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COMPREHENSIVE CANCER CENTER

Protocol Title: A Phase I, Multicenter Study of CD4- directed chimeric antigen receptor engineered T-cells (CD4CAR) in patients with Relapsed or Refractory CD4+ Lymphoid Hematological Malignancies

Protocol Number: CTO-IUSCCC-ICG122-101

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PROTOCOL SIGNATURE PAGE

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VERSION DATE: 10/28/2025

I confirm I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable guidelines for good clinical practices, or the applicable laws and regulations of the country of the study site for which I am responsible, whichever provides the greater protection of the individual. I will accept the monitor's overseeing of the study. I will promptly submit the protocol to applicable ethical review board(s).

Instructions to the investigator: Please **SIGN** and **DATE** this signature page. **PRINT** your name and title, the name and location of the facility in which the study will be conducted, and the expected IRB approval date. Scan and email the completed form to Indiana University Simon Comprehensive Cancer Center and keep a record for your files.

_____ _____

Signature of Investigator Date

_____

Investigator Name (printed)

_____

Investigator Title

_____

Name of Facility

_____

Location of Facility (City and State)

_____ □ Not Submitting to IRB

Expected IRB Submission Date

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List of Abbreviations

APC	antigen presenting cell
aAPC	artificial APC
AE	adverse event
AML	acute myeloid leukemia
ASTCT	American Society for Transplantation and Cellular Therapy
B-ALL	B cell acute lymphoblastic leukemia
CART-19 cells	redirected autologous T cells
CIR	chimeric immune receptor
CFR	code of federal regulations
CMV	cytomegalovirus
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse events
CTRC	clinical and translational research center
CTL	cytotoxic T lymphocyte
CD137	4-1BB co-stimulatory molecule
DFS	disease free survival
DLCO	diffusing capacity of the lungs for carbon monoxide
DSMB	data safety and monitoring board
EF	ejection fraction
FACT	Foundation for the Accreditation of Cellular Therapy
FDA	Food and DAdrug dministration
GCP	good clinical practices
GMP	good manufacturing practices
GVHD	graft versus host disease
HAMA	human anti-mouse antibodies
HSCT	hematopoietic stem cells transplant
ICANS	immune effector cell associated neurotoxicty
ICE	Immune Effector-Cell Associated Encephalopathy
IBC	Institutional Biosafety Committee
IRB	Institutional Review Board
MRD	minimal residual disease
NCI	National Cancer Institute
PBMC	peripheral blood mononuclear cells

RAC	NIH Office of Biotechnology Recombinant DNA Advisory Committee
RCL	replication competent lentivirus
SAE	serious adverse event
scFv	single chain Fv fragment
T-ALL	T cell acute lymphoblastic leukemia
TCR	T cell receptor
TCR-ζ	Signaling domain found in the intracellular region of the TCR zeta, gamma and epsilon chains
Vβ	A rearranged T cell specific gene that can be used to determine clonality of a T cell population

Protocol Synopsis

Title	A Phase I, Multicenter Study of CD4- directed chimeric antigen receptor engineered T-cells (CD4CAR) in patients with Relapsed or Refractory CD4 Positive Lymphoid Hematological Malignancies
Short Title	CD4 redirected autologous T cells CARs
Protocol Number	CTO-IUSCCC-ICG122-101
Phase	Phase 1 / Pilot
Methodology	Open-label dose escalation with lab correlates
Study Duration	Approximately 36 months
Study Center(s)	Multicenter 1. Indiana University Simon Comprehensive Cancer Center (Coordinating Center) 2. Stony Brook Cancer Center 3. University of Miami 4. Albert Einstein Health Network 5. University of Texas MD Anderson Cancer Center
Objectives	The primary objectives are 1. To identify dose limiting toxicities of CD4CAR and the recommended phase II dose. 2. Describe toxicity profile of CD4CAR within the scheduled dose escalation Secondary objectives are to describe in vivo behavior of CD4CAR
Number of Subjects	Up to 30 subjects will be enrolled
Diagnosis and Main Inclusion Criteria	Inclusion criteria include patients aged ≥ 12 years old with CD4+ T cell hematologic malignancies with either relapsed or refractory disease (including those patients who have undergone a prior transplant (if allogeneic, subjects are eligible if there are no remaining donor cells) and patients with an inadequate response after 4-6 cycles of standard chemotherapy) are eligible. Response criteria for each disease subset will be evaluated based on Standard of Care Guidelines.
Study Product, Dose, Route, Regimen	Autologous T cells transduced with a lentiviral vector to express the scFv nucleotide sequence of the anti-CD4 molecule derived from humanized monoclonal ibalizumab and the intracellular domains of CD28 and 4-1BB co-activators fused to the CD3zeta T-cell activation signaling domain administered. To be given by IV infusions as a single dose (total dose of up to ~$2x10^6$ T Cells/Kg)

Duration of administration	Approximately 5-30 minutes; drug is expected to persist at detectable levels in circulation for an average of 28 days
Reference therapy	None. The experimental product described in this protocol will be given to subjects with unmet medical needs for which there are no effective therapies known at this time.

Schedule of Events

If any tests/procedures diverge from those listed on the Schedule of Events below due to Institutional Policies (i.e. MUGA in lieu of an ECHO, etc.), those policies will be followed with approval of the Sponsor Investigator. These will not constitute a deviation.

Schedule of Events	Screening (Section 5.3 and 7.2)*****	Leukapheresis for 1st manufacturing attempt*****	Second Screening (Section 5.3)*****	Conditioning Chemotherapy *****	Third Screening (Section 5.5)	CD4CAR Infusion	Follow-Up****								
	-42 to -7	-42 to -35	~Day -7 to -5	~Day -4 to -2	~Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 through discharge*	Post-discharge follow up to 2 years[A]	Up to twice a year for up to 15 years
Informed Consent Form	X														
Demographics & Medical History	X		X	X											X[I]
Leukapheresis		X**													
Conditioning Chemotherapy				X											
Administer CD4CAR						X[4]									
Hospital Stay				X	X										
Cytokine release syndrome evaluations[K]					X[K]										
Neurological Examination (ICANS/ICE)[L]					X[L]										
Physical Examination	X			X	X	X[5]	X	X	X	X	X	X	X	X	X[AN]
Vital Signs including temperature, respiratory rate, blood pressure, and pulse and oxygen saturation						X[5]									
Psychosocial Assessment[J]	X														
ECOG Performance Status	X			X	X									X	
EKG (12 lead)	X**				X[U]			X[AN]						X[B]	
Cardiac function assessment[10,13]	X**		X[AN]				X[AN]								
CBC w/diff [2,10]	X**		X	X	X	X[4]	X	X	X	X	X	X	X	X, **SEE NOTE[Ai]**	
CMP (chemistry)[2,7,10]	X**		X	X	X	X[4]	X	X	X	X	X	X	X	X	

Schedule of Events	Screening (Section 5.3 and 7.2)*****	Leukapheresis for 1st manufacturing attempt*****	Second Screening (Section 5.3)*****	Conditioning Chemotherapy *****	Third Screening (Section 5.5)	CD4CAR Infusion	Follow-Up****								
	-42 to -7	-42 to -35	~Day -7 to -5	~Day -4 to -2	~Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 through discharge*	Post-discharge follow up to 2 years[A]	Up to twice a year for up to 15 years
Creatinine Clearance Assessment	X**			X[AN]		X[AN]									
Uric Acid (Serum Urate)[T]	X**			X		X[4,7]	X[7]	X[7]	X[AN]					X[C]	
Serum β2-microglobulinT	X														
Ig (IgA, IgM, IgG, IgD, IgE)[10]	X**												X[AN]	X[D]	X[AN]
PT, PTT	X**				X	X	X	X	X	X	X	X	X	X[E]	
CRP[1] and Ferritin Level	X**				X		X	X	X	X	X	X	X	X[F]	
Pregnancy Test***	X		X												
Infectious Disease Markers (incl. hepatitis)	X[M, **]														
Viral and Fungal Markers	X[N]					X[G]								X[G]	
Syphilis[10,R]	X**														
Urinalysis (routine)				X[O]										X[AN]	
Disease assessment[3,10]	X[AN,**]													X[AN]	X[AN]
ABO & Rh Screen[10]	X**														
Bone Marrow Aspirate/Biopsy	X[AN,9]				X[AN]					X[AN]				X[AN]	
Spinal Tap and CSF analysis[3]			X[AN]												
Tissue Sample[3]			X[AN]												
Clinical Flow Cytometry[10,Q]	X**				X[Q]	X							X[AN]	X[AN]	
Survival		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X[I]	X[I]
Follow up Questionaire															X
Concomitant Medications[S]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Correlative Studies											
Schedule of Events	Screening (Section 5.1 and 7.2)*****	Leukapheresis For 1st manufacturing attempt*****	Conditioning Chemotherapy *****	CD4CAR Infusion	Follow-Up****						
	-42 to -7	-42 to -35	~Day -4 to Day -2	Day 0	Day 1	Day 3	Day 4	Day 5	Day 7 through discharge	Post-discharge follow up to 2 years[A]	Up to twice a year for up to 15 years
Research Flow Cytometry[P]			X[6]			X		X	X[11]	X[F]	
Transgene copy number (TCN)			X[6]			X		X	X[11]	X[F]	
HAMA			X[6]						X[12]	X[F]	
Replication competent lentivirus (RCL)[H]			X[6]							X[H]	X[H]
Cytokine Panel[1]			X[6]			X[1]		X[1]	X[1,11]	X[F]	
Optional blood samples for future research[8]			X[6,8]						X	X[8]	

* Repeat procedures listed under Day 7 until subject is discharged. Subjects will undergo approx. once a week thereafter for four weeks (until Day 28) the following: physical exam, documentation of adverse events and blood draws for hematology, chemistry, and engraftment and research labs.

** The following screening tests must be completed and subject is confirmed eligible prior to leukapheresis: CMP, CBC, Cardiac function assessment, creatinine clearance, research flow cytometry, clinical flow cytometry, Infectious disease Markers, Syphilis, PT, PTT, ABO & rh screen, EKG, uric acid, Ig (IgA, IgM, IgG, IgD, IgE), and ferritin level.

*** Females of childbearing potential only: required at screening, and within 7 days of beginning of conditioning chemotherapy.

**** All actionable tests will be done in a clinical lab. None of the research lab results will be utilized for treatment decisions at any point in the trial.

***** These windows may be exceeded with the necessity of repeat manufacturing and/or manufacturing delays. Leukapheresis will only occur **once** per subject. Falling outside these windows will not constitute a deviation.

AN. As clinically indicated or determined/required by the PI/clinical team/institutional policy

1 If CRS occurs while subject is hospitalized, repeat CRP and cytokine panel(research and clinical panel) every 8 +/- 2 hours, as feasible; in consideration of subject availability, and clinical/research staff operations, until CRS is resolved. Include IL-2, IL-12, IL-6, IL-15 (research only), IFNgamma and CRP. Bank two aliquot for future studies.

2 Chemistry values recorded in the database consists of Albumin, Total protein,Calcium,Glucose (non-fasting), BUN, Serum Uric Acid, Creatinine, Alk Phos, Total Bilirubin, Potassium, Sodium, Chloride, ALT, AST, CO2, LDH, Magnesium and Phosphorous.

CBC w diff values to be recorded in the database consist of WBC count, RBC count, Hgb, PLT, Hematocrit %, Lymphocytes absolute and manual/automated diff %, Monocytes absolute and manual/automated diff %, Neutrophils absolute and manual/automated diff %, and atypical lymphocytes manual differential. Manual differential will only be required if a % diff value indicates a grade 2 or greater AE.

3 Screening assessments should be completed within 7 days of Leukapheresis. Perform as clinically indicated, as deemed necessary by physician at other times post-infusion. Submission of tissue samples to the sponsor is not required. Assessments will be done at Day 14 and 28 post CD4CAR infusion, and according to standard of care and practices every 3 months for 2 years after CD4CAR cell infusions or until the subject requires alternative therapy for their disease per current NCCN guidelines for the subject's specific disease. Data related to disease assessments will be collected after 2 years as they are performed and data available per standard of care for up to 15 years. Tumor assessments will depend on the subject's underlying disease and may be performed by Imaging (any technique), Physical Exam, Bone Marrow Biopsy, or Spinal Tap/cerebrospinal fluid analysis. See section 7.8 for details.

4 To be obtained the day before or within 12 hours prior to CD4CAR infusion: CBC with diff, assessment of CD3, CD4, and CD8 counts, and serum potassium, and uric acid.

5 To include vital signs (including temperature, respiratory rate, pulse and oxygen saturation, and blood pressure) prior to infusion, at the end of infusion, and every 15 minutes (+/-5 minutes) for 1 hour post infusion. If vital signs values are abnormal, continue to monitor every 15 minutes (+/- 5 minutes) until stable (may be up to 6 hours post-infusion).

6 To be drawn Day -4 prior to start of conditioning chemotherapy

7 Uric acid and potassium to be drawn 3 times a day, for 48 hours post infusion

8 Optional blood samples: to be obtained at Day -4 prior to start of conditioning chemotherapy, and again approximately on Day 7, Day 14 (+/-3 days) and Day 28 (+/-3 days), day 60 (+/- 7 days), 3 months (+/- 7 days), 6 months (+/- 30 days) and 2 years (+/- 30 days) post CD4CAR infusion . The timing of this test should be decided in consultation with the principal investigator. Refer to ICF to verify subject's agreement before obtaining the sample.

9 Bone marrow biopsy/aspirate will only be done if clinically indicated (i.e. depending on the subject's diagnosis). Screening bone marrow biopsy/aspirates must occur no later than 30 days prior initiation of conditioning chemotherapy. Indolent skin lymphomas and other CD4 positive T cell hematological malignancies with very low incidence of marrow involvement may be excluded from screening bone marrow biopsy/aspirate requirement at the discretion of the treating physician. If subject is receiving a Bone Marrow Aspirate or any other tissue sampling, send 2ml in a purple top tube from the sample to Dr. Salman's lab for research purposes.

10 The following tests do not need to be repeated if completed within 28 days of Day -7: disease assessments, clinical flow, CMP, CBC, Cardiac function assessment, Syphilis, Ig (IgA, IgM, IgG, IgD, IgE), or ABO & Rh screen.

11 Subjects will have blood drawn for cytokine levels (twice a week from day 7 to day 14, then only as needed), CD4CAR Transgene Copy Number (PCR) and flow cytometry (refer to footnotes A, F and P) in order to evaluate the presence of CD4CAR cells on day 3, 5, 7, 14 and 28 post infusion, or as clinically indicated.

12 HAMA sample will be drawn a single timepoint on day 7.

13 Cardiac function assessment may include ECHO or MUGA.

A. Subjects will be followed for a total of 15 years after infusion. The initial follow up phase will occur the first 2 years after infusion. Subjects will be evaluated on at least a quarterly basis until 2 years post infusion Follow up will continue an additional 13 years. Short term and intermediate follow up visits will be scheduled approximately as follows (compatibly with clinic operations, weekends and holidays) until either new carcinogenesis, bone marrow / stem celltransplant or death occur. Unscheduled follow up visits and tests can occur more frequently as clinically indicated. Unless otherwise indicated by a footnote, tests included in this column will occur at every follow up visit, including unscheduled visits.

   i. **Approx. once a week up to day 28 (+/-3 days) post infusion to assess hematologic dose limiting toxicity**; or as clinically indicated, then
   ii. Once a month (+/-7 days) from 2-6 months post-CD4CAR infusion, or more often as clinically indicated, then
   iii. Once every 3 months after month 6 (+/-30 days), for up to 2 years post-CD4CAR infusion, or more often as clinically indicated, then
   iv. Following evaluation at 2 years, subjects will be followed up to twice a year (+/-30 days) by phone and questionnaire (to include adverse events, quality of life, and disease status) for an additional thirteen (13) years to assess for the diagnosis of long-term health problems, such as development of new malignancy

B. Follow up EKG required at ~Day 28 visit only

C. Follow up uric acid will be performed at each outpatient visit occurring up to Day 14, and as clinically indicated thereafter

D. Follow up immunoglobulin testing occur monthly for up to 6 months, then quarterly up to year 2, or more often as clinically indicated.

E. Follow up PT/PTT test will be performed at a follow up visit occurring approximately on Day 14 post CD4CAR infusion, or more often if clinically indicated

F. These tests are performed until CD4CAR is undetected by two consecutives TCN and flow cytometry tests and at principal investigator and treating physician discretion

G. Viral (CMV, HHV6 and EBV) and fungal (β-D-glucan and aspergillus galactomannan) markers to be performed at least once weekly up to Day 28, and as clinically indicated thereafter.

H. RCL to be performed at Day -4 prior to start of conditioning chemotherapy, month 3, 6 and 12 months after CD4CAR infusion. If all samples for an individual subject are negative at one year, testing can be discontinued and annual review of medical history will suffice. If any sample is positive, additional evaluation will be required and continued monitoring beyond 12 months may be required after discussion with FDA. This will be performed by the Indiana University Gene Therapy Testing Laboratory.

