sequence for clarity. All other applicable aspects of the clinical protocol apply to all patients.

13.8.1 Screening Participant Eligibility Checklist: Two Stages for Screening/Enrollment

I. Screening and Enrollment for Leukapheresis

Protocol Title:	Phase I/Ib Clinical Trial of Autologous CD22 Chimeric Antigen Receptor (CAR) T cells in Adults with Recurrent or Refractory B Cell Malignancies
Protocol Number:	CCT5029 / IRB-50836
Protocol Director:	Matthew Frank, M.D., Ph.D.

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

III. Study Information:

SRC Approved ☐ IRB Approved ☐ Contract signed ☐

IV. Inclusion/Exclusion Criteria for Stage 1: Leukapheresis

INCLUSION CRITERIA	Yes	No	Supporting Documentation†
1. Disease Status a. <u>Disease Status of ALL</u> • Must have chemotherapy refractory disease defined as progression or stable disease after a standard line of therapy, or relapsed disease after achieving CR. • Subjects with persistent or relapsed minimal residual disease (MRD) (by flow cytometry, PCR, FISH, or next generation sequencing) require verification of MRD on two occasions at least 2 weeks apart. • Subjects with Philadelphia Chromosome positive acute lymphoblastic leukemia (Ph+ALL) are eligible if they progressed after receiving a tyrosine kinase inhibitor (TKIs). • Subjects with recurrence of isolated CNS relapse after achieving complete remission (CR) are eligible.	☐	☐	☐ **NA**

INCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
b. Disease Status of aggressive B-cell NHL • Histologically confirmed aggressive B cell NHL including the following types defined by WHO 2008: o DLBCL not otherwise specified; T cell/histiocyte rich large B cell lymphoma; DLBCL associated with chronic inflammation; Epstein-Barr virus (EBV)+ DLBCL of the elderly; OR o primary mediastinal (thymic) large B cell lymphoma; OR transformation of follicular lymphoma, marginal zone lymphoma or chronic lymphocytic leukemia/small lymphocytic lymphoma to DLBCL o Follicular Lymphoma Grade 3B o Subjects with DLBCL , Follicular Lymphoma Grade 3B –or– Subjects with transformed FL, MZL, or CLL/SLL who HAVE NOT received chemotherapy prior to transformation: Must have received an anthracycline regimen and an anti-CD20 monoclonal antibody (unless documented CD20-negative) and be refractory or relapsed after second line of DLBCL treatment. Subjects with a partial response to second line therapy must be ineligible for autologous transplant. o Subjects with transformed FL, MZL, or CLL/SLL who HAVE received anthracycline-containing chemotherapy prior to transformation: Must have progressed, had SD or recurred with transformed disease after initial treatment for DLBCL.	☐	☐	☐ **NA**
2. CD22 expression • Subjects with ALL: CD22 positive expression on malignant cells is required and must be detected by immunohistochemistry or flow cytometry. The choice of whether to use flow cytometry or immunohistochemistry will be determined by what is the most easily available tissue sample in each subject. CD22 expression must be demonstrated subsequent to any anti-CD22 targeted therapy (e.g. Moxetumomab pasudotox or inotuzumab ogozamicin) in subjects with ALL. • Subjects with aggressive B-cell NHL: CD22 expression at any level, including undetectable, will be acceptable. Subjects must have archival tissue available for analysis of CD22 expression or must be willing to undergo a biopsy of easily accessible disease.	☐ ☐	☐ ☐	
3. Prior Bone Marrow-Stem Cell Transplant Subjects who have undergone autologous SCT with disease progression or relapse following SCT are eligible. Subjects who have undergone allogeneic SCT will be eligible if, in addition to meeting other eligibility criteria, they have no evidence of GVHD	☐	☐	