I. Long-term safety follow-up will include monitoring for infections occurrence for up to 15 years, immunoglobulin (Ig) levels and need for intra-venous immunoglobulin for 2 years after CD4CAR infusion. Long term follow-up will be limited to documentation in medical records of secondary carcinogenesis and RCL for subjects who receive additional routine or experimental anticancer therapy, including medical, radiation or surgical intervention (with the exclusion of hematopoietic stem cells transplant

J. A note should be made in subject's chart confirming subject has adequate support and a documented caregiver.

K. Assessment for and grading of cytokine release syndrome (CRS) will be done at least daily and whenever clinically indicated (for example, with a change in clinical status). See section 10.3.1

L. The nursing staff will conduct focused neurologic examinations approx. every 8 hours for at least 7 days (or earlier if the subject is deemed stable for discharge prior to 7 days post-infusion) post CAR T cell infusion for ICANS using the American Society for Transplantation and Cellular Therapy's Immune Effector Cell-Associated Encephalopathy (ICE) score grading scale. See Section 10.3.2. See Table 4 and Table 5 for more details

M. Infectious disease markers to include Hepatitis B Core (HBC), Hepatitis B Surface Antigen (HBSAG), Hepatitis C Virus (HCV), NAT Triplex (if positive serology) (or institutional equivalent):, Human Immunodeficiency Virus (HIV), Human T-lymphotropic virus 1 (HTLV), Screening Test for Syphilis, West Nile Virus (WNV), Trypanosoma cruzi (Chagas, CGS), Cytomegalovirus (CMV), Zika Virus, Epstein Barr Virus, Toxoplasma, Herpes Simplex Virus (HSV) and Varicella.

N. Viral and Fungal Disease markers include Aspergillus Galactomannan Ag, CMV qPCR, EBV qPCR Fungitell-D, HHV-6 qPCR.

O. Urinalysis to be performed at least once during days -4 through -2 or more as clinically indicated

P. Research Flow Cytometry will evaluate the presence of CD4CAR expression. The conditioning chemotherapy collection will occur on **D-4 only** (as per standard of care).

Q. Clinical Flow Cytometry will include lymphocyte subset quantitation, including CD4, CD8, and CD3 cell determination (t-cell subsets) **and** a leukemia/lymphoma assay. Collection of the clinical flow will coincide with disease assessment collection timepoints (see footnote 3), as well as a mandatory collection within 12 hours of CD4CAR infusion (see section 9.1); **with the exception of**: if a subject has circulating tumor cells. If this occurs, the clinical flow will be collected on Day-1 (no collection on day 0 if day-1 sample is collected), and on Day 28. The clinical flow may be repeated more frequently as necessary at investigator discretion.

R. Syphilis to be performed at screening and repeated at investigator discretion as needed.

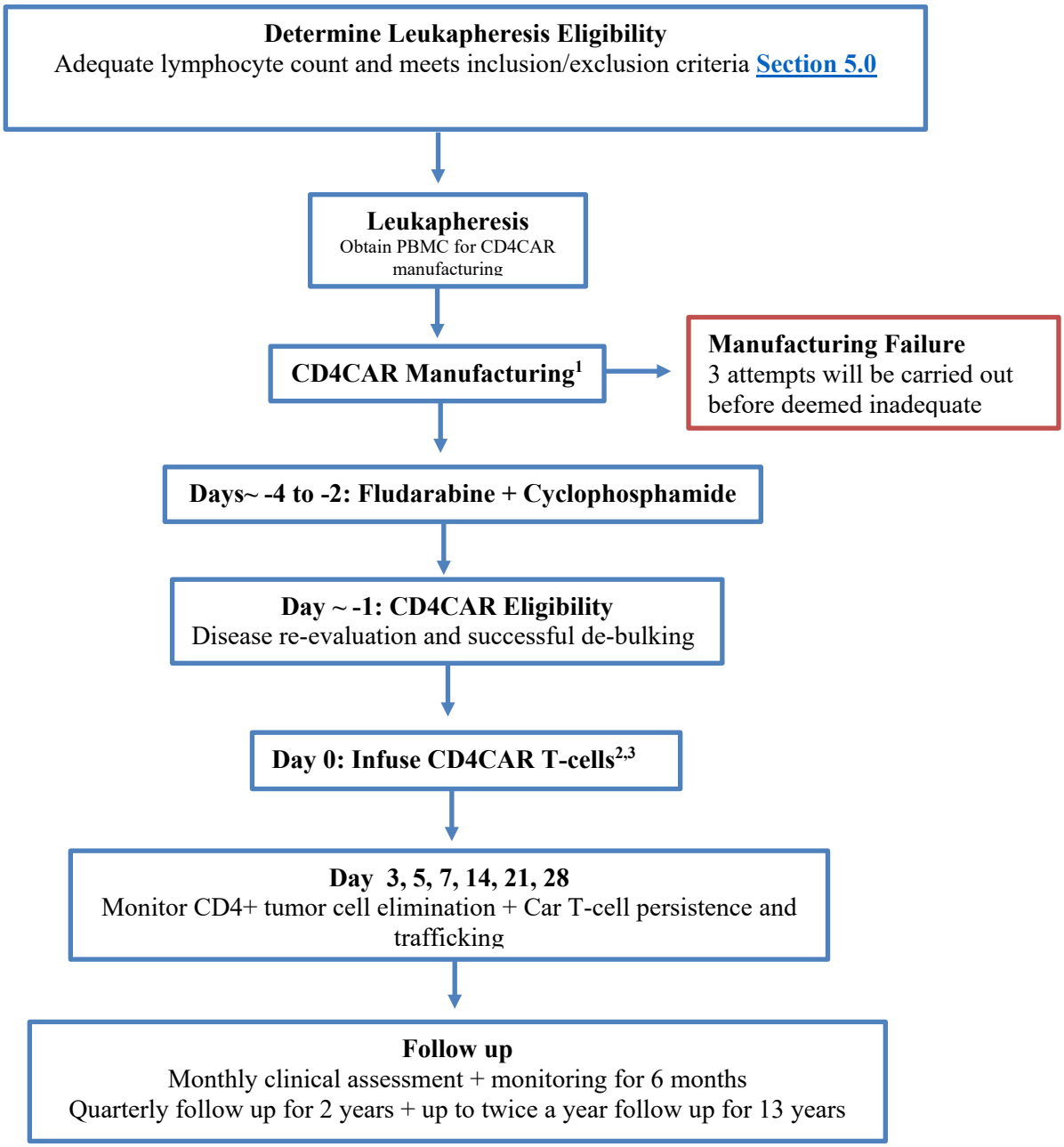
S. All prescription and nonprescription medications, vitamins, herbal and nutritional supplements, taken by the subject during the 30 days prior to screening will be recorded at the screening visit. At every visit following the conditioning chemotherapy up to the year 15 post CD4CAR infusion, concomitant medications will be recorded in the medical record and on the appropriate CRF. After year 2, concomitant medications will only be collected if the subject comes in-person for their follow-up visit. Any additions, deletions, or changes of these medications will be documented.

T. These tests to be completed per institutional guidelines.

U. EKG to be performed on day -1 or more as clinically indicated.

Figure 1: Study Schema

Subject Population: CD4 + lymphoid malignancies with no available curative treatment options



1. The cell product is expected to be ready for release approximately 4 weeks after single apheresis. Any additional manufacturing attempts will be made by using product stored from original apheresis collection.
2. CD4CAR infusions begin 3 to 4 days after completion of chemotherapy unless toxicities related to conditioning chemotherapy of ≥ grade 3 are present
3. To allow for full spectrum toxicity duration evaluation and reporting, no patients within the same or a different cohort will be initiated on conditioning chemotherapy sooner than **28 days** from the CD4CAR date of the preceding patient

1. INTRODUCTION

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of good clinical practice (FDA Title 21 part 312 and International Conference on Harmonization Guidelines), applicable government regulations and institutional research policies and procedures.

1.1 Background

CD4 positive hematological malignancies: CD4 is a glycoprotein found on the surface of immune cells such as T cells, monocytes, macrophages, and dendritic cells. It was discovered in the late 1970s and was originally known as leu-3 and T4 (after the OKT4 monoclonal antibody that reacted with it) before being named CD4 in 1984[1]. In humans, the CD4 protein is encoded by the CD4 gene. Like many cell surface receptors/markers, CD4 is a member of the immunoglobulin superfamily. CD4 assists the T cell receptor (TCR) in communicating with an antigen-presenting cell. Using its intracellular domain, CD4 amplifies the signal generated by the TCR by recruiting an enzyme, the tyrosine kinase Lck, which is essential for activating many molecular components of the signaling cascade of an activated T cell. Various types of T cells are thereby produced. CD4 also interacts directly with MHC class II molecules on the surface of the antigen-presenting cell using its extracellular domain. The extracellular domain adopts an immunoglobulin-like beta-sandwich with seven strands in 2 beta sheets.[2] CD4 is uniformly expressed on peripheral T cell lymphoma (PTCL), angioimmunoblastic T cell lymphoma (AITL) and on 60% of T-ALL[3] and 40% of AML[4]. While rarer than the B cell malignancies, T cell tumors pose significant treatment challenges for affected adults and children. Current management of PTCL, for example, comes with a progression-free survival rates of 40% to 50%.[5] And although acute lymphoblastic leukemia (ALL) is now curable in 80–90% of cases, patients with T-ALL experience a higher frequency of induction failure and early relapses[4] compared with B-ALL patients. Most children and adults with relapsed T-ALL will not be cured. AML induction failures and chemo refractoriness remain a challenge to qualify patients for definitive therapy in the form of allogeneic stem cell transplant.[6]

Adoptive immunotherapy: Adoptive transfer is a term created by Medawar to describe acquired immunologic tolerance. Adoptive immunotherapy denotes the transfer of immunocompetent cells for the treatment of cancer or infectious diseases. Substantial progress has been made in our understanding of the molecular and cellular basis of T-cell-mediated antitumor responses[7, 8,9,10.] CD8+ T cells have been identified as potent effectors of the adaptive antitumor immune response.[7,8] The target antigens that are recognized by tumor-reactive CD8+ T cells have been shown to be mostly non-mutated self-antigens that are also expressed by tumor cells (these antigens are denoted here as self/tumor antigens). However, clinical targeting of T-cell lymphomas by adoptive immunotherapy is currently non-existent.

The adoptive transfer of engineered T cells with redirected specificity to express chimeric antigen receptors (CARs): The concept of CARs was first described 25 years ago as a means of introducing tumor specificity into adoptive cell therapy[11]. The initial design joined an antibody-derived scFv to the CD3ζ intracellular signaling domain of the T-cell receptor through hinge and transmembrane domains. The first CARs showed evidence of function in preclinical studies and had limited responses in clinical trials[7, 8]. The development of the "first generation" CAR lead to optimization of both in vitro and in vivo T-cell proliferation and persistence. Subsequently, "second-generation" CARs were developed by incorporating an additional domain, CD28 or 4-1BB, to supply a costimulatory signal. "Third generation" CARs were then developed by incorporating two costimulatory domains, a combination of CD27, CD28, 4-1BB, ICOS, or OX40[12, 13]. First-, second-, and third-generation CARs are depicted in Figure 2.

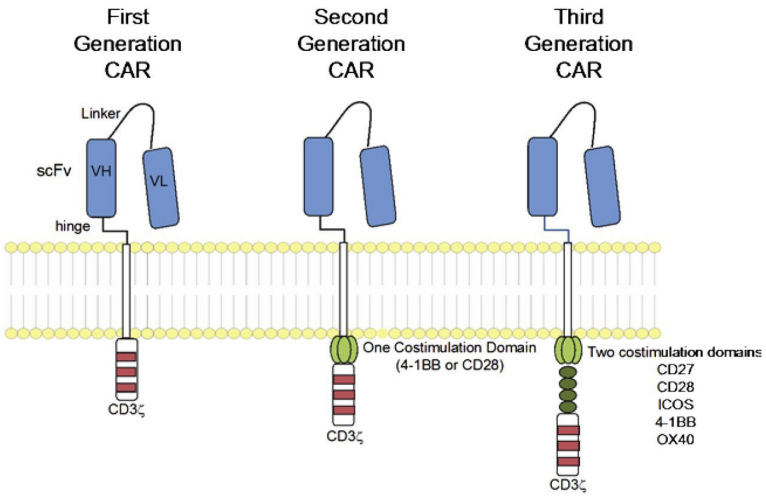


Figure 2. CAR Structure

CAR structure according to signaling domains. CAR molecules link an extracellular single-chain variable fragment (scFv) to intracellular signaling domains. The intracellular component includes the CD3ζ intracellular signaling domain of the T-cell receptor either alone (first generation) or in combination with 1 (second generation) or 2 (third generation) costimulatory domains.

CAR designs differ not only in their components but also in their functional properties. The CD3ζ signaling domain of the T-cell receptor, when engaged, will activate and induce proliferation of T cells but can lead to anergy. The addition of a costimulatory domain in second-generation CARs improved replicative capacity and persistence of modified T cells. The optimal costimulatory domain remains unknown. Similar antitumor effects are seen in vitro with CD28 or 4-1BB CARs, but preclinical in vivo studies suggest that 4-1BB CARs may produce superior proliferation and/or persistence.[13] Moreover, clinical trials suggest that both of these second-generation CARs are capable of inducing substantial T-cell proliferation in vivo, but CARs containing the 4-1BB costimulatory domain appear to persist longer. [14, 15, 16]

Another variable that may influence replicative capacity and persistence is the ex vivo cell culture system used to manufacture large quantities of engineered T cells. While various systems have been developed, current systems employ antibodies and/or artificial antigen-presenting cells to engage CD3 with costimulation provided by a second signal or cytokine.[17, 18] It appears that the in vitro culture system for T-cell expansion plays an important role in influencing the final composition of effector, naive, and memory T cells in the manufactured product. Although effector T cells may mediate potent cytotoxicity, they are terminally differentiated with minimal replicative capacity; comparatively, naive and memory T cells have significant replicative capacity and potential for long-term persistence, respectively. Several groups currently use an anti-CD3/CD28 antibody-coated magnetic bead system, developed by Levine *et al*[17], that allows manufactured T cells to maintain a memory phenotype and long-term persistence.[19, 20]

T cells that express CARs are engineered using gene transfer technologies, often simultaneously coupled with expansion techniques. Various modes of gene transfer can be employed, from retroviral and lentiviral vector methods resulting in permanent modification of the genome, to RNA-based methods leading to transient gene expression. Retro- or lentiviral approaches have the advantage of long-term gene expression and, therefore, the potential for long-term disease control from a single infusion of engineered T cells (if those T cells persist). The disadvantages of permanent modification are persistent on-target toxicity, as well as the theoretical risk of transformation, if gene insertion were to result in dysregulation of an oncogene. This has been reported with retroviral modification of hematopoietic stem cells, but not with lentiviral transduction and not when mature T cells are used as the target[21, 22]. Gene transfer using messenger RNA yields transient expression without integration into the genome, obviating any concern of transformation. Short-term expression may also be desirable for CARs directed against antigens expressed on normal cells when sustained on-target toxicity is a concern.

Lymphocyte Costimulation: Maximal activation, proliferation and persistence of T cells that respond to antigenic stimuli are dependent on the receipt of two discrete signals mediated by cell surface receptors. The primary "activation" signal is generated by ligation of the TCR with antigen in the form of peptides presented

in the groove of HLA class I molecules and the second signal by ligation of a costimulatory molecule with its cognate ligand. T cell costimulatory molecules which have been identified to date include members of the immunoglobulin super-family (CD28) and members of the tumor necrosis factor (TNF) super-family (e.g. CD40L, CD134, CD137 [4-1BB]).[23, 24] Signaling through the cytosolic domain of the usual scFv-TCRζ single chain construct does not fully replicate the multichain TCR signaling complex.[25, 26]. 4-1BB is a T cell co-stimulatory receptor induced by TCR activation and evokes various T cell responses. [27] Its signals are critical for long-term proliferation of CD8 cells. CD28 signals are essential for sustained CD4 cell proliferation[28, 29]. The requirement for costimulation was investigated in the CD19-CAR studies by incorporating additional signaling modules in the cytoplasmic domain of the chimeric receptor. Preclinical data[29], indicates that "bipartite receptors" comprised of TCRζ and either CD28 or 4-1BB signaling modules substantially improve the function and proliferation of T cells. The coalescence of improved T cell culture methods and lentiviral vector technology have made it possible to test this novel concept, which is now ready for clinical evaluation, and thereby advancing the field of cancer gene therapy by adoptive immunotherapy.

Rationale for Lymphodepletion: Host lymphodepletion may enhance the effectiveness of adoptively transferred T cells. Homeostatic T cell proliferation can result in the induction of autoimmunity, providing a clue to improved antitumor strategies. T cells can undergo up to seven rounds of cell division after being deprived of contact with APC. This homeostatic response of T cells is directed largely towards self-antigens. Lymphodepletion eliminates regulatory T-cells and other elements of the immune system that may act to down regulate necessary cytokines production, which enhances the availability of cytokines such as IL-7 and IL-15**.** This hypothesis has been tested clinically in patients with metastatic melanoma refractory to conventional treatments. In this scenario, the patients received a lymphodepleting conditioning regimen consisting of cyclophosphamide and fludarabine prior to adoptive transfer of T cells.[30] This lymphodepleting chemotherapy has improved engraftment. In addition, in the setting of lymphopenia, combined vaccine therapy and adoptive T-cell transfer fosters the development of enhanced memory T-cell responses.[31] These data indicate that the increased antitumor efficacy of adoptive transfer following host lymphodepletion is more than simply "making room" for cellular expansion.

1.2 Investigational Agent

The investigational agent in this protocol is a CD4-specific chimeric antigen receptor engineered T-cell (CD4CAR T-cell) (Figure 3)[32] which is an autologous human T-cell engineered to express an anti-CD4scFV domain derived from the monoclonal antibody (mAb) ibalizumab (Hu5A8 or TNX-355). In order to effectively bind CD4, this is paired with an antigen receptor with an intracellular tyrosine-based activation motif from the TCR by lentiviral transduction of a CD4CAR gene. This redirects specificity of the transduced T cells for cells that express CD4, which may be malignant or normal T cells. In addition to CD4scFv, the cells will be transduced to express an intracellular signaling molecule comprised of a tandem signaling domain comprised of 4-1BB and TCRζ. The scFv is derived from a mouse monoclonal antibody and the signaling domains are entirely of the native human sequences.

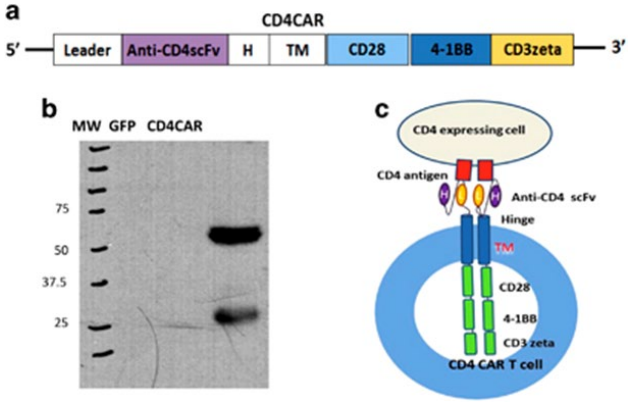


Figure 3:CD4CAR expression.

(a) Schematic representation of recombinant lentiviral vectors encoding CD4CAR. CD4CAR expression is driven by a SFFV promoter. The third generation CD4CAR contains a leader sequence, the anti-CD4 scFv, a hinge domain (H), a transmembrane domain (TM) and intracellular signaling domains as follows: CD28, 4-1BB (both co-activators), and CD3zeta. (b) 293FT cells were transfected with lentiviral plasmids for GFP (lane 1) and CD4CAR (lane 2) for western blotting analysis at 48 h after transfection and probed with mouse anti-human CD3z antibody, with the CD4CAR band observed at the expected size (59.16 kDa), and a breakdown product observed at ~26 kDa. (c) Illustration of the components of third-generation CAR T cells

The CAR constructs were developed by iCellGene Therapeutics and the clinical grade vector will be manufactured at

Indiana University. The CD4CAR cells will be manufactured in the GMP facility at Indiana University according to the process shown in Figure 4. At the end of cell cultures, the cells are cryopreserved in infusible cryomedia bags. Each bag will contain an aliquot (volume dependent upon dose) of cryomedia containing the following infusible grade reagents: 10% DMSO, and 4.50% human serum albumin with each planned dose level of autologous T cells per bag.

Absorption, distribution and metabolism. Lymphocytes have complex trafficking and survival kinetics, and after adoptive transfer several fates have been demonstrated: 1) margination; 2) exit from the peripheral blood trafficking to lymphoid tissues; and 3) death by apoptosis. Following an intravenous dose, retrovirally modified and adoptively transferred T cells have been shown to persist in the circulation for at least 10 years in severe combined immunodeficiency disease (SCID) patients due to the replicative competence of T cells [33]. Human CD8+ CTLs have an elimination half-life from the peripheral blood of about 8 days and this increases to about 16 days when low doses of IL-2 are given [34]. In patients with HIV infection, the mean half-life of lentivirally modified CD8+ T cells in the circulation of 5 patients following a single infusion was 23.5 (± 7.7) days.[35]

Drug interactions. CD4CAR cells are expected to retain many of the properties of natural T cells. As such, they will be expected to be susceptible to immunosuppressive agents such as corticosteroids, immunophilins such as cyclosporine and tacrolimus, methotrexate, mycophenolate mofetil, mTOR inhibitors such as rapamycin, alemtuzumab, daclizumab, and denileukin diftitox. Lymphocytes are especially susceptible to cytotoxic and chemotherapeutic agents that are commonly administered for hematologic malignancies such as cyclophosphamide and fludarabine.

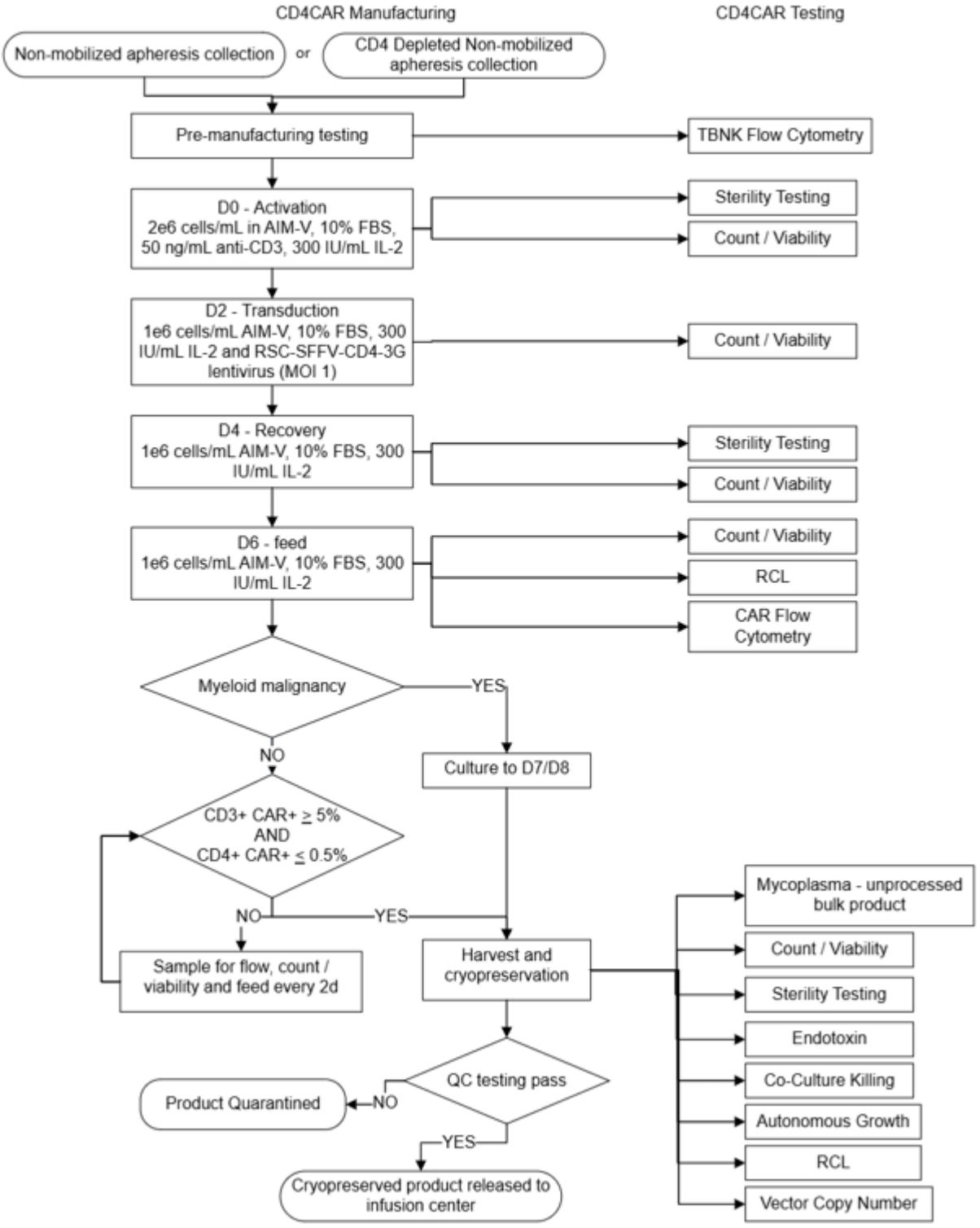


Figure 4 CD4CAR Manufacturing Process

Immune elimination. In general, the CAR can be immunogenic, either because foreign sequences such as antibiotic selection genes or mouse antibody sequences are expressed, or because novel epitopes are created at the fusion joint of human signaling domains that are not normally juxtaposed. Immunogenicity of the CAR can

lead to the rejection of the adoptively transferred T cells. The basis for this supposition is that human retrovirally-modified CTLs expressing a fusion protein consisting of hygromycin: HSV thymidine kinase were eliminated by host CTLs in patients with advanced HIV infection[35]; importantly, this immune mediated elimination was not accompanied by adverse effects and required 6 to 8 weeks to occur. Another possible problem with immunogenicity relates to the fact that some of the scFvs incorporated in the CARs used in current clinical trials are of murine origin and can thus elicit a human anti-mouse protein immune response. This can be a cellular immune response that eliminates the transduced T cells after 4–6 weeks, but can also result in the generation of anti-murine scFv IgG or even IgE antibodies. In a recent mRNA mesothelin-CAR trial, Newick and colleagues observed an anaphylactic reaction when CARs expressing a murine scFv were re-administered to a patient after a period of 6 weeks.[36] Therefore, most groups are now using human or humanized scFvs in their CAR constructs. The potential immunogenicity of transduced genes must be considered with the CD4CAR T cells and so, there is the possibility that CD4CAR T cells will be rejected in our patients. We expect the cells to persist for a sufficient period of time to determine safety, T cell subset specific persistence, and effects on tumor burden and tumor specific immunity at 4 weeks following the first infusion.

1.3 Preclinical Data

Details of our CD4CAR active moiety have been published.[32] Briefly, for our CD4CAR the scFv (single-chain variable fragment) is derived from humanized monoclonal ibalizumab (i.e., Hu5A8 or TNX-355) investigated in Phase I and II clinical trials.[37, 38] Our CD4CAR redirects the antigen specificity of CD8+ cytotoxic T cells to CD4-expressing cells with CD4CAR T cells derived from human PBMCs and cord blood effectively redirecting T-cell specificity to CD4+ cells in *vitro*.

Characterization of CD4CAR. As illustrated and detailed in Figure 2, our CD4CAR expression is driven by a SFFV promoter. (a) This third generation CD4CAR contains a leader sequence, the anti-CD4 scFv, a hinge domain (H), a transmembrane domain (TM) and intracellular signaling domains as follows: CD28, 4-1BB (both co-activators), and CD3zeta. (b) 293FT cells were transfected with lentiviral plasmids for GFP (lane 1) and CD4CAR (lane 2) for western blotting analysis at 48 h after transfection and probed with mouse anti-human CD3z antibody, with the CD4CAR band observed at the expected size (59.16 kDa), and a breakdown product observed at ~26 kDa. Using flow cytometry (n=3 experiments) we have demonstrated that 96% of the expanded T cells were CD45RO+, ~83% were CD62L+ and ~80% were CD8+CD45RO+CD62L+ whereas fewer than 4% were CD45RA+. This indicates that CD4CAR T cells are highly enriched for CD8+ T cells and most of them bear a central memory T cell like immunophenotype. Recent studies have shown that memory CD8+ cells are responsible for engraftment, and these cells are critical 'active ingredient' for CARs.[39]

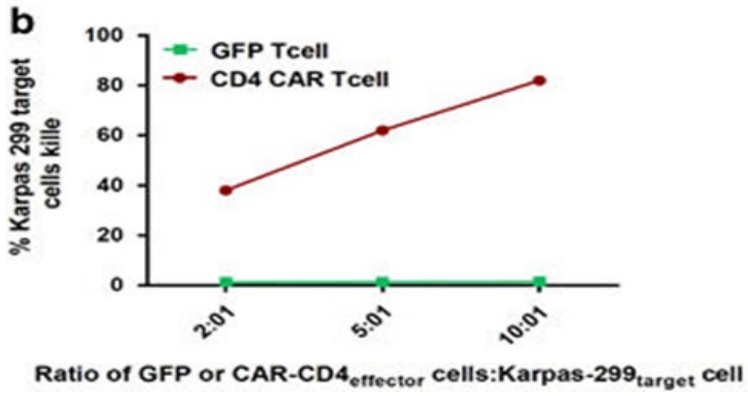


Figure 5. CD4CAR T cells specifically kill KARPAS 299 cells. PBMC T cells transduced with either GFP control or CD4CAR lentiviral supernatant were incubated with CFSE-stained KARPAS 299 at the ratios of 2:1, 5:1 and 10:1. After overnight incubation at 37 °C, dye 7AAD was added, and the cells were analyzed by flow cytometry. Percentage of killing of target cells is measured by comparing survival of target cells relative to the survival of negative control cells (SP53 cells, a B-cell lymphoma cell line stained with CMTMR).

CD4CAR T cells specifically eradicate CD4-expressing cell lines and primary patient cells without effects on CD4 negative non-target cells. CD4+ KARPAS, Sezary, and PTCLS were lysed at >95% cytotoxicity whereas CD4- Sp53 cells were not affected. Significant tumor lysis was observed for most co-cultures at low E: T ratios of 2:1 (Figure 5) consistent with CD4+ cells targeting.

CD4CAR T cells exhibit significant antitumor activity in vivo. We developed a xenogeneic mouse model using the KARPAS 299 cell line. Multiple different settings were used to test CD4CAR T-cell efficacy in vivo n=5 in each

group. Prior to the injection, modified T cells displayed ~40–50% of cells expressing CD4CAR as demonstrated by flow cytometry analysis. Mice received intradermal injections of KARPAS 299 cells, and then a low dose (2 million) of single systemic injection (intravenous administration) of CD4CAR T cells was given. A single low dose of systemic CD4CART cells administration to leukemia-bearing mice caused only transient regression or delayed the appearance of leukemic mass (Figure 6A). When leukemia growth increased, an additional course of administration of 8×10^6 CD4CAR T cells arrested the leukemic growth (Figure 6A). To further test the efficacy of CD4CAR anti-leukemia activity, we administered two courses of large doses of CD4CAR T cells.

Similarly, two injections totaling 13.5×10^6 CD4CAR T cells caused pronounced leukemia growth arrest compared with a lower CD4CAR dose but eventually the leukemic cell population recovered (Figure 6B). Finally, we investigated the efficacy of multiple course injections of a low dose of CD4CAR T cells (each 2.5 × 10^6 cells). We treated the mice bearing repeat subcutaneous leukemia with CD4CAR T intravenous injections of for total of cells, once every 4 or 5 days courses of four injections. After four treated mice CD4CAR T-cell no toxic administration, one of the four CD4CAR was tumor free and exhibited more appearance. Multiple-dose effect T-cell-treated mice displayed (Figures 6A significant anti-leukemic with compared with single dose prolonged and C). Moreover, treatment KARPAS CD4CAR T cells significantly treatment the survival of mice bearing control T 299 lymphoma compared with with the GFP-transduced cells (Fig. 6D).