INCLUSION CRITERIA	Yes	No	Supporting Documentation†
and have been without immunosuppressive agents for at least 30 days.			
4. Prior Therapy Wash-out At least 2 weeks or 5 half-lives, whichever is shorter, must have elapsed since any prior systemic therapy at the time the subject is planned for leukapheresis, except for systemic inhibitory/stimulatory immune checkpoint therapy, which requires 5 half-lives. Exceptions: a. There is no time restriction with regard to prior intrathecal chemotherapy provided there is complete recovery from any acute toxic effects of such; b. Subjects receiving hydroxyurea may be enrolled provided there has been no increase in dose for at least 2 weeks prior to starting apheresis; c. Subjects who are on standard ALL maintenance type chemotherapy (vincristine, 6-mercaptopurine or oral methotrexate) may be enrolled provided that chemotherapy is discontinued at least 1 week or 5 half-lives, whichever is shorter, prior to apheresis. d. Subjects receiving steroid therapy at physiologic replacement doses only are allowed provided there has been no increase in dose for at least 2 weeks prior to starting apheresis. e. For radiation therapy: Radiation therapy must have been completed at least 3 weeks prior to enrollment for apheresis, with the exception that there is no time restriction if the volume of bone marrow treated is less than 10% and also the subject has measurable/evaluable disease outside the radiation port or the site of radiation has documented progression.	☐	☐	
5. Prior CAR Therapy Subjects who have undergone prior CAR therapy will be eligible if < 5% of circulating levels of CD3+ cells express the previous CAR if a qualified assay exists; 30 days must have elapsed post CAR infusion prior to apheresis.	☐	☐	
6. Age greater than or equal to 18 years of age	☐	☐	
7. Eastern cooperative oncology group (ECOG) performance status of 0, 1, or 2; or Karnofsky ≥ 60% (See **section 13.1, Appendix A**)	☐	☐	
8. Normal Organ and Marrow Function (supportive care is allowed per institutional standards, i.e. filgrastim, transfusion) • ANC ≥ 750/uL* • Platelet count ≥ 50,000/uL* • Absolute lymphocyte count (ALC) ≥ 150/uL*	☐ ANC Platelet ALC	☐	

INCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
Adequate renal and hepatic function defined as: • Creatinine ≤ 2 mg/dL OR creatinine clearance (as estimated by Cockcroft Gault Equation) ≥ 60 mL/min • Serum ALT/AST ≤ 10x Upper limit of normal (ULN) (Elevated ALT/AST related to leukemia involvement of the liver will not disqualify a subject). • Total bilirubin ≤ 1.5 mg/dL, except in subjects with Gilbert's syndrome. * ALL subjects will not be excluded because of pancytopenia ≥ Grade 3 if it is felt by the investigator to be due to underlying leukemia.	Creatinine AST/ALT Bilirubin		
9. CNS Status Subjects with CNS involvement are eligible as long as there are no overt signs or symptoms that in the evaluation of the investigator would mask or interfere with the neurological assessment of toxicity.	☐	☐	
10. Females of childbearing potential must have a negative serum or urine pregnancy test within 7 days prior to leukapheresis (females who have undergone surgical sterilization or who have been postmenopausal for at least 2 years are not considered to be of childbearing potential)	☐	☐	
11. Ability to give informed consent. Must be able to give informed consent. Legal authorized representative (LAR) is permitted if subject is cognitively able to provide verbal assent.	☐	☐	

Subjects meeting any of the following criteria are not eligible for participation in Stage 1 (Leukapheresis) of the study:

EXCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
1. Recurrent or refractory ALL limited to isolated testicular disease.	☐	☐	
2. History of other malignancy, unless disease free for at least 3 years. At the discretion of the Principal Investigator, subjects in remission for 1-2 years prior to enrollment may be deemed eligible after considering the nature of other malignancy, likelihood of recurrence during one year following CAR therapy, and impact of prior treatment on risk of CD22-CAR T cells. Subjects in remission <1 year are not eligible. • Exception: Nonmelanoma skin cancer or carcinoma in situ (e.g. cervix, bladder, breast) is eligible.	☐	☐	

EXCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
• Hormonal therapy in subjects in remission > 1 year will be allowed.			
3. Presence of active fungal, bacterial, viral, or other infection that is uncontrolled requiring intravenous antimicrobials. Simple UTI and uncomplicated bacterial pharyngitis are permitted if responding to active treatment.	☐	☐	
4. Current knowledge of infection with: • HIV, • Hepatitis B (HBsAg positive) or • Hepatitis C virus (anti-HCV positive). A history of hepatitis B or hepatitis C is permitted if the viral load is undetectable per quantitative PCR and/or nucleic acid testing.	☐	☐	
5. CNS disorder such as cerebrovascular ischemia/hemorrhage, dementia, cerebellar disease, or any autoimmune disease with CNS involvement that in the judgment of the investigator may impair the ability to evaluate neurotoxicity.	☐	☐	
6. History of myocardial infarction, cardiac angioplasty or stenting, unstable angina, or other clinically significant cardiac disease within 12 months of enrollment.	☐	☐	
7. History of severe immediate hypersensitivity reaction to any of the agents used in this study.	☐	☐	
8. Women who are pregnant or breastfeeding.	☐	☐	
9. In the investigator's judgment, the subject is unlikely to complete all protocol-required study visits or procedures, including follow-up visits, or comply with the study requirements for participation.	☐	☐	
10. Primary immunodeficiency or history of autoimmune disease (e.g. Crohns, rheumatoid arthritis, systemic lupus) requiring systemic immunosuppression/systemic disease modifying agents within the last 2 years.	☐	☐	

†All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

IV. Statement of Eligibility

By signing this form of this trial I verify that this subject is [☐ **eligible** / ☐ **ineligible**] for participation in **Stage 1: Leukapheresis** on the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Treating Physician Signature:	Date:
Printed Name:	
Secondary Reviewer Signature:	Date:
Printed Name:	
Study Coordinator Signature:	Date:
Printed Name:	

I. Screening and Enrollment for Lymphodepleting Chemotherapy and CD22-CAR T cell infusion

Protocol Title:	**Phase I/Ib Clinical Trial of Autologous CD22 Chimeric Antigen Receptor (CAR) T cells in Adults with Recurrent or Refractory B Cell Malignancies**
Protocol Number:	**CCT5029 / IRB-50836**
Protocol Director:	**Matthew Frank, MD, PhD**

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☐ Female

III. Study Information:

SRC Approved ☐ IRB Approved ☐ Contract signed ☐

IV. Inclusion/Exclusion Criteria for Stage 2: Lymphodepleting Chemotherapy and CD22-CAR T cell infusion

INCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
1. Measurable Disease • Subjects with ALL: must have evaluable or measurable disease (MRD positive by flow cytometry, NGS, or PCR is acceptable). • Subjects with aggressive B-cell NHL: must have evaluable or measurable disease according to the revised IWG Response Criteria for Malignant Lymphoma[38]. Lesions that have been previously irradiated will be considered measurable only if progression has been documented following completion of radiation therapy.	☐ ☐	☐ ☐	
2. Toxicities due to prior therapy must be stable or resolved (except for clinically non-significant toxicities such as alopecia or cytopenias)	☐	☐	
• Adequate renal, hepatic, pulmonary and cardiac function defined as: • Creatinine ≤ 2 mg/dL • Cardiac ejection fraction ≥ 45%, no evidence of physiologically significant pericardial effusion as determined by an ECHO, MUGA or Cardiac MRI [performed **within 180 days or after most recent anthracycline based treatment or mediastinal**	☐ ANC Platelet Creatinine	☐	

INCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
radiation therapy (whichever is most recent)] • No clinically significant ECG findings • No clinically significant pleural effusion • Baseline O_2 saturation > 92% on room air * A subject will not be excluded because of pancytopenia ≥ Grade 3 if it is felt by the investigator to be due to underlying leukemia/lymphoma.	LVEF ECG Pleural SaO_2		
3. CNS Status Subjects with CNS involvement are eligible as long as there are no overt signs or symptoms that in the evaluation of the investigator would mask or interfere with the neurological assessment of toxicity.	☐	☐	
4. Females of childbearing potential must have a negative serum or urine pregnancy test within 7 days of lymphodepleting chemotherapy (females who have undergone surgical sterilization or who have been postmenopausal for at least 2 years are not considered to be of childbearing potential)	☐	☐	
5. Contraception Subjects of child-bearing or child-fathering potential must be willing to practice birth control from the time of enrollment on this study and for four (4) months after receiving the preparative lymphodepletion regimen.	☐	☐	
6. Ability to give informed consent. Must be able to give informed consent. Legal authorized representative (LAR) is permitted if subject is cognitively able to provide verbal assent.	☐	☐	

Subjects meeting any of the following criteria are not eligible for participation in Step 2 (Lymphodepleting Chemotherapy and CD22-CAR T cell infusion) on the study:

EXCLUSION CRITERIA	**Yes**	**No**	**Supporting Documentation†**
1. Recurrent or refractory ALL limited to isolated testicular disease.	☐	☐	
2. Hyperleukocytosis (≥ 50,000 blasts/μL) or rapidly progressive disease that in the estimation of the investigator and sponsor would compromise ability to complete study therapy.	☐	☐	

EXCLUSION CRITERIA	Yes	No	Supporting Documentation†
3. Presence of active fungal, bacterial, viral, or other infection that is uncontrolled requiring intravenous antimicrobials. Simple UTI and uncomplicated bacterial pharyngitis are permitted if responding to active treatment.	☐	☐	
4. Women who are pregnant or breastfeeding.	☐	☐	
5. In the investigator's judgment, the subject is unlikely to complete all protocol-required study visits or procedures, including follow-up visits, or comply with the study requirements for participation.	☐	☐	
6. Acute neurological toxicity > Grade 1 (with the exception of peripheral sensory neuropathy)	☐	☐	

†All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

IV. Statement of Eligibility

By signing this form of this trial I verify that this subject is [☐ **eligible** / ☐ **ineligible**] for participation in **Step 2 Lymphodepleting Chemotherapy and CD22-CAR T cell infusion** in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Treating Physician Signature:	Date:
Printed Name:	
Secondary Reviewer Signature:	Date:
Printed Name:	
Study Coordinator Signature:	Date:
Printed Name:	

13.8.2 Informed Consent Process

All participants must be provided a consent form prior to Stage 1 Leukapheresis (if leukapheresis is to be performed on this study) and prior to Stage 2 Lymphodepleting Chemotherapy and Cell Infusion describing the study parts with sufficient information for subjects to make an informed decision regarding their participation. The investigational nature and research objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts and potential benefits, and alternative therapies will be carefully explained to the subject, and the subject/LAR asked to review the consent form and to ask questions prior to agreeing to participate in each stage of this protocol. The subject is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences.

The original signed copy of the consent documents must be retained in the research file and a copy placed in the medical record.

13.8.3 Subject Screening Using Two Stages for Consent and Enrollment

13.8.3.1 Screening for Step 1

(a) CD22 expression

For R/R ALL subjects: Expression will be evaluated by immunohistochemistry or by flow cytometry in a CLIA approved laboratory and documentation at screening will be required (testing is permitted to be conducted at any time since diagnosis, unless subject has received anti-CD22 targeted therapy, such as Moxetumomab pasudotox or inotuzumab ozogamicin, in which case the specimen must be collected after such therapy).

For R/R aggressive B-cell NHL: CD22 expression at any level, including undectable, will be acceptable. Subjects must have archival tissue available for analysis of CD22 expression or must be willing to undergo a biopsy of easily accessible disease.

(b) Medical history and concomitant medication

The subject's complete history through review of medical records and by interview will be collected and recorded. Concurrent medical signs and symptoms must be documented to establish baseline severities. A disease history, including the date of initial diagnosis and list of all prior treatment including BMSCT and CAR therapies, responses, and duration of response to the prior treatment also will be recorded. Most current disease status will be determined to include presence and location of current disease. Prior therapy washout periods will be calculated. History of known infection with HIV, hepatitis B and hepatitis C will be elicited, but viral testing is not necessary, as this will be performed standard of care prior to the leukapheresis procedure.

(c) Physical examination

A complete physical examination will be performed. The exam will include general appearance of the subject, height and weight, examination of the skin, eyes and ears, nose, throat, lungs, heart, abdomen, extremities, musculoskeletal system, and nervous system.

(d) Vital signs, including blood pressure, heart rate, oxygen saturation and temperature

(e) Performance status (ECOG or Karnofsky) see Appendix A, Section 13.1

(f) β-HCG pregnancy test on all women of child-bearing potential

(g) General Laboratory Tests: The following will be obtained during the screening process for Step 1:

- Chemistries: sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), creatinine, glucose, calcium, AST, ALT, alkaline phosphatase, bilirubin, albumin, total protein
- CBC with differential

13.8.3.2 Screening for Step 2

(a) Physical examination will be performed and changes from Step 1 noted.