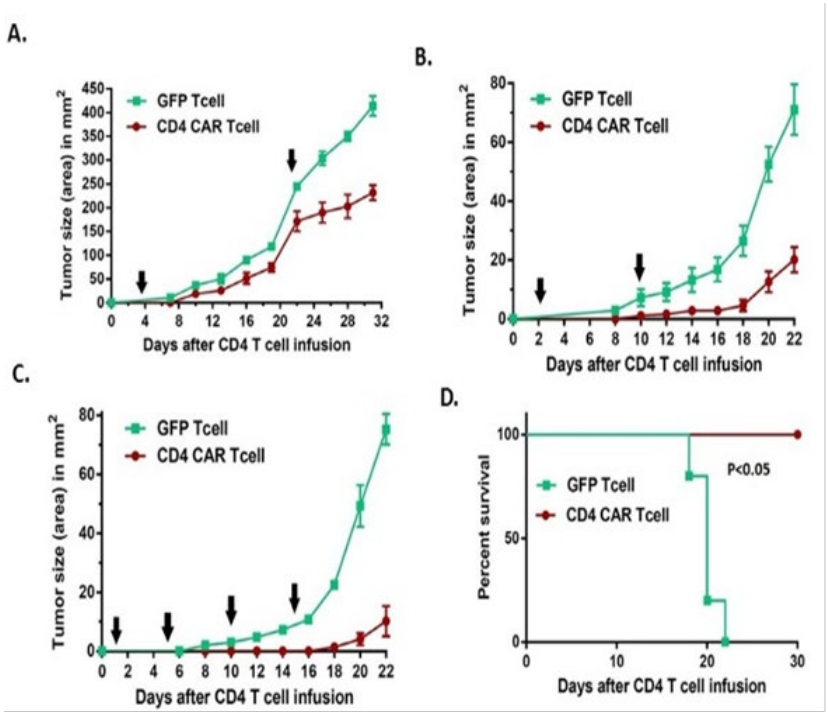


Figure 6: CD4CAR T cells efficiently mediate anti-leukemic effects in vivo with different modes. NSG mice received 2.5 Gy for sub-lethal irradiation. Twenty-four hours after irradiation, mice were injected subcutaneously with either 1 x10^6 (in A) or 0.5 x10^6 (in B and C) KARPAS 299 cells. Injected mice were treated with different courses and schedules of CD4CAR T cells or control T cells. N=5 for each group of injected mice. (A), a low dose of 2x10^6 of CD4CAR T cells were injected on day 3 and then a large dose, 8x10^6 of CD4CAR T cells on day 22 after tumor growth is accelerated. (B), two large doses of CD4CAR T cells, 8x10^6 and 5.5x10^6 were injected on day 3 and 10 respectively. (C), four relatively low doses of CD4CAR T cells (each 2.5 x 10^6) were injected every 5 days. (D), the overall survival of mice treated with the indicated CD4CAR T cells or GFP T cells. N=10

CD4CAR T-cells eradicate systemic T-cell leukemia. We further investigated the ability of CD4CAR T cells to eradicate systemic T cell tumors instead of focal involvement using the Jurkat leukemic line where 40-60% of the cells express CD4. Following treatment with CD4CAR T-cells (Day 6), mice injected with CD4CAR T cells had 50% less tumor burden than control mice; 72% on Day 8 and 80% by Day 13 (Figure 4) with significant ($p<0.01$) differences between groups.

1.4 Clinical Data

CD4 as a therapeutic target: Targeting CD4 for immunotherapy has been investigated before in phase I and II clinical trials for both of the treatment and prevention of HIV. It was shown that Ibalizumab (Hu5A8 or TNX-355), a monoclonal antibody that binds CD4 (the primary receptor for HIV) inhibits the viral entry process. Studies have confirmed that it is a potent HIV-1 neutralizing antibody that can be used safely.[40,41] Ibalizumab was proven to have an avid and specific binding ability to CD4 over the course of many years of testing, which

makes it an ideal tool for our purposes of designing a chimeric construct.[42,43] Anti-CD4 antibodies have also been extensively used for CD4+ cell depletion in non-human primate models,[44] as well as in numerous clinical trials for lupus,[45-47] psoriasis,[48-50] and cutaneous and peripheral T cell lymphoma.[51-54] These studies have provided valuable clinical insight and data about adverse events and toxicity during hematologic states of transient CD4+ cell depletion. Many anti-CD4 antibody studies have already shown that this pharmacological approach is safe with uncommon off-target effects and that the depletion of CD4+ cells is both well-tolerated and reversible with the return of normal CD4 function after drug elimination. The targeting and removal of CD4+ cells for the treatment of cutaneous T-cell lymphoma with chimeric anti-CD4 monoclonal antibodies have been evaluated in Phase I and II clinical trials, where one study showed significant suppression of CD4 counts that lasted from one week to 22 weeks in duration. Zanolimumab, another anti-CD4 monoclonal antibody, has been recently evaluated for the treatment of T-cell lymphoproliferative disorders, including peripheral T cell lymphoma and cutaneous T cell lymphoma. The median depletion of CD4+ T cells was 75-80% in peripheral blood samples with recovery of CD4 counts after treatment. Moreover, opportunistic infections were not reported in the zanolimumab trials, which is most likely due to the protective functions of the CD8+ T cells and natural killer cells (NK cells) against pathogens because these cells did not decline in number during treatment.[50, 51]

The preservation of the CD8+ cells and NK cells, while there is concomitant CD4+ cell ablation, provides insight into the extraordinary pharmacologic specificity of the commercially available anti-CD4 monoclonal antibodies and how this may contribute to minimal off-target effects. Nonetheless, autoimmunity and immune suppression are still of concern with long-term CD4+ depletion therapy. Regulatory T-cells (Treg cells) are also CD4+ and essential in regulating immune function. At present, there is tremendous clinical interest in the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and the programmed cell death protein 1 (PD-1) and PD-ligand (PD-L) regulatory path.

CD4CAR cells with a 4-1BB:TCRζ have not been previously tested in humans. CART-20 cells with a TCRζ reported safety in patients with mantle cell lymphoma.[55] CD19-targeted T cells rapidly induced molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia.[56] They also reported complete responses with acceptable toxicity without chronic B-cell aplasia in children with relapsed or refractory acute lymphoblastic leukemia even after allogeneic hematopoietic stem cell transplantation.[57] Results from related approaches testing CARs in humans are available from patients with HIV infection and cancer.

CD4: TCRζ experience in HIV infection: CD4ζ is a genetically engineered, MHC– unrestricted receptor composed of the ζ subunit of the CD3 T cell receptor, the cytoplasmic domain involved in signal transduction, fused to the transmembrane and extracellular domains of human CD4, which targets HIV *env* expressed on the surface of infected cells.[58] Upon binding to the HIV envelope, CD8+ T cells engineered to express the CD4ζ fusion protein proliferate and initiate effector functions such as cytokine secretion and HIV-specific cytolytic activity.[59,60] The survival of co-stimulated gene–marked T cells was published in 3 studies.[61-63] CD4ζ modified T cells were detected by DNA PCR in the peripheral blood of all patients following infusion. Sustained mean levels of 1% to 3% of T cells were detected at all-time points after infusion. In extended follow-up, CD4ζ was detected in the blood of 17 of 18 patients 1 year post infusion. In the only published phase II HIV gene therapy trial, 40 patients were randomized to receive an infusion of CD4ζ–modified T cells or non-transduced co-stimulated T cells. The treatment was found to be safe and associated with treatment-related increases in CD4+ T cell counts.[60] Together, these clinical results indicate that CD4ζ CAR anti-HIV vector is safe and has stable engraftment and persistence in lymphopenic patients with HIV infection.

G250:TCRζ experience in renal cell carcinoma (RCC) Lamers and colleagues reported the interim results of a trial testing T bodies in 3 patients with metastatic renal cell carcinoma.[64] The T bodies were targeted with a scFv specific for carboxy-anhydrase-IX (the G250 antigen) that is overexpressed on clear cell RCC and in the biliary tract. The T body was expressed in autologous T cells using retroviral transduction. The subjects were

treated with a dose-escalation scheme of intravenous doses of 2 x 10^7 T cells on day 1; 2 x 10^8 T cells on day 2; 2 x 10^9 T cells on days 3 to 5 (treatment cycle 1); and 2 x 10^9 cells on days 17 to 19, in combination with human recombinant IL-2, given subcutaneously at 5 x10^5 U/m^2 twice daily administered at days 1 to 10 and days 17 to 26. Infusions of these G250 T bodies were initially well tolerated. However, after four to five infusions, liver enzyme disturbances reaching NCI CTC grades 2 to 4 developed. These toxicities necessitated the cessation of treatment in subjects 1 and 3, corticosteroid treatment in patient 1, and reduction of the maximal T-cell dose in subjects 2 and 3. All three patients developed low levels of anti-scFv (G250) antibodies between 37 and 100 days after the start of T-cell therapy. The liver enzyme elevations resolved. The T bodies circulated from 32 to 53 days after infusion, and the authors demonstrated anti-tumor activity of the G250 T body cells in the peripheral blood of the patients after infusion. The authors concluded that the liver toxicity was most likely due to the reactivity of G250 T bodies against the target antigen expressed on normal tissue, that is, the epithelial cells lining the bile ducts. Thus, this is interpreted as a form of "on target, off organ" toxicity.

α-CD20-ζ expressing CD8 cells for follicular lymphoma Press and colleagues reported a clinical trial evaluating escalating doses of CD8 T cells modified by electroporation to express a chimeric immune receptor targeting the CD20 B cell marker and linked to the CD3ζ activation domain for the treatment of refractory follicular lymphoma and mantle cell lymphomas.[65] A total of 7 subjects were enrolled in the trial who each received 3 infusions of escalating doses of CD20 specific CARTs of 10^8 cell/m^2, 10^9 cell/m^2, and 3.3 x 10^9 cell/m^2 spaced 2 to 5 days apart. The first three patients had poor persistence of the cells post infusion which was improved in the last 4 patients whom received subcutaneous low dose (500,000 IU/m^2) IL-2 twice daily for 14 days. No subjects developed an immune response to the infused cells, which was evaluated by a chromium release assay against targets expressing the scFv and neomycin resistance gene products (for cellular immunity), as well as by ELISA for antibody responses to the anti-CD20 antibody. Two patients became positive for HAMA 3 and 6 months after infusion. Since the chimeric immune receptor does not contain the murine Fc region, the conversion to HAMA positivity may reflect immune reconstitution and response to prior therapy. Clinical responses were modest, with no grade 3 or 4 toxicities. Toxicities were restricted to the four patients receiving IL-2, and were attributed to an IL-2 induced flu like syndrome.

Lentiviral vector in clinical experience Considerable data in phase I/II studies in well-controlled patients show that HIV-based lentiviral vectors are safe.[63-65] No serious adverse events related to the study product have been observed to date; All SAE reported were unrelated to study treatment. No replication competent lentivirus has been detected in viral vector lots or in patients post infusion. It was also shown that longitudinal evolution of cells transduced with a lentiviral vector after infusion of gene modified CD4+ cells provided no evidence for abnormal expansions of cells due to vector-mediated insertional activation of proto-oncogenes.[66]

1.5 Dose Rationale:

The primary objective of this protocol is to test the safety and feasibility of administering CD4CAR cells to patients with advanced refractory hematologic malignancies. Therefore, we must administer a dose of cells that is 1) safe and 2) feasible to produce and can be tracked in the subject after infusion in order to determine persistence and trafficking. The starting dose was based on our preclinical data converted to human doses. Our preclinical studies showed anti-tumor efficacy at an injection number of 2 x 10^6 CD4CAR cells per mouse. A typical mouse used for CAR preclinical studies weighs around 25 grams. An average adult male weighs ~70 kg – a factor of 2800x. Scaling to a human dose level comes out to 5.6 x 10^9 CD4CAR cells. However, we anticipate that human T-cells expand at anywhere from 10-100x better in humans than in mice (as a conservative assumption), so the dose level is revised to ~5.6 x 10^6 CD4CAR cells per patient. This dose level corresponds to dose level 1 in clinical trial dosing of 8 x 10^4 CD4CAR cells/kg for a 70 kg patient. Since there are about 10^{12} T-cells in a healthy adult, the proposed highest dose is equivalent to 0.0056% of the total body mass of T-cells. While the number seems low compared to clinical equivalents of a CD19 CAR, recent data presented at ASH 2017 shows that a total cell dose of 1X10^6 /kg is non inferior to 2x10^6 /kg and might be less toxic (ASH abstract

888). Furthermore, pre-treated patients will have variable but much less total T-cells than a healthy adult, and this number may provide safety consideration in anticipation of T-cell proliferation. Furthermore and because this is the first in human CAR T against CD4, we plan to adopt a relatively low dose as a starting point.

To document that this dose is sufficient and safe we will *first)* dose escalate in a 3+3 traditional design with the intention to stop at either a DLT or when maximum effective dose that eliminated CD4 + tumor cells is established, whichever comes first and *second)* track the CAR cells *in vivo* at multiple time points for each dose variable in relation to tumor elimination (see trafficking below).

Following tumor burden reassessment and when eligibility for infusion is met, CD4CAR T-cells will be thawed and infused. Each infusion bag will constitute approximately 10-50 mL containing approximately the planned dose level for that particular infusion as explained in Table 1**.**

Table 1. Dose Escalation Schedule

Dose Level	Transfected Cell dose/Kg
1	8.0×10^4 /kg
2	1.6×10^5 /kg
3	4.0×10^5 /kg
4	1.0×10^6 /kg
5	2.0×10^6 /kg

1.6 Risk/Benefits:

Known potential risks: Recent studies from Memorial Sloan Kettering Cancer Center (MSKCC), University of Pennsylvania, and the National Cancer Institute have shown the complete remission (CR) rates ranging from 71 percent to 90 percent in patients with B-ALL using slightly different versions of CD19 CARs. A systematic review of efficacy and safety in clinical trials of CD19 and CD20 CAR T-cells showed that the most common adverse events were temporary. [57, 65, 67]

1.6.1 Immediate Risks

Central nervous system toxicity: The development of neurologic toxicities including confusion, delirium, expressive aphasia, obtundation, myoclonus, and seizure has been reported in patients receiving CD19-specific CAR T cells.[57, 65, 67] The causative pathophysiology of these neurologic side effects is unknown, though given similar events reported with blinatumomab administration[68], it is plausible that elevated cytokine levels are partly responsible for the neurologic sequelae. Conversely, direct CAR T-cell toxicity on the central nervous system is possible but has not been demonstrated.[69, 70] Patient correlates have not been informative as reports have conflicted on the correlation between the number of engineered or non-engineered T cells in spinal fluid and status of central nervous system leukemia with neurological complications. Similarly, EEG traces have not reliably identified seizure activity despite clinical symptoms demonstrating such activity. To date, the neurologic toxicity has been reversible in a majority of cases and it is unclear if this toxicity is restricted to CD19-specific CAR T cells or will be exhibited by the targeting of other tumor-associated antigens.

Risk of tumor lysis related to cytoreductive therapy: The risk of tumor lysis syndrome (TLS) is dependent on the type of disease and burden of disease, but in most cases, this risk is expected to be manageable. Patients with the highest risk for TLS have aggressive lymphomas (e.g. Burkitt's lymphoma or other 'high-grade" NHL) and acute lymphoblastic leukemia (ALL). For the purpose of this study, all patients will receive

CD4CAR T-cells only after attempting tumor debulking with the lymphodepleting conditioning chemotherapy. Patients will be closely monitored both before and after CD4CAR infusions, including routine blood testing for potassium and uric acid. Patients will also be treated with allopurinol for prevention of TLS. If the uric acid prior to CD4CAR administration is > 8 or acute tumor lysis occurs, patients will be immediately given the necessary hydration, and possibly rasburicase, according to standard indications to minimize TLS toxicity.

Cytokine Release Syndrome: Cytokine release syndrome (CRS) is an acute systemic inflammatory syndrome characterized by fever and multiple organ dysfunction that is associated with chimeric antigen receptor (CAR)-T cell therapy, therapeutic antibodies, and haploidentical allogeneic transplantation. CRS is a supraphysiologic response to immune therapy that activates or engages T cells and/or other immune effector cells. The systemic reaction is associated with increased levels of inflammatory cytokines and activation of T lymphocytes, macrophages, and endothelial cells. However, the contributions of the individual cellular components and cytokines to the cause and severity of CRS are not well defined. CRS is a common AE associated with CAR therapy.[55, 56, 65] Emergency medical equipment (i.e., emergency cart) will be available during the infusion in case the subject has an allergic response, severe hypotensive crisis, or any other reaction to the infusion. Vital signs (temperature, respiration rate, pulse and oxygen saturation, and blood pressure) will be taken immediately before (+/-5 minutes) and after infusion(+/-5 minutes), then every 15 minutes(+/-5 minutes) for at least one hour and until these signs are satisfactory and stable (in the event the infusion lasts more than 15 minutes, vital signs are taken every 15 minutes during infusion). Side effects following T cell infusions related to CRS include transient fever, chills, and/or nausea. Subjects will be pre-medicated with acetaminophen and diphenhydramine prior to the infusion of CD4CAR cells. These medications may be repeated every six hours as needed. A course of non-steroidal anti-inflammatory medication may be prescribed if the patient continues to have fever not relieved by acetaminophen, and empiric antibiotic administration will be strongly considered. It is recommended that patients not receive systemic corticosteroids such as hydrocortisone, prednisone, prednisolone or dexamethasone at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on T cells (Table 3). Monitoring cytokines level at variable time points is planned as a biological correlate to this trial. IL-2, IL-12, IL-6, IL-15, CRP and IFNγ levels will be monitored on Day 3, 5, and 7 during hospitalization and every 8 +/- 2 hours or as feasible, in consideration of participant availability, and clinical/research staff operations, during CRS. These will also be tested twice a week up to day 14 after CD4CAR infusion, then once a week up to day 28, and per schedule of events thereafter until CD4CAR is undetectable (e.g. two consecutive negative CD4CAR tests either by research flow cytometry or by transgene copy number). An aliquot of serum or plasma (at least 0.4 mL) will be banked for additional testing needs at each cytokine testing time point.

All patients will be regularly monitored with blood testing for C-reactive protein concentration as a surrogate biomarker for increased Il-6 concentration at the same time points for cytokines levels, for quick results in case cytokines are not resulting soon enough for clinical guidance of management. If IL-6 and interferon-γ levels are due to potential CRS, anti-IL-6 receptor antibody, tocilizumab, will be immediately utilized (with or without steroids) to reverse the syndrome according to approved indications.[70] Appropriate cultures and medical management of presumptive infectious etiology will be initiated. If a contaminated CD4CAR T cell product is suspected, the product can be retested for sterility using archived quality check samples per FACT accredited lab standards.

1.6.2 Intermediate/Long Term Risks

Viral/bacterial/fungal infection risk due to transient CD4+ depletion. Infections are a concern with long-term CD4 cell depletion. We speculate that similar to CD4 lymphodepletion observed with anti-CD4 antibodies and from our preclinical data, CD4 lymphopenia will be temporary and is expected to resolve by 6 months post infusion, but in some cases may be prolonged. Nonetheless, all patients at risk will be closely

followed during CD4 cell lymphopenia per the current standard of clinical care in order to document AEs by physical examination and frequent blood testing. The clinical monitoring for these patients will follow the guidelines used for HIV patients with CD4 cell lymphopenia; patients will receive prophylaxis for fungal, viral and opportunistic infections during the lymphopenia period where clinically indicated.

Uncontrolled T cell proliferation In pre-clinical studies, CD4CAR cells have only proliferated in response to physiologic signals or upon exposure to CD4.[74] In the context of this protocol, it is possible that the T cells will proliferate in response to signals from the malignant tumor or normal T cells. This could be beneficial or harmful, depending on the extent of related manifestations. These manifestations include CRS (see section10.3.1), clonal dominance of adoptively transferred T cells has been associated with tumor reduction in adoptive transfer trials. Safe persistence of infused CAR T cells in this trial and others, is essential for tumor control.

Drug interactions*:* CD4CAR cells are expected to retain many of the properties of natural T cells. As such, they will be expected to be susceptible to immunosuppressive agents such as corticosteroids, cyclophilins such as cyclosporine and tacrolimus, methotrexate, mycophenolate mofetil, and mTOR inhibitors such as rapamycin, alemtuzumab, daclizumab, denileukine diftitox. Lymphocytes are especially susceptible to cytotoxic and chemotherapeutic agents that are commonly administered for hematologic malignancies such as cyclophosphamide and fludarabine. It is of utmost importance to allow for a wash out period for these drugs that depends on their specific half-life before CD4CAR infusion, to not compromise the efficacy of the incoming CAR T cell infusion.

1.6.3 **Long Term Risks**

Secondary carcinogenesis (development of new malignancy) The occurrence of adverse events caused by insertional mutagenesis in three patients in a gene therapy trial for X-linked SCID following stem cell therapy emphasizes the potential for problems in translating this approach to the clinic.[71] To date, malignancy due to insertional mutagenesis has not been reported following adoptive of engineered T cells. Lentiviral vectors may have a lower risk than oncoretroviral vectors based on several considerations.[72,73] Clonal dominance will be an exploratory objective for this trial. Monitoring for T cell clonal outgrowth will be performed by flow cytometric analysis for CD4CAR expressing cells, and by CBC count. If the number of chimeric immune receptor cells continues to increase after 6 weeks, a Vβ repertoire analysis will be performed to evaluate clonality, or if the CBC analysis reveals abnormal T cell counts, then the Vβ analysis will be performed earlier. If a subject's Vβ repertoire is found to be monoclonal or oligo clonal, the subject's T cells will be evaluated for the pattern of vector insertion site. If the site of insertion is found to favor a single dominant insertion site pattern the clinical trial will be placed on hold to allow evaluation of the subject in consultation with gene therapy experts, study investigators, DSMC, FDA and NIH. Further evaluation of the subject will comprise of confirmation of the persistence of the clonality within a 3 month period, and monitoring of the subject for hematologic malignancies..

Replication competent lentivirus (RCL) Replication-competent lentivirus (RCL) may be generated during the CD4CAR manufacturing phase or subsequently after introduction of vector transduced cells into the patient. However, an RCL resulting from the production phase is highly unlikely since elements are incorporated in the design of the vector system that minimize vector recombination and generation of RCL. Furthermore, the vector used to transduce the product undergoes sensitive assays for detection of RCL before it can be released to a subject. Nevertheless, generation of an RCL following infusion of the vector product remains a theoretical possibility. The consequences of such replication events in subjects without a known lentiviral infection are unknown, and therefore subjects with coexistent HIV infection are excluded from participation in this study in order to minimize this possibility. The development of RCL could pose a risk to both the subject and their close contact(s), and therefore, monitoring for RCL will be conducted during the course of the trial.

Regulatory agencies and the gene therapy community have previously discussed measures to be taken should an RCL be confirmed in a subject. However, because the probability and characteristics of an RCL are unknown, no guidelines have been put in place. Nevertheless, all agree that the subject must be isolated until an understanding of how to manage the subject becomes clear. Some considerations are

- Intensive follow-up of subject in consultation with gene therapy experts, study investigators, FDA and NIH.
- Inform local public health officials and CDC.
- Identify sexual partners and provide appropriate counseling and intervention.

1.6.4 Known Potential Benefits

This is a first in–human, as CD4CAR cells have not been previously tested in humans and represent a potential curative therapy for patients who have little or no other options for treatment. This study will follow the current standards of care for CD4+ T-cell malignancies, which entails that in the case that several clinically established chemotherapy regimens prove unsuccessful, CD4CAR T-cells will be offered.

2. STUDY OBJECTIVES

2.1 Primary Objectives:

3. To identify dose-limiting toxicities of CD4CAR and the recommended phase II dose.
4. Describe toxicity profile of CD4CAR within the scheduled dose escalation

2.2 Secondary Objectives:

1. To determine the duration of in vivo persistence of the CD4CAR, including
   a) Evaluate trafficking of CD4CAR cells to tumor sites in bone marrow and lymph nodes and relation to efficacy.
   b) Determine if cellular or humoral host immunity develops against the murine anti- CD4, and assess correlation with loss of detectable CD4CAR (loss of engraftment).
   c) Evaluate the relative subsets of CD4CAR T cells (Tcm, Tem, and Tregs), when feasible
2. Describe CD4CAR associated cytokine production.
3. Determine the effect of undergoing HSCT post CD4CAR in patients who become eligible to undergo the procedure
4. Tissue banking for future research when available
5. Document any preliminary evidence of objective antitumor activity.
6. Evaluate biomarkers and cytokines in relation to toxicity and efficacy including CSF levels (when tested).
7. Examine for T cell function upon T cell recovery, when feasible and if no transplant has been done

3. STUDY ENDPOINTS

3.1 Primary Endpoints

This pilot study is designed to test the safety and relative engraftment of the autologous T cells transduced with the anti-CD4 lentiviral vector in patients with relapsed, refractory and incurable CD4+ hematological malignancies.

Primary safety, feasibility and engraftment endpoints include:

Occurrence of study adverse events, defined as NCI CTCAE signs/symptoms, laboratory toxicities and clinical events on study, including but not limited to:

1. Fevers
2. Rash
3. Neutropenia, thrombocytopenia, anemia, marrow aplasia
4. Hepatic dysfunction
5. Pulmonary infiltrates or other pulmonary toxicity
6. Cytokine release syndrome
7. Neurotoxicity

3.2 Secondary Endpoints

1. Describe anti-tumor responses to CD4CAR cell infusions per response criteria.
2. For subjects with active disease, typical response criteria for partial response (PR) or complete response (CR) will be determined based on disease type. Response determination will be only "descriptive" given the small number of subjects to be treated.
3. For subjects treated with MRD (identified by PCR analysis of blood or marrow), determine elimination of MRD scored as yes/no.
4. Describe overall survival and cause of death
5. Feasibility to manufacture CD4CAR cells from this patient population apheresis products. The number of manufactured products that do not meet release criteria for vector transduction efficiency, T cell purity, viability, sterility and tumor contamination will be determined as manufacturing failures.
6. Duration of *in vivo* survival of CD4CAR cells is defined as "persistence." Persistence will be measured using flowcytometry as indicated in the schedule of events.
7. New onset malignancies post CD4CAR treatment.
8. Determine if host immunity develops against the murine anti-CD4 or other elements of the transgene or vector, and assess correlation with loss of detectable CD4CAR (loss of engraftment).
9. Determine the subsets of CD4CAR cells (Tcm, Tem, and Treg), when feasible

3.3 Exploratory Endpoints

Even though we recognize that CD4+ malignancies significantly vary for example, peripheral T-cell leukemia, T-cell prolymphocytic leukemia, and CD4+ AML are different diseases, we intend to target the CD4 positive cells. Even though there are microscopic, flow cytometric, cytogenetic, immunohistochemical, and molecular evaluations that for ultimate hematopathologic diagnosis and classification, targeting CD4+ receptor is the common treatment goal. Nonetheless, we will document and analyze CD4CAR T-cell responses in each sub-classified disease entity and category for differences in terms of partial and complete remission and DFS.

Even though CNS toxicity is a major side effect reported in previous CAR T cell trials, CAR trafficking to the CNS was not studied at depth. In this study we will determine CD4CAR trafficking to CNS if CNS toxicity >/= G3 is documented.

For the purposes of comparing grading systems and improving understanding of differences of grading systems within a study, cytokine release syndrome grading data will be collected using both the ASTCT consensus grading[81] (which is used to guide clinical management of trial subjects) and the Lee (2014) grading systems.

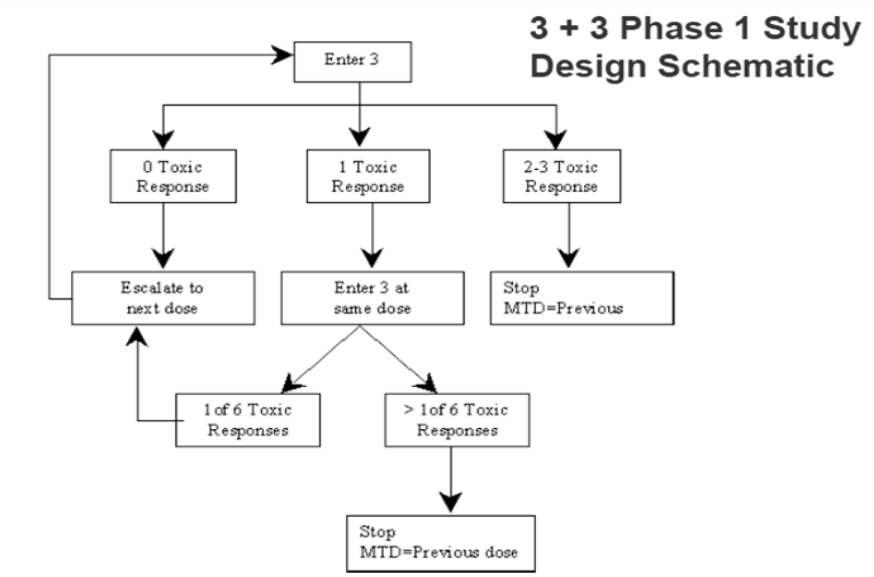
We will also describe timing of absolute neutrophil and platelet engraftment in participants who receive alloHSCT within 90 days of CD4CAR infusion

4. STUDY DESIGN

4.1 General Design

This study is designed as a single arm open label Phase I, 3x3, multicenter study of CD4-directed chimeric antigen receptor engineered T-cells (CD4CAR) in patients with relapsed or refractory T-cell leukemia and lymphoma. Specifically, the study may be conducted at several academic cancer centers to evaluate the safety and tolerability of CD4CAR T-cells.

4.1.1 Figure 7: 3+3 Phase 1 Study Design Schematic



At entry, subjects will be staged and the suitability of their T cells for CD4CAR manufacturing will be determined by documenting that they have the minimal T cell number adequate for apheresis (screening step). Subjects who have adequate T cells will be leukapheresed to obtain large numbers of peripheral blood mononuclear cells (PBMC) for the manufacturing. The T cells will be purified from the PBMC, transduced with CD4CAR lentiviral vector, expanded in vitro and then frozen for future administration. Next, conditioning chemotherapy will be given. Following tumor burden reassessment, CD4CAR cells will be thawed and infused on Day 0 of treatment.

Subjects will have blood tests to assess safety, engraftment and persistence of the CD4CAR cells at regular intervals through Day 28 of the study. Planned biological correlates will be performed at variable time points including trafficking. Following the 6 months of intensive follow-up, subjects will be evaluated quarterly for two years with a medical history, a physical examination, and blood tests. Following this evaluation, subjects will enter annual long-term follow-up by phone and questionnaire for an additional 13 years to assess for the diagnosis of long-term health problems, such as secondary carcinogenesis (development of new malignancy) and replication competent lentivirus (RCL). If all RCL tests are negative during the study period to the first annual evaluation, then plasma may be archived for analysis on an as needed basis.

4.2 Description of the Study Design

The study will be performed as a dose-escalation protocol. Due to the relatively low incidence and prevalence of CD4+ hematological malignancies and the associated aggressive nature of these diseases and the sequel of treatment failure, we expect to recruit up to 30 patients with an expected dropout rate of 25% primarily due to rapid progression or death and screen and or manufacturing failure.The study will utilize autologous CD4CAR T-cells that are engineered to express a chimeric antigen receptor (CAR) targeting CD4 that is linked to the CD28, 4-1BB, CD3ζ signaling chains (third generation CAR).

At entry, patients will be staged per the current standard of care and the suitability of their T-cells for CD4CAR-T manufacturing will be evaluated primarily by T cell counts. Patients will be leukapheresed to obtain peripheral blood mononuclear cells (PBMC) for CD4CAR manufacturing. A 12-15-liter apheresis procedure will be performed at the apheresis center with the intention to harvest nucleated cells to manufacture CD4CAR T-cells. The T-cells will be purified from the PBMC, transduced with CD4CAR lentiviral vector, expanded *in vitro*, and then frozen for administration, Figure 4. The cell product is expected to be ready for release approximately 4 weeks after apheresis. Disease progression in the interim will be deemed as excluded. A single dose of CD4CAR transduced T cells will consist of the cell number for the dose level to be infused. Each dose will be stored in either one or two bags. The route of administration is by IV infusion and the duration of infusion will be approximately 20 to 30 minutes. Each bag will contain an aliquot (30-50mLs) of cryomedia containing the following infusible grade reagents (% v/v): 4.5% human serum albumin with 10% DMSO.

4.3 Dose of CD4CAR description

A principal goal of Phase I portion is to establish a recommended dose and/or schedule of CD4CAR. The guiding principle for dose escalation in phase I is to avoid unnecessary exposure of patients to sub-therapeutic doses (i.e., to treat as many patients as possible within the therapeutic dose range) while preserving safety and maintaining rapid accrual. We will use the rule-based traditional "3+3" design for evaluation of safety during Phase I. Based on lab experience in mice the starting dose (dose level 1) for the first cohort of three patients in the phase I portion of the study will be 8.0 $x10^4$ transfected cell dose/kg. The dose escalation will follow a modified Fibonacci sequence as below. One month must elapse between cohorts.

If 2 of 3 patients out of the first cohort of three patients at dose level 1 experience DLT, the trial will be placed on hold. If one out of three patients in the first cohort of dose level 1 experience DLT, three more patients will be enrolled at dose level 1; the dose escalation continues until at least two patients among a cohort of six patients experience DLT (i.e., ≥33% of patients with a DLT at this dose level)

- If one of the first three patients in dose level 1 experiences a DLT, three more patients will be treated at dose level 1 (see below).
- If none of the three patients or only one of the 6 patients in the dose level 1 experiences a DLT, the dose escalation continues to the dose level 2
- If one of the first four patients in dose level 2 experience a DLT, three more patients will be treated at dose level 2 (see below).*
- If none of the four patients or only one of the 6 patients in the dose level 2 experiences a DLT, the dose escalation continues to the dose level 3*
- If one of the first three patients in dose level 3 experiences a DLT, three more patients will be treated at dose level 3
- If none of the three patients or only one of the 6 patients in the dose level 3 experiences a DLT, the dose escalation continues to the dose level 4

- If one of the first three patients in dose level 4 experiences a DLT, three more patients will be treated at dose level 4
- If none of the three patients or only one of the 6 patients in the dose level 4 experiences a DLT, the dose escalation continues to the dose level 5
- If one of the first three patients in dose level 5 experiences a DLT, three more patients will be treated at dose level 5
- If none of the three patients or only one of the 6 patients in the dose level 5 experiences a DLT, dose level 5 will be declared MTD

*At Dose level 2, 1 patient received a dose lower than the defined dose level due to an error in the volume calculation of the cryo media. The patient did not experience a DLT. However, since this patient did not receive the full dose of the CD4CAR, it was decided to expand this dose level to add an additional patient to fully assess the safety of dose level 2 before consideration of escalating to the next dose level.