(b) Vital signs, including blood pressure, heart rate, oxygen saturation and temperature

(c) Performance status (ECOG or Karnofsky) see Appendix A, Section 13.1

(d) Electrocardiogram (ECG)

(e) ECHO, MUGA or Cardiac MRI for LVEF and pericardial effusion assessment [performed **within 180 days or after most recent anthracycline based treatment or mediastinal radiation therapy** (whichever is most recent)]

(f) Creatinine clearance: A measured 24 hour urine creatinine clearance test may be performed if the serum creatinine is elevated, and the measured value will be recorded in the CRF and may be used to qualify the subject for study participation.

(g) β-HCG pregnancy test on all women of child-bearing potential (within 7 days prior to starting chemotherapy)

(h) Coagulation tests including prothrombin time (PT)/ partial thromboplastin time (PTT)

(i) General Laboratory Tests: The following will be obtained during the screening process for Stage 2 (unless previously performed within 28 days with no subsequent treatment initiated):

- Chemistries: sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), creatinine, glucose, calcium, AST, ALT, alkaline phosphatase, bilirubin, albumin, total protein
- Phosphorus and magnesium
- CBC with differential
- C-reactive protein (CRP), ferritin and Triglyceride level
- Urinalysis

(j) Additional Tests:

The investigator may order additional tests in some subjects if needed to fully assess clinical status and obtain baseline results. Examples of such tests include: haptoglobin, soluble IL2R, immunoglobulin levels, and viral serology or PCR for cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV), and Epstein-Barr virus (EBV).

(k) Disease Evaluation (methods will be determined by investigator based on subjects' location of disease, not all are required on all subjects), including determination of measurable disease.

- Imaging Studies appropriate to sites relevant to subject's disease: subjects with aggressive B-cell NHL will undergo PET/CT; other imaging studies (e.g. MRI of the brain) will be performed as determined by investigator
- Bone marrow aspirate/biopsy: subjects with ALL will undergo bone marrow biopsy, subjects with bone marrow involvement prior to therapy or if new abnormalities in the peripheral blood counts or blood smear cause suspicion of bone marrow involvement will undergo bone marrow aspirate and biopsy
- Lumbar puncture, if determined as clinically necessary by the investigator

(a) Concomitant Medication

A list of concomitant medications will be captured at the end of screening at the time of confirmation of eligibility. Medications that are stopped during the screening period prior to eligibility sign off do not need to be recorded. The concomitant medication list will be updated at each clinic visit.

13.8.4 Study Calendar Using Two Stages for Enrollment

	Stage 1: Leukapheresis		Stage 2: Lymphodepleting Chemotherapy and CD22-CAR T Cell Infusion										
				Preparative Regimen and Cell Infusion			Post Treatment Assessment				Long Term Follow-Up[L]		Long Term Gene Therapy Follow Up (until year 15)
Procedure	**Screening[A] (≤ 28 days of enrollment, unless specified)**	**Apheresis[D] (up to 24h prior unless noted)**	**Screening[A] (≤ 28 days of enrollment, unless specified)**	**Day -5, Day -4, Day -3**	**Prior to cell infusion (< 24 h)**	**Day 0**	**5x per week[I], Day 1 to Day 14 (± 2 d)**	**Twice weekly, Day 15 to Day 27 (± 4 d)**	**Day 28 (± 4 d)**	**Monthly, Month 2-3 (±2 weeks)**	**Month 6 (±1 mo) 9, (±1 mo) 12 (±2 mo) q6-12 months to 5 years**	**Annually ,Year 6 to 15 (±3 mo)**	
Medical History	X												
Physical Exam	X		X		X	X	X[I]	X[I]	X	X	X		
Vital signs	X	X	X	X	X	X[F]	X[F]	X[F]	X	X	X		
Neurologic exam			X		X	X[H]	X[H]	X[H]	X[H]	X[H]	X[H]		
Performance Status	X		X						X	X	X		
Height	X			X[S]									
Weight	X	X		X[S]					X	X	X		
Labs[c]													
• CBC with diff	X	X[D]	X	X	X	X	X[I]	X[I]	X	X	X		
• PT/PTT			X										
• Chemistries[B]	X	X[D]	X	X	X	X	X[I]	X[I]	X	X			
• Phosphorus		X[D]	X	X[S]		X	X[I]	X[I]					
• Magnesium		X[D]	X	X[S]	X	X	X[I]	X[I]					
• CRP		X[D]	X	X[S]	X	X	X[I]	X[I]	X				
• Ferritin			X	X[S]			X[I]	X[I]	X				
• Uric acid and LDH				X[S]		X	X[I]	X[I]					
• CD22 staining by IHC or flow cytometry	X[P]												
• Quantitative IgG immunoglobulins									X[M]	X[M]			
• Urine analysis			X										
• β-HCG pregnancy test on all females of child-bearing potential	X	X[C]	X	X[C]									
• RCL blood sample					X[N]					X[N]	X[N]		
ECG			X										
ECHO, MUGA or cardiac MRI			X[Q]										
Correlative Research Studies	Correlative samples outlined in Section 13.7, Appendix G												
• Leukapheresis		X							X[J]	X[J]	X[J]		
Disease Evaluation													