In summary, the dose escalation continues until at least two patients among a cohort of six patients experience DLT (i.e., ≥33% of patients with a DLT at that dose level). The recommended dose for phase II trials is defined as one dose level just below this toxic dose level. ***There will be no intra-patient dose escalation or reduction.***

To allow for full spectrum toxicity duration evaluation and reporting, no patients within the same or a different cohort will be initiated on conditioning chemotherapy sooner than **28 days** from the CD4CAR date of the preceding patient. Based on FDA recommendations, there will be a 28 day observation period between the first pediatric subject and enrollment of subsequent pediatric subjects.

4.4 Dose Limiting Toxicity (DLT)

Except for the CAR T Cell therapy specific toxicities of CRS, ICANS, Hemophagocytic lymphohistiocytosis (HLH)/ macrophage-activation syndrome (MAS) and T cell depletion, all other AEs will be defined and graded according to the **NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0.** All AEs that meet the criteria below will be considered a DLT except those that are clearly due to disease progression or documented extraneous causes. DLTs will be evaluated up to day 28 for dose escalation decisions, AND it is consisting of:

1. Grade 3-5 allergic reactions.
2. Grade 3 cytokine release syndrome which does not improve to ≤ Grade 2 within 72 hours (graded according to ASTCT CRS: See Table 3)
3. Grade 4 CRS of any duration (graded according to ASTCT CRS: See Table 3)
4. Grade ≥ 3 immune effector cell-associated neurotoxicity syndrome (ICANS) of any duration (graded according to ASTCT ICANS Consensus grading for Adults: See Table 5)
5. Any Grade 4 hematologic toxicity that does not resolve to Grade ≤ 2 within 28 days (4 weeks) from CD4CAR infusion <u>except for the following</u>:
    a. <u>Lymphopenia</u>, the complication from which rather than the lymphocyte count in itself is defined as a DLT if this complication is a grade 3 or 4 event and does not resolve within the washout period. These complications include fungal infections, disseminated viral infections, or organ failure that is attributed to the infectious agent itself.

b. Cytopenia, which is judged to be secondary to the underlying malignancy or alternative etiology (e.g., lymphodepletion chemotherapy, nutritional deficiencies, viral infection, prior lines of therapy).

Note: Grade 4 toxicities persisting on Day 28 in patients with complete remission (both intra- and extra-medullary) and no identifiable alternative etiology will qualify as a DLT.

6. Any Grade 5 toxicity not due to the underlying malignancy.

In patients who undergo allogeneic stem cell transplant at any time after receiving the CD4CAR, post-transplant hematological toxicities, including lymphopenia, will be documented as transplant-related and do not constitute a DLT.

The DSMC and Phase I Review Committee may recommend de-escalation or expansion of a cohort based on any clinically significant events or the totality of safety experience.

4.5 Post infusion monitoring:

Samples for cytokines quantification will be obtained during hospitalization and after discharge per schedule of events, as well as every 8 +/- 2 hours, or as feasible, in consideration of participant availability, and clinical/research staff operations, during active CRS and as needed per treating physician.

Patients will be monitored for fungal, bacterial, viral and opportunistic infections as needed per the clinical team and/or institutional protocol.

We plan to collect data about clinical and radiological measurements of residual tumor burden starting on day 6 and weekly afterwards until remission and then monthly for 6 months. This will be followed by quarterly clinical evaluations for the next two (2) years with review of symptoms, physical examination, and blood testing per schedule of events. Photographs of skin disease might be part of this monitoring process.

Adverse event follow-up will include evaluation of SAEs, AEs ≥ Grade 3, infections requiring intervention, RCL, and carcinogenesis according to protocol specific time points or their occurrence, whichever occurs first. After these short- and intermediate-term evaluations are performed, these patients will enter annual long-term follow-up to assess for disease-free survival (DFS), relapse, and the development of other health problems or malignancies. This long-term follow-up will be by phone and a questionnaire for an additional thirteen (13) years (participants will be encouraged to visit the clinical site one to two times per year).

The treating physician will decide to proceed to allogeneic or autologous transplant when needed.

RCL and vector-related carcinogenesis monitoring will be performed according with current and future FDA guidelines for lentiviral vectors.

5. SUBJECT SELECTION

Eligible population: Adults and children (12 years and older) males and females regardless of their ethnicity, social background or country of citizenship, who have CD4 positive T cell malignancies that have relapsed after or are refractory to second line of standard therapy for this this disease will be given an equal opportunity to be enrolled on this study if they meet the eligibility criteria and do not fit any of the exclusion criteria. Patients will be enrolled to study cohorts, mainly one patient per month to allow enough time for toxicity monitoring. They will screened, and if eligible, enrolled on the next available dose level on a first come first served bases with no discrimination.

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual will be enrolled if they meet the following criteria:

1. Patients must voluntarily sign and date informed consent forms that state his or her willingness to comply with all study procedures and availability for the duration of the study.
2. Age 12 years old or older
3. Subjects with **any** documented CD4+ T cell hematologic malignancies. Male and female subjects with CD4+ T-cell hematologic malignancies with either relapsed or refractory disease (including those patients who have undergone a prior transplant (if allogeneic, subjects are eligible if there are no remaining donor cells) and patients with an inadequate response after 4-6 cycles of standard chemotherapy) are eligible. Response criteria for each disease subset will be evaluated based on Standard of Care Guidelines.
4. Creatinine clearance of > 60 ml/min (or otherwise non clinically-significant, per study investigator)
5. ALT/AST < 3 x ULN
6. Bilirubin < 2 x ULN
7. No supplemental oxygen at rest

   Note: Pulmonary Function Test (PFT) only required per treating physician discretion
8. Adequate cardiac function with EF of ≥50%
9. Adequate venous access for apheresis and no other contraindications for leukapheresis

5.2 Exclusion Criteria

1. Pregnant or lactating women. The safety of this therapy on unborn children is not known. Female study participants of reproductive potential (see definition below) must have a negative serum or urine pregnancy test prior to initiation of conditioning chemotherapy, per research sites' clinical policy.
2. Uncontrolled active infection necessitating systemic therapy.
3. Active hepatitis B or hepatitis C infection. Active hepatitis C is defined as the hepatitis C antibody is positive while quantitative HCV RNA results exceed the lower detection limit.

   Note the following subjects will be eligible:

   - Subjects with a history of hepatitis B but have received antiviral therapy and have non-detectable viral DNA for 6 months prior to enrollment are eligible
   - Subjects seropositive for HBS antibodies due to hepatitis B virus vaccine with no signs or active infection (Negative HBs Ag, HBc and HBe Ags) are eligible
   - Subjects who had hepatitis C but have received antiviral therapy and show no detectable hepatitis C virus (HCV) viral RNA for 6 months are eligible
   - If hepatitis C antibody test is positive, then patients must be tested for the presence of antigen by reverse transcription-polymerase chain reaction (RT-PCR) and be hepatitis C virus ribonucleic acid (HCV RNA) negative.
4. Concurrent use of systemic glucocorticoids in greater than replacement doses (unless as a part of a standard of care salvage therapy or conditioning protocol), or steroid dependency defined in rheumatological and pulmonary diseases as uninterrupted corticosteroid intake for more than a year at a

dosage of 0.3 mg/kg/day or greater, and where the underlying disease worsens on temporary stoppage of steroid therapy, with symptoms of steroids withdrawal (eg, lethargy, headache, weakness, pseudorheumatism, emotional disturbances, etc) precipitated by the temporary stoppage.

Subjects who receive daily corticosteroids in replacement doses can be included in the study. The replacement doses are defined as following:

a. Hydrocortisone 25mg/day or less
b. Prednisone 10mg/day or less
c. Dexamethasone 4mg or less

Note: Recent or current use of inhaled glucocorticoids is not exclusionary, as this route pertains extremely minimal systemic penetration.

5. Any uncontrolled active medical disorder that would preclude participation as outlined in the opinion of the treating investigator and/or study chair
6. HIV infection.
7. Subjects declining to consent for treatment
8. Subjects who have received or will receive live vaccines within 30 days before the first experimental cell treatment. Inactivated seasonal flu vaccination is allowed.
9. Subjects with active autoimmune diseases who need systematic treatments (such as disease modifying agents, corticosteroids and immunosuppressive drugs) in the last 2 years.
   Note: Replacement therapy (thyroxine, insulin or physiological corticosteroid replacement therapy (up to10 mg of oral daily prednisone or equivalent in hydrocortisone and dexamethasone) to treat adrenal dysfunction or pituitary dysfunction) is not considered as systematic therapy. Subjects who need inhalation corticosteroid therapy can be included in this trial. Subjects with vitiligo or in long-term remission of pediatric asthma or allergic diseases can be included in this trial.
10. Subjects with a history of mental disorders or drug abuse that may influence treatment compliance.
11. Active malignancy not related to a T-cell malignancy that has required therapy in the last 3 years or is not in complete remission. Exceptions to this criterion include successfully treated non-metastatic basal cell or squamous cell skin carcinoma, or prostate cancer that does not require therapy. Other similar malignant conditions may be discussed with and permitted by the Principal Investigator.
12. Treatment with any investigational cell/gene therapy within the past 6 months
13. Treatment with any investigational anticancer agent within 14 days of study entry or 5 half-lives (whichever is shorter)

5.2.1 Eligibility for Conditioning Chemotherapy

1. Specific organ function criteria for cardiac, renal, and liver function must be similar to initial inclusion values.

2. Review of co-morbidities to confirm no major changes in health status (examples of major changes include heart attack, stroke, and any major trauma).

3. Planned infusion dose was successfully manufactured and met release criteria.
4. Negative pregnancy testing (if applicable).

5.3 Eligibility for CD4CAR infusion:

5.3.1 Inclusion

1. Afebrile and not receiving antipyretics, and no evidence of active infection.
2. Specific organ function criteria for cardiac, renal, and liver function must be similar to initial inclusion values. The following test does not need repeated: EF if obtained within 6 weeks of initial assessment.
3. If previous history of corticosteroid chemotherapy, subject must be off all but adrenal replacement doses 3 days before the CD4CAR infusion.

5.3.2 Exclusion

Note: ***A subject may still receive the CD4CAR infusion up to 10 days post conditioning chemotherapy as long as they do not meet any of the following at time of infusion:***

1. Requirement for supplemental oxygen to keep saturation greater than 95% or presence of radiographic abnormalities on a clinically indicated chest x-ray that are progressive.
2. New cardiac arrhythmia not controlled with medical management.
3. Hypotension requiring pressor support.
4. Positive blood cultures for bacteria, fungus, or virus within 48-hours of T cell infusion.

5.4 Contraception and Reproductive Potential Guidelines

Female subjects of reproductive potential (women who have reached menarche or women who have not been post-menopausal for at least 24 consecutive months, i.e., who have had menses within the preceding 24 months, or have not undergone a sterilization procedure [hysterectomy or bilateral oophorectomy]) must have a negative serum or urine pregnancy test prior to conditioning chemotherapy.

Due to the high-risk level of this study, while enrolled, all subjects must agree not to participate in a conception process (e.g., active attempt to become pregnant or to impregnate, sperm donation, in vitro fertilization). Additionally, if participating in sexual activity that could lead to pregnancy, the study subject must agree to use reliable and double barrier methods of contraception from time of consent through at least **90 days** after CD4CAR infusion.

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

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

Subjects who are not of reproductive potential (women who have been post-menopausal for at least 24 consecutive months or have undergone hysterectomy, salpingotomy, and/or bilateral oophorectomy or men who have documented azoospermia) are eligible without requiring the use of contraception. Acceptable documentation of sterilization, azoospermia, and menopause is specified next:

Written or oral documentation communicated by clinician or clinician's staff of one of the following:

- Physician report/letter
- Operative report or other source documentation in the subject record (a laboratory report of azoospermia is required to document successful vasectomy)
- Discharge summary
- Laboratory report of azoospermia
- Follicle stimulating hormone measurement elevated into the menopausal range

6. SUBJECT REGISTRATION

Participants MUST be enrolled and registered with Indiana University Simon Comprehensive Cancer Center (IUSCCC) Clinical Trials Office (CTO) prior to the start of protocol treatment. After eligibility is confirmed by the outside participating site staff, a completed eligibility checklist, supporting source documentation, and signed consent will be sent to IUSCCC for verification. The Multicenter Project Manager, or designee, will assign the patient a study number and return the enrollment information to the site. The site staff will then register the patient in OnCore®. Additional details of this process can be found in the Study Procedure Manual. Issues that would cause treatment delays should be discussed with the overall Study Principal Investigator. If a participant does not receive protocol therapy following registration within the allowed time period, the participant's registration on the study may be canceled. The Project Manager should be notified of cancellations as soon as possible. All patients must meet eligibility criteria. Request for eligibility exceptions will not be entertained.

Regulatory files will be maintained by the IUSCCC Clinical Trials Office. Each participating site must submit regulatory documents (informed consents, 1572s, Financial Disclosures, IRB approval documents, Continuing Reviews, Amendments, patient brochures or recruitment material etc.) to the Coordinating Center. The Coordinating Center will provide each site with a comprehensive list of the required documents prior to study start-up, throughout the duration of the study and upon study closeout. It is the responsibility of the participating site to maintain copies of all documentation sent to the Coordinating Center. Applicable regulatory documents must be completed and on file prior to registration of any patients. Potential patients will be identified in the Oncology outpatient clinics or by referrals from outside physicians. Patients who appear to be eligible for this trial will undergo the Informed Consent Process and be screened for eligibility utilizing the Eligibility Criteria. The original signed IRB approved Informed Consent Document and completed eligibility checklist will be forwarded to the Clinical Trials Office designee for eligibility verification and registration in the OnCore® database. Notification will be sent to the principal investigator, treating physician and research nurse when registration is complete to confirm registration and inform them of patient ID number.

7. STUDY PROCEDURES

7.1 Overview:

The study consists of 1) consenting, 2) a screening phase, 3) an intervention/treatment phase consisting of apheresis, chemotherapy and infusions of CD4CAR cells 4) follow up.

7.2 Screening Assessment & Enrollment

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

To be eligible, subjects must have an adequate number of T cells that can be successfully transduced and expanded with the anti-CD4 lentivirus vector, as determined from a sample of PBMC obtained by phlebotomy

at the first screening visit (~week -8). The purpose of this screening procedure is to exclude subjects from participation who would otherwise undergo a futile apheresis and restaging, without the possibility of having the source T cells obtained by apheresis returned as redirected T cells.

7.2.1 Baseline assessment. Subjects will be consented and elibility will be reviewed. Eligible subjects who have signed an informed consent and have adequate pre-screening evaluation will undergo a routine lymphoma/leukemia staging workup including:

- β2 Microglobulin Level
- Serum immunoglobulin levels
- Cardiac function assessment, and blood organ function testing
- Syphilis

Screening procedures other than safety tests indicated below may not need to be repeated if initially conducted out of the time window indicated in the schedule of events. The following "safety" screening procedures should be repeated closer to the date of enrollment (i.e. start of conditioning chemotherapy), if and when clinically indicated based on the participant's disease, and at investigator's discretion:

- History and Physical Examination (including height, weight, body surface area)
- ECOG Performance Status Assessment
- Complete Blood Count, Differential and Platelet Count.
- Chemistry Panel, including Lactate Dehydrogenase Level
- Viral serologies (CMV, HIV EBV, Hepatitis B/C)
- Fungal Markers
- Bone marrow aspirate and lymph node biopsy (if accessible). Samples are sent to hematopathology for MRD assessment and CD4 expression.
- Imaging completed as part of standard of care done within 42 days of study entry (time of second screening) if required for disease evaluation.
- Clinical Flow cytometry lymphocyte subset quantitation, including CD4 and CD3 cell determination

7.3 Intervention/Treatment

7.3.1 Bridging Therapy

It is anticipated that many patients might progress between time of enrollment to conditioning chemotherapy. Prior to CD4CAR cell infusion, bridging therapy (examples may include but are not limited to: steroids, chemotherapy, radiation therapy, or targeted therapies) might be planned, per treating physician discretion. Any bridging therapy conducted will conclude within 3 days prior to start of conditioning chemotherapy.

7.3.2 Conditioning Chemotherapy for CD4CAR infusion

The purpose of the chemotherapy is to induce lymphopenia in order to facilitate engraftment and homeostatic expansion of CD4CAR cells. The chemotherapy may also to help to further reduce disease tumor burden. Chemotherapy is started approximately 4 days before infusion so that CD4CAR cells may be given 2 days after completion of the chemotherapy. Fludarabine (25 mg/m^2/day x 3 days) and cyclophosphamide (500 mg/m^2/day x 3 days) are the agents of choice, as there is the most experience with the use of these agents in facilitating adoptive immunotherapy. Bendamustine is now an approved alternative for both approved therapies and on

clinical trials and hence we would like to allow our trials to utilize Bendamustine as an alternative per PI discretion.

The following tests wil be completed prior to initiating conditioning chemotherapy:

- RCL testing
- Baseline human anti-murine antibody (HAMA)
- Research flow cytometry
- Transgene copy number
- Baseline Cytokine levels
- Optional blood samples for future unspecified research

A limited restaging is done at the completion of conditioning chemotherapy in order to provide baseline tumor burden measurements, if applicable. This may include imaging, physical examination, and MRD assessments.

7.3.3 CD4CAR Infusion

For the following organ toxicities, the following specific temporary exclusion criteria may apply (see section 5.4.2). Infusion of T cell infusions will be delayed in case of: 1) Pulmonary: Requirement for supplemental oxygen to keep saturation greater than 95% or presence of radiographic abnormalities on clinically indicated chest x-ray that are progressive; 2) Cardiac: New cardiac arrhythmia not controlled with medical management. 3) Hypotension requiring pressor support. 4) Active Infection: Positive blood cultures for bacteria, fungus, or virus within 48-hours of T cell infusion. A subject may still receive the CD4CAR infusion up to 10 days post conditioning chemotherapy if the condition(s) above change and the exclusion(s) no longer apply. Any subject unable to receive infusion will be withdrawn and replaced.

Note: Hematologic toxicities are an expected outcome from the preconditioning chemotherapy and therefore are excluded from toxicities that may delay infusion and will not delay receipt of T cell infusion.

Subjects will receive infusion in BMT unit in an isolated HEPA filtered room. The cells are thawed at the patient's bedside as described above. The thawed cells will be given at an infusion rate so that the duration of the infusion will be approximately 20 to 30 minutes, including the backflush/rinse of the product bag and line (refer to section 9.2) . Subjects will be pre-medicated, and cells will be infused as described. Subjects' vital signs will be assessed immediately prior to infusion (+/- 5 minutes), every 15 minutes during infusion (+/- 5 minutes), at the end of the infusion(+/- 5 minutes), and every 15 minutes(+/- 5 minutes) thereafter for 1 hour and until these are stable and satisfactory.

7.4 Assessment of engraftment and persistence:

While inpatient, subjects will have blood drawn for cytokine levels, CD4CAR Transgene Copy Number (PCR) and research flow cytometry in order to evaluate the presence of CD4CAR cells on day 3, 5, 7, 14, and 28 post infusion, or more often as clinically indicated. Other procedures required as outlined in the Schedule of Events.

Once discharged, subjects will undergo approx. once a week (+/-3 days) thereafter until Day 28 the following: physical exam, ECOG, documentation of adverse events and concomitant medications; and blood draws for hematology, chemistry, viral and fungal markers, engraftment and persistence of CART-4 cells and research

labs (cytokine levels will be tested twice a week from day 7 to day 14). Refer to Schedule of Events for full list of procedures.

7.5 Post discharge evaluations for 6 months post infusion

All subjects with fever regardless of time of onset during CD4CAR persistence will be hospitalized until delayed/unexpected onset of CRS is ruled out.

Stable subjects will return on a monthly basis during months 2 to 6 (+/- 7 days) post CD4CAR cell infusion. At these study visits, subjects will undergo the following: concomitant medication, physical exam, documentation of adverse events and blood draws for hematology, chemistry, engraftment and persistence of CD4CAR cells and research labs. Testing for replication competent lentivirus (RCL) will be performed pre-treatment, 3, 6 and 12 months according to the current FDA Guidance (issued January 2020).

7.6 Quarterly evaluations for up to 2 Years Post Infusion

Subjects will be evaluated on at least a quarterly basis (+/-30 days) until 2 years post infusion. At these study visits, subjects will undergo the following: concomitant medication, physical exam, documentation of adverse events and blood draws for hematology, chemistry, engraftment and persistence of CD4CAR cells and research labs. Testing for replication competent lentivirus (RCL) will be performed pre-treatment, 3, 6 and 12 months according to the current FDA Guidance (issued January 2020), then discontinued for the individual subject if all are negative. Documentation of adverse events will include evaluation of SAEs, Aes ≥ Grade 3, with particular emphasis on infections, immunoglobulin levels, intravenous immunoglobulin administration, RCL, and carcinogenesis.

7.7 Annual evaluations for up to 15 Years Post Infusion (optional in person visits)

Following this evaluation at 2 years, subjects will be followed up to twice a year (+/-30 days) by phone and questionnaire (to include adverse events, quality of life, and disease status) for an additional thirteen (13) years to assess for the diagnosis of long-term health problems, such as development of new malignancy. After the initial follow up phase of two years, subjects will be encouraged to visit the treatment site one to two times per year for a physical exam and medical history (including adverse events, and concomitant medications) with careful attention to features possibly related to oncoretroviral diseases such as cancer, neurologic disorders or other hematologic disorders. Visiting the research center for annual evaluations is optional. Adverse events in this follow-up period include documenting intercurrent infections requiring intervention (e.g. intravenous immunoglobulin therapy), immunoglobulin values when clinically indicated, RCL and carcinogenesis. If CD4CAR continues to be detected, labs will be drawn to evaluate persistence by transgene copy number at in person annual visits, if they occur. If all RCL tests are negative during the study period to the first annual evaluation, then plasma may be archived for analysis on an as needed basis.

7.8 Tumor Response Assessments

Although this is a phase 1 dose finding study, we still will conduct tumor response assessments. This assessment will be done according to standard of care and practices every 3 months for 2 years after CD4CAR cell infusions or until the patient requires alternative therapy for their disease.

Tumor assessments will depend on the patients underlying disease as follows:

For lymphomas presenting with solid tumor masses/lymph nodes, we will perform imaging (any technique) and possible biopsies of disease sites. Samples will be tested by flow cytometry, immunohistochemistry and molecular studies when applicable

For Leukemias: CBCs will be done at each visit and if any suspicious changes of recurrence occur, such as new onset cytopenia or abnormal circulating cells, a bone marrow biopsy will be performed to examine for disease

recurrence. All standard morphological, flow cytometry and molecular profiling will be requested on all bone marrow biopsies.

Table 2. Example Tumor Assessments

Disease	Physical Exam*	CBC with differential	Bone marrow aspirate +/- biopsy	Lymph node biopsy	Imaging scans
TALL	+	+	+/-		+/-
T cell NHL	+	+	+/-	+	+

*For patients with skin involvement, clinical measurements as well as photographs of the lesions will be added to the evaluation methods. For applicable participants, a study physician or nurse will take photographs of skin disease in accordance to research sites' clinical policies and procedures, and stored in the participants' medical records as well as in participants' study binder.

A sufficient number of pictures should be taken to assess disease status at each visit. New lesions should also be documented. Pictures will be annotated to include subject ID, name, MRN, lesion location (e.g. right upper thigh), date, and time collected.

8. DRUG INFORMATION

8.1 Description

The investigational agent in this protocol is a CD4-specific chimeric antigen receptor engineered T-cell (CD4CAR T-cell) (Fig.3), which is an autologous human T-cell engineered to express an anti-CD4scFV domain derived from the monoclonal antibody (mAb) ibalizumab (Hu5A8 or TNX-355). In order to effectively bind CD4, this is paired with an antigen receptor with an intracellular tyrosine-based activation motif from the TCR by lentiviral transduction of a CD4CAR gene. This redirects specificity of the transduced T cells for cells that express CD4, which may be malignant or normal T cells. In addition to CD4scFv, the cells will be transduced to express an intracellular signaling molecule comprised of a tandem signaling domain comprised of 4-1BB and TCRζ. The scFv is derived from a mouse monoclonal antibody and the signaling domains are entirely of the native human sequences. The CAR constructs were developed at the Stony Brook campus by iCellGene, and the clinical grade vector is manufactured at Indiana University. The CD4CAR cells will be manufactured in the GMP facility at Indiana University according to the process shown in figure 4. At the end of cell cultures, the cells are cryopreserved in infusible cryomedia.

8.2 Treatment Regimen

A period of **28** days must be allowed between subjects from the start date of CD4CAR infusion until the following participant's conditioning chemotherapy can start. Based on FDA recommendations, there will be a 28 day observation period between the first pediatric subject and enrollment of subsequent pediatric subjects. The following cohort plan assumes no DLTs occur in the study:

- Patients 1-3
    - This cohort will be treated with Dose Level 1, $8x10^4$ /kg total transfected CD4CAR T cells. The infusion will be scheduled to occur approximately 3 to 4 days following chemotherapy**.**
- Patients 4-6
    - This cohort will be treated with Dose Level 2, which consists of 1.6 $x10^5$ /kg total transfected

CD4CAR T cells. The infusion will be scheduled to occur approximately 3 to 4 days following chemotherapy.

- Patients 7-9
    - This cohort will be treated with Dose Level 3, which consists of $4x10^5$ /kg total transfected CD4CAR T cells. The infusion will be scheduled to occur approximately 3 to 4 days following chemotherapy.
- Patients 10-12
    - This cohort will be treated with Dose Level 4, 1 $x10^6$/kg total transfected CD4CAR T cells. The infusion will be scheduled to occur approximately 3 to 4 days following chemotherapy.
- Patients 13-15
    - This cohort will be treated with Dose Level 5, 2.0 $x10^6$ /kg total transfected CD4CAR T cells. The infusion will be scheduled to occur approximately 3 to 4 days following chemotherapy.

8.3 Preparation, packaging, shipping and receiving Study Drug

8.3.1 Preparation

8.3.1.1 Manufacturing and Cryopreservation

The CAR construct used in this study was developed by iCell Gene Therapeutics. The clinical grade vector is manufactured at Indiana University, a GMP facility. The CD4CAR T-cells will be manufactured at the Indiana University Cell Immunotherapy and Transduction Facility. Manufacturing and facility information is on file with the FDA.

8.3.1.2 Apheresis

The apheresis procedure is carried out to obtain PBMCs for CD4CAR manufacturing. Our intention is to harvest approximately $5x10^8$ white blood cells to manufacture CD4CAR T-cells from a single leukapheresis. Lower yields are acceptable.

The numbers of liters of blood volume to be processed would be determined based on patient's weight and height (e.g. 12-15-liters).

At IUSCCC, the apheresis product will be transferred to the Cell Immunotherapy and Transduction facility, but may not begin the same day as collection. **The cell product is expected to be ready for release approximately four (4) weeks after apheresis**.

8.3.1.3 Cryopreservation

At the end of cell culture, the cells are cryopreserved in infusible cryomedia that will be shipped to the investigator.

As a single infusion is planned, each bag will contain an aliquot (volume dependent upon dose) of cryomedia containing the following infusible grade reagents: 4.5% human serum albumin with 10% DMSO.

One bag of CD4CAR modified cells will be delivered in a cryoshipper with all necessary documentation to be filled out at the time of administration including the Cellular Therapy Infusion Record. Additional bags will remain at the GMP facility as back up infusions. If a back-up bag cannot be made from the original manufacturing run, a back-up product will be created from an additional manufacturing run. If needed, they will be shipped to the site per above.

8.3.1.4 Thawing

The product will be thawed at bedside in a water bath at 37 degrees Celsius shortly prior to infusion, at

subjects' bedsides.

8.3.2 Packaging

Infusion bags will contain the appropriate volume of infusion mix to obtain the desired concentration of CD4CAR cells. Each bag will contain an aliquot (volume dependent upon dose) of cryomedia containing the following infusible grade reagents: 4.5% human serum albumin with 10% DMSO.

8.3.3 Shipment and Storage

The manufactured CD4CAR cellular product will be shipped according to the manufatoring site procedure in a qualified dry shipper, either within the institution or outside of the institution.

8.3.4 Receiving, Storage and Thawing

Upon release, the cells will be delivered to the research site's Stem Cell Therapy Lab, where an inventory and inspection (including, but not limited to, physical evaluation, temperature evaluation, labeling, etc.) of the study treatment supplies will be performed and a drug receipt log will be filled out and signed by the person accepting the shipment. The cells will be then delivered to the stem cell facility clinical lab in each institution. A chain of custody document will be maintained to document movement of the cells in the facility.

It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory included in the shipper. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. Sites may use their own forms per their institutional policy or use templates provided by the coordinating center. If needed, examples of forms can be provided to research sites.

8.3.5 Dispensing of Study Drug

After logging the cells in the research site facility, bags/vials (20 to 100 ml capacity) containing CART-4-transduced T cells will be stored in site's cell processing facility conditions in a monitored -150°C freezer. Infusion bags will be stored in the freezer until needed. CART-4-transduced T cells will be delivered and stored in accordance with each site's policy.

On the day of infusion, frozen CD4CAR cells will be transported in dry shipper to the subject's bedside. The cells will be thawed at the bedside one bag at a time using a water bath maintained at 36°C to 38°C. The bag will be gently massaged until the cells have just thawed. There should be no frozen clumps left in the container. If the CD4CAR T cell product appears to have a damaged or leaking bag, or otherwise appears to be compromised, it should not be infused, and should be returned to the site's cell processing facility as specified below.

8.3.6 Return or Destruction of Study Drug

CD4CAR T cells may require return to the site's cell processing facility for a variety of reasons, including but not limited to: 1) Mislabeled product; 2) Condition of patient prohibits infusion/injection, and 3) Subject refuses infusion/injection; any unused frozen product will be returned to the coordinating center for disposal or storage as per facility policy.

If a product is manufactured for a subject but is unable to be administered, (including but not limited to previous situations mentioned) the product will be stored for a year, and if at that time the product still cannot be administered to that subject it may be utilized for research purposes.

There will be an ongoing reconciliation of drug shipped, drug consumed, and drug remaining, performed by the BMT lab. This information is submitted on an annual basis to the FDA in annual reports. Drug destroyed on site will be documented in the study files.

9. CD4CAR TREATMENT PLAN

Infusion bags will have affixed to them a label containing the following: "FOR AUTOLOGOUS USE ONLY." In addition, the label will have at least two unique identifiers such as the subject's initials, birth date, and study number. Prior to the infusion, two individuals will independently verify all this information in the presence of the subject and confirm that the information is correctly matched to the participant.

Emergency medical equipment (i.e., emergency trolley) will be available during the infusion in case the subject has an allergic response, or severe hypotensive crisis, or any other reaction to the infusion. Vital signs (temperature, respiration rate, pulse and oxygen saturation and blood pressure) will be taken before (+/-5 minutes) and after infusion (+/-5 minutes), then every 15 minutes (+/-5 minutes) for at least one hour and until these signs are satisfactory and stable (in the event the infusion lasts longer than 15 minutes, vital signs are also taken every 15 minutes during infusion(+/-5 minutes)).

9.1 Pre-Infusion Premedication and lab tests

- Subjects will be pre-medicated with acetaminophen 650 mg by mouth and diphenhydramine hydrochloride 25-50 mg by mouth or IV prior to the infusion.
- Within 12 hours before the first infusion, all patients will have a blood count with differential, and an assessment of CD3, CD4, and CD8 counts since chemotherapy is given in part to induce lymphopenia.
- Within 12 hours before the first infusion, blood chemistry including uric acid and potassium will be collected.
- Within 12 hours before first infusion PT, PTT will be collected.

9.2 Procedure to be followed for cell infusion*:*

Each research site will follow their institutional policy for infusion of CAR-T products. Below is an example on how the process could be performed.

9.2.1 Day of Infusion:

On the day of the infusion, the RN will assemble supplies for infusion:

- One 1 liter bag of Plasma-Lyte A injection pH 7.4
- One BD SmartSite Gravity set or equivalent
- One secondary admin set with bag hanger (Note: secondary admin set is not applicable if gravity set has dual spikes)
- Vital signs cycling every 15 minutes

9.2.2 Prior to infusion:

- Prime one spike and line of tubing with approx. 500ml of Plasmalyte A. This will connect to central line on subject. Clamp after primed.
- Spike CAR T- cell bag with the non-primed side of tubing and prime with CART-T cells to drip chamber. Remainder of tubing should already be primed with Plasmalyte A.
- Infuse the entire contents of the CAR-T cell bag by gravity. Gently agitate the CAR-T cell bag during infusion to prevent cell clumping.
- Vital signs, including temperature, respiratory rate, blood pressure, and pulse and oxygen saturation will

be taken:

- Prior to the start of infusion,
- ***Only applicable if total infusion last longer than 15 minutes:*** Every 15 minutes throughout infusion(+/-5 minutes)
- Completion of infusion (+/-5 minutes)
- Every 15 minutes (+/-5 minutes) thereafter for at least one hour until vital signs are satisfactory and stable (may be up to 6 hours post-infusion)

- Following infusion, repeat backflush process by adding 20 mL of Plasmalyte A into the the IP bag. Leave the bag inverted and seal above the bag so that the bag can be aseptically removed.