	Stage 1: Leukapheresis		Stage 2: Lymphodepleting Chemotherapy and CD22-CAR T Cell Infusion										
				Preparative Regimen and Cell Infusion			Post Treatment Assessment				Long Term Follow-Up[L]		Long Term Gene Therapy Follow Up (until year 15)
Procedure	**Screening[A] (≤ 28 days of enrollment, unless specified)**	**Apheresis[D] (up to 24h prior unless noted)**	**Screening[A] (≤ 28 days of enrollment, unless specified)**	**Day -5, Day -4, Day -3**	**Prior to cell infusion (< 24 h)**	**Day 0**	**5x per week[I], Day 1 to Day 14 (± 2 d)**	**Twice weekly, Day 15 to Day 27 (± 4 d)**	**Day 28 (± 4 d)**	**Monthly, Month 2-3 (±2 weeks)**	**Month 6 (±1 mo) 9, (±1 mo) 12 (±2 mo) q6-12 months to 5 years**	**Annually ,Year 6 to 15 (±3 mo)**	
• PET/CT chest/abdomen/pelvis, brain MRI (or other appropriate imaging)			X[K]						X[K]	X[K]	X[K]		
• Lumbar puncture			X[K]						X[K]	X[K]	X[K]		
• Bone marrow aspirate			X[K]						X[K]	X[K]	X[K]		
• MRD blood sample (Subjects with ALL only with identifiable clones)			X[K]						X[K]	X[K]	X[K]		
• Lymph node biopsy			X[R]				X[R]	→	X[R]		X[R]		
Treatment Regimen													
•													
• Lymphodepleting chemotherapy				X[E]									
• CD22-CAR T cells infusion						X[G]							
Response Evaluation[K,L]									X	X	X	X	
Adverse Events[U]		X		X	→	→	→	→	X	X[O]	X[O]		X[O]
Concomitant Medications	X	X	X	→	→	→	→	→	X	X[O]	X[O]		X[O]
Long term follow up questionnaires												X[T]	X[T]

A: Within 28 days prior to enrollment on this step, unless otherwise specified.
B: Laboratory evaluation to include; Chemistries: Sodium (Na), Potassium (K), Chloride (Cl), Total CO_2 (bicarbonate), Urea nitrogen (BUN), Creatinine, Glucose, Calcium (Ca) total, ALT/GPT, AST/GOT, Alkaline Phosphatase, Bilirubin, Albumin, Total Protein); creatinine clearance may be performed if the serum creatinine is elevated. The PI may order additional tests in some subjects if needed to fully assess clinical status and obtain baseline results. Examples of such tests include: haptoglobin, soluble IL2R, immunoglobulin levels, and viral serology or PCR for cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV), and Epstein-Barr virus (EBV).
C: Within 7 days prior to leukapheresis procedure and Day -5 (not repeated if screening labs performed within 7 days).
D: For cell acquisition for product development. Visit may be skipped if subject has cryopreserved cells that meet IND criteria. Labs required within 7 days prior to leukapheresis.
E: Subjects will receive hydration and prophylactic medications according to institutional standard practice prior to, during and after lymphodepletion chemotherapy.
F: Vital signs (blood pressure, heart rate, respiratory rate, pulse oximetry, temperature) collected on Day 0: prior to infusion, at the conclusion of the infusion (±10 min), 60 min. (±10 min) and as clinically indicated, then Day 1-28: every shift (± 2 hrs) during hospitalization, and with each visit after discharge.
G: Premedications will be provided as described in protocol **Section 6.6**. Subject may be offered additional CAR T cell treatments if enough cells were manufactured from the initial preparation, the subject experienced at least partial response to the first infusion, and the subject meets all eligibility criteria. Subsequent infusions will follow the same procedures as the first.
H: Neurologic exam daily and ICE score every shift (± 2 hrs) during hospitalization, then neurologic exam with each visit after discharge. After Day 28, continue until end of neurotoxicity or at investigator discretion.

I: Five (5) times per week from Day 1 to Day 14 (± 2 days) (unless otherwise indicated) with visits no more than 48 hours apart, and then twice weekly until Day 28 (± 4 days). LDH testing only required if elevated at baseline (prior to lymphodepletion chemotherapy). Uric acid only at baseline (Day -5 or -6). Phosphorus, magnesium, CRP, Ferritin, and LDH daily while hospitalized during initial hospitalization, then Day 14 (± 3 days) and Day 21 (± 3 days).

J: Subjects may undergo a small volume apheresis (approx. one to two blood volumes) in lieu of standard blood draw to obtain peripheral blood lymphocytes for correlative studies including Day 28 (±7 days), and 3 (± 2 weeks) , 6 (± 1 month) , 9 (± 1 month) , and 12 (± 2 months) months.

K: Subjects with leukemia will undergo bone marrow biopsy and patients with aggressive B-cell NHL will undergo PET/CT (or PET at the investigator's discretion) at screening, Day 28 (±4 days) and Month 3 (± 1 month), 6 (± 1 month), then per standard of care frequency. Disease evaluations may be repeated if >28 days passes between enrollment and start of lymphodepletion chemotherapy or if bridging anti-neoplastic therapy is used post-enrollment (per **Section 5.3.1**). Other methodologies including lumbar puncture, x-rays, MRI, MRD evaluation, lymphocyte subsets, etc determined by investigator based on sites and type of disease may be used; consistency throughout protocol evaluations is necessary. Day 28 will be considered the response evaluation for subjects with ALL. Month 3 will be considered the response evaluation for subjects with aggressive B-cell NHL.

L: Subjects will be monitored by physical exams, disease evaluations, vitals, CBC with diff, Chemistries, and IgG levels until relapse or subject proceeds to other therapies at investigator's discretion; at which time Long-Term Gene Therapy follow-up will proceed as per **Section 5.5.5.** Subjects with progressive disease or who start alternative therapies will undergo evaluations for long term gene therapy follow up ONLY; additional testing (i.e. correlative samples, quantitative immunoglobulins, etc) may be performed at the investigator's discretion. Evaluations may be performed by an outside physician or oncologist as long as required measures are included and maintained in the subject's research record.

M: Immunoglobulin levels every 3-12 weeks post infusion until Month 3 (±2 weeks).

N: RCL samples collected and sent to IU GTTL: prior to cell infusion; and at 3 (± 1 month), 6 (± 1 month), and 12 months (± 2 months) post cell infusion, unless FDA approved for archived specimens. Subsequent RCL blood samples will be stored annually for 4 additional years if all RCL in first year negative.

O: Targeted adverse/serious adverse event reporting and concomitant medication collection until Month 24, disease progression, or start of alternate therapy (whichever occurs first).

P: For R/R ALL: CD22 expression can be any time since diagnosis but must be re-demonstrated if patient has received targeted anti-CD22 therapy. For R/R aggressive B-cell NHL: CD22 expression is not required. Subjects must have archival tissue available or must be willing to undergo a biopsy of easily accessible disease.

Q: Testing performed within 180 days or after most recent anthracycline based treatment or mediastinal radiation therapy may be used for confirmation of eligibility

R: If feasible and easily accessible, lymph node biopsy will be obtained from subjects with lymphoma at baseline, and once between Day 7 and Day 28. A PBMC sample (for CAR T cells, etc) and lymph node biopsy may be collected at the time of progression, prior to starting any subsequent anticancer therapy, if clinically feasible.

S: Height within 30 days prior to Day -5. Others collected once, Day -5 or Day -6 only.

T: After 5 years, health status data will be obtained from surviving subjects via telephone contact or mailed questionnaires. The long term follow up period for lentiviral vectors is 15 years.

U: All adverse events will be recorded and reported from the start of lymphodepletion chemotherapy and conclude 30 days after the last study treatment. Prior to the start of lymphodepletion chemotherapy, only unexpected serious adverse events related to study procedures will be collected (see **Section 7.3.1**).

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