9.3 Post-Infusion Clinical Guide and recommendations for supportive care and treatment of side effects in the immediate post-infusion period:

Daily review of patient history or physical exam will be documented post infusion while inpatient. Assessment for and grading of cytokine release syndrome (CRS) will be done at least daily and whenever clinically indicated (for example, with a change in clinical status).

9.3.1 Constitutional

Administer acetaminophen for symptomatic management of fevers in patients with normal hepatic function; NSAIDS may be considered in fevers refractory to acetaminophen. Provide cooling blankets for fevers 40° C or greater; avoid meperidine; avoid corticosteroids unless used as management of severe CRS.

9.3.2 Cardiovascular

Stop or taper antihypertensive medications prior to cell infusion. Telemetry monitoring will begin, at the latest, on the day of CAR T cell infusion and continue until at least day 7 post infusion or until resolution of CRS (or until discharge if the subject is deemed stable for discharge prior to 7 days post-infusion). Vital signs (including temperature, heart rate, blood pressure, respiratory rate, and pulse and oxygen saturation) will be done and documented just prior to CAR T cell infusion. During CAR T cell infusion, monitor and document vital signs every 15 minutes for the duration of the infusion, then immediately following infusion, and every 15 minutes for at least one hour and stable and satisfactory. After this initial monitoring period, monitor and document vital signs at least every 4 hours on an inpatient unit for at least 7 days following infusion (unless the patient is deemed stable for discharge prior to 7 days); monitor and document vital signs at least every 2 hours in patients with fevers and/or tachycardia (at least 20 bpm above subject's average baseline heart rate). Strict fluid input and urine output will be measured. Initiate replacement intravenous fluids for patients with poor oral intake or high insensible losses to maintain net even fluid balance. Administer a 20mL/kg normal saline bolus for patients with hypotension defined by: SBP ≤ 80% of pre-infusion SBP with <100 mmHg or a SBP ≤ 90mmHg. A second bolus of normal saline may be given if SBP remains ≤ 90mmHg. Patients with persistent hypotension after adequate intravenous fluid resuscitation will be initiated on vasopressor support. Norepinephrine is the preferred first-line vasopressor[78].

9.3.3 Hematologic

Prior to conditioning chemotherapy, allopurinol will be initiated for tumor lysis syndrome (TLS) prophylaxis in patients without a contraindication. TLS will be treated according to institutional standards. Monitor complete blood count with differential, complete metabolic panel, CRP, ferritin and coagulation panel at least daily for at least 7 days post CAR T cell infusion, or for the duration of hospitalization. Transfuse packed

red blood cells to maintain a goal hemoglobin of ≥ 8.0g/dL. Transfuse platelets to maintain a goal platelet count of ≥ 10,000/mL; a higher goal may be appropriate pending clinical situation. Monitor fibrinogen and consider cryoprecipitate transfusions to maintain fibrinogen ≥ 100 mg/dL. If bleeding occurs or an invasive procedure is needed, a higher level of fibrinogen should be maintained.

9.3.4 Neurologic

Anti-seizure prophylaxis with levetiracetam 500mg administered twice daily will be initiated 24 hours before cell infusion and will continue for at least 30 days post CAR T infusion. The nursing staff will conduct focused neurologic examinations approx. every 8 hours for at least 7 days (or earlier if the patient is deemed stable for discharge prior to 7 days post-infusion) post CAR T cell infusion for Immune effector cell-associated neurotoxicity syndrome (ICANS) using the American Society for Transplantation and Cellular Therapy's Immune Effector Cell-Associated Encephalopathy (ICE) score grading scale[81]. Neurologic examinations will be intermittently done thereafter. A brain MRI will be performed in any patient experiencing neurologic toxicity as clinically feasible. A lumbar puncture will be performed to evaluate for infectious pathogens, cytokine levels, and CAR T-cell levels in patients experiencing neurologic toxicity whenever feasible. The neurology service will be consulted for any patient experiencing neurologic toxicity. Standard antiepileptic medications are used for patients having active seizures (refer to Table 5).

9.3.5 Infectious disease

Preferred prophylactic antimicrobials are trimethoprim-sulfamethoxazole (TMP-SMX) DS tablet three times weekly for pneumocystis jirovecci and cryptococcal prophylaxis (beginning within one week prior to cell infusion), acyclovir 400mg q8h for herpes virus prophylaxis (beginning on day of cell infusion), azithromycin 1200mg PO weekly for bacterial prophylaxis (beginning within one week of cell infusion), and appropriate fungal prophylaxis (beginning day of cell infusion), consistent with NIH guidelines for infection prophylaxis in patients with HIV[83, 87]. Alternatives may be used per clinical judgment based on patient condition. Levofloxacin may be added for patients with severe neutropenia (ANC <500). The duration of prophylactic antimicrobials will be at the discretion of the PI. For the duration of CD4 aplasia, prophylaxis will follow current NIH guidelines for HIV infected patients with AIDS[87]. Patients experiencing fever (temperature ≥ 38°C) will have blood cultures drawn (from peripheral and central lines), urinalysis and urine culture performed, and chest radiography and may undergo organ specific scans or pan scan as determined by PI. Broad-spectrum antibiotic coverage will be initiated in patients with fever and/or those with signs consistent with sepsis.

9.3.6 Stable subjects:

Subjects' vital signs (temperature, heart rate, respiratory rate, pulse and oxygen saturation, and blood pressure) will be assessed and telemetry monitoring will be performed immediately after the end of the infusion and every 15 (+/-5) minutes thereafter for one hour and will continue until these vital signs are stable and satisfactory.

9.4 Post-infusion laboratory testing to assess engraftment and persistence

- Potassium and uric acid will be collected3 times a day for 48 hours post-infusion to monitor for TLS.
- CD4CAR T-cell flow cytometry and transgene copy number (PCR) will be collected according to the schedule of events.
- Cytokine levels will be evaluated per schedule of events in addition to and as needed approximately every 8 +/- 2 hours as feasible, in consideration of participant availability, and clinical/research staff

operations, if/when CRS occurs and until resolution.

- Bone marrow aspirate and other disease site biopsies, if feasible and applicable, will be collected to analyze CAR T-cell trafficking and engraftment as needed, if the procedure can be performed safely as determined by the treating physician. If done, bone marrow aspirate and other disease site biopsies will be analyzed by flow cytometry with Fab labeling and by PCR specific to CD4CAR transgene.
- Optional blood samples for future unspecified research will also be collected at the time of estimated maximum engraftment, per the study lab manual. Patients will return to the research site per schedule of events or more often as clinically indicated.

9.5 Prior and Concomitant Medications

All prescription and nonprescription medications, vitamins, herbal and nutritional supplements, taken by the subject during the 30 days prior to screening will be recorded at the screening visit. At every visit following the conditioning chemotherapy up to the year 15 post CD4CAR infusion, concomitant medications will be recorded in the medical record and on the appropriate CRF. After year 2, concomitant medications will only be collected if the subject comes in-person for their follow-up visit. Any additions, deletions, or changes of these medications will be documented.

10. TOXICITIES TO BE MONITORED AND GENERAL MANAGEMENT GUIDELINES

10.1 Replication-competent lentivirus

Follow-up of subject in consultation with gene therapy experts, study investigators, FDA and NIH.

- Inform local public health officials and CDC.
- Identify sexual partners and provide appropriate counseling and intervention.

10.2 Clonality and insertional oncogenesis

- Monitoring for T cell clonal outgrowth will be performed by flow cytometric analysis for CAR expression. If an abnormal clone of T cells is detected at any time during CAR persistence, insertional and VB studies will be considered.

10.3 Management of Toxicity

10.3.1 Cytokine Release Syndrome (CRS):

One of the most common acute toxicities of CAR T cells is CRS. CRS is due to the rapid proliferation in vivo of the CAR T cells. The cytokines implicated in CRS may be directly produced by the infused CAR T cells, or other immune cells such as macrophages that might produce cytokines in response to the infused CAR T cells. A wide variety of cytokines including interleukin-6 (IL-6), interferon-γ, tumor necrosis factor, IL-2, IL-2–receptor-a, IL-8, and IL-10 are elevated in the serum of patients experiencing fever, tachycardia, hypotension, and other toxicities after CAR T cell infusions. [75] Criteria for severe CRS are fevers of 38°C or greater for at least 3 consecutive days and elevation of two serum cytokines by 75-fold, or of at least one serum cytokine by at least 250-fold, as well as one clinical sign of severe toxicity (Davila et al, 2014). Although this criteria reliably identifies patients who will need intensive monitoring and intervention for CRS, obtaining real-time cytokine levels may not be possible at some facilities. IL-6 has emerged as key mediator of the pro-inflammatory effects seen in CRS. IL-6 induces proliferation of CRP by hepatocytes and has been correlated with increased levels of IL-6 in CRS. While elevation of CRP >20 mg/dL has been correlated with severe CRS with a specificity of 100%, the prognostic value of this marker is still unknown[76] however, it may be helpful for identifying the peak point of toxicity and predicting toxicity resolution.

Any concurrent end-organ toxicities will be graded in accordance with Common Terminology Criteria for Adverse Events v5. Grading of CRS is outlined in Table 2[81]. Recommendations for the management of CRS are outlined in Table 3. [82, 83] It is recommended that at least two doses of tocilizumab (patient specific) are on site for all patients to be treated with CD4CAR T cells and available for immediate use.

Table 3: Grading of CRS (ASTCT CRS Consensus Grading) [81]

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

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 antipyretic or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driving by hypotension and/or hypoxia.

[1]CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with temperature of 39.5°C, hypotension requiring 1 vasopressor, and hypoxia requiring low-flow nasal cannula is classified as grade 3 CRS.

[2]Low-flow nasal cannula is defined as oxygen delivered at ≤6 L/minute. Low flow also includes blow-by oxygen delivery. High-flow nasal cannula is defined as oxygen delivered at >6 L/minute.

Table 3: Guidelines for the management of CRS [82, 83]

CRS Grade	CRS Parameter	Management Guidelines		
		Diagnostic Work-Up	Supportive Care	Anti-IEC Therapies
Grade 1	Fever	• Assess for infection with blood and urine cultures, and chest radiography • Cardiac telemetry and pulse oximetry	• Acetaminophen and hypothermia blanket as needed for the treatment of fever • Ibuprofen if fever is not controlled with above; use with caution or avoid with thrombocytopenia or renal dysfunction • Empiric broad-spectrum antibiotics, and consider filgrastim products if neutropenic • Maintenance IV fluids for hydration • Symptomatic management of constitutional symptoms and organ toxicities as per standard guidelines • If not on seizure prophylaxis, initiate levetiracetam 500mg PO twice daily	• Consider tocilizumab* for 1 dose for persistent fever lasting greater than 3 days
Grade 2	Hypotension	• Cardiac telemetry • Fever work-up if not previously performed (assess for infection with blood and urine cultures, and chest radiography)	• IV fluid bolus of 500-1000mL normal saline; repeat once as needed to maintain normal BP • If hypotension persists after IV fluids, tocilizumab, and dexamethasone, start vasopressors, transfer patient to ICU, obtain ECHO, and refer to further management as in Grade 3 or 4 CRS • Symptomatic management of fever as in Grade 1 CRS	• Administer tocilizumab* for 1 dose and consider dexamethasone 4-10mg IV for 1 dose (or methylprednisolone equivalent) and reassess in 6 hours or earlier if clinically indicated (Tocilizumab may be repeated every 8 hours for up to 3 additional doses in a 24 hour period)

CRS Grade	CRS Parameter	Management Guidelines		
		Diagnostic Work-Up	Supportive Care	Anti-IEC Therapies
			• Symptomatic management of constitutional symptoms and organ toxicities as per standard guidelines	
	Hypoxia	• Pulse oximetry • Fever work-up if not previously performed (assess for infection with blood and urine cultures, and chest radiography)	• Use supplemental oxygen as needed • If hypoxia persists after above interventions, but oxygen requirement is stable with low-flow nasal cannula, continue close monitoring. If oxygen requirement increases to high-flow nasal cannula, face mask, or positive pressure ventilation, refer to further management as in Grade 3 or 4 CRS • Symptomatic management of fever as in Grade 1 CRS • Symptomatic management of constitutional symptoms and organ toxicities as per standard guidelines	• Administer tocilizumab* for 1 dose and consider dexamethasone 4-10mg IV for 1 dose (or methylprednisolone equivalent) and reassess in 6 hours or earlier if clinically indicated (tocilizumab may be repeated every 8 hours for up to 3 additional doses in a 24 hour period)
Grade 3	Hypotension	• Obtain ECHO if not performed already • Cardiac telemetry • Fever work-up if not previously performed (assess for infection with blood and urine cultures, and chest radiography)	• Consider transfer of patient to ICU (per institutional guidelines) • IV fluid boluses as needed as in Grade 2 CRS • Use vasopressors as needed • Symptomatic management of fever as in Grade 1 CRS	• Tocilizumab* as in Grade 2 CRS if not administered previously; tocilizumab may be repeated every 8 hours for up to 3 additional doses in a 24 hour period • If on one vasopressor: tocilizumab as in Grade 2 CRS and dexamethasone 10mg IV every 6 hours (or

CRS Grade	CRS Parameter	Management Guidelines		
		Diagnostic Work-Up	Supportive Care	Anti-IEC Therapies
			• Symptomatic management of constitutional symptoms and organ toxicities as per standard guidelines	methylprednisolone equivalent) • If on two vasopressors: tocilizumab as in Grade 2 CRS and dexamethasone 20mg IV every 6 hours (or methylprednisolone equivalent) • If vasopressin and norepinephrine equivalent is ≥15mcg/minute, follow as in Grade 4 CRS • Once CRS improves to Grade 1 or less, taper and/or stop corticosteroids depending on clinical situation
	Hypoxia	• Pulse oximetry • Fever work-up if not previously performed (assess for infection with blood and urine cultures, and chest radiography)	• Supplemental oxygen including high-flow nasal cannula, face mask, non-rebreather mask, or Venturi mask as needed • Symptomatic management of fever as in Grade 1 CRS • Symptomatic management of constitutional symptoms and organ toxicities as per standard guidelines	• Tocilizumab* and dexamethasone 10mg IV every 6 hours (or methylprednisolone equivalent) if not administered previously; tocilizumab may be repeated every 8 hours for up to 3 doses in a 24 hour period) • If there is no improvement in hypoxia within 24 hours, or there is rapid progression of pulmonary infiltrates or sharp increase in FiO2 requirements, increase dexamethasone to 20mg IV every 6 hours (or methylprednisolone equivalent) • Once CRS improves to

CRS Grade	CRS Parameter	Management Guidelines		
		Diagnostic Work-Up	Supportive Care	Anti-IEC Therapies
				Grade 1 or less, taper and/or stop corticosteroids depending on clinical situation
Grade 4	Hypotension	• Obtain ECHO if not performed already • Cardiac telemetry • Fever work-up if not previously performed (assess for infection with blood and urine cultures, and chest radiography)	• Transfer patient to ICU • IV fluid boluses as needed as in Grade 2 CRS • Vasopressors as in Grade 3 CRS • Use vasopressors as needed • Symptomatic management of fever as in Grade 1 CRS • Symptomatic management of constitutional symptoms and organ toxicities as per standard guidelines	• Tocilizumab* as in Grade 2 CRS if not administered previously; tocilizumab may be repeated every 8 hours for up to 3 additional doses in a 24 hour period • Methylprednisolone 1000mg/day in divided doses IV for 3 days followed by rapid taper as per clinical situation • If hypotension is refractory for >24 hours or if patient is deteriorating rapidly, consider additional therapies including activation of safety switches if applicable (address with PI)
	Hypoxia	• Monitor oxygen saturation while on mechanical ventilation • Fever work-up if not previously performed (assess for infection with blood and urine cultures, and chest radiography)	• Transfer patient to ICU • Positive pressure ventilation including CPAP, BiPAP, mechanical ventilation • Symptomatic management of fever as in Grade 1 CRS • Symptomatic management of constitutional symptoms and organ toxicities as per standard guidelines	• Tocilizumab* as in Grade 2 CRS if not administered previously; tocilizumab may be repeated every 8 hours for up to 3 additional doses in a 24 hour period • Methylprednisolone 1000mg/day in divided doses IV for 3 days followed by rapid taper as per clinical situation • If hypoxia is refractory for >24 hours or if patient is deteriorating

CRS Grade	CRS Parameter	Management Guidelines		
		Diagnostic Work-Up	Supportive Care	Anti-IEC Therapies
				rapidly, consider additional therapies, including activation of safety switches if applicable (address with PI)

*When tocilizumab is required for treatment of CRS, approved doses will be used. All doses will be capped at 800mg per dose. If clinical improvement does not occur, the same dosage may be repeated for up to 3 additional doses in a 24 hour period, separated by at least 8 hours. Tocilizumab dosing: Patients <30kg: Give 12mg/kg x 1 dose (maximum 800mg/dose) in 50mL normal saline over 1 hour. Patients ≥30kg: Give 8mg/kg x 1 dose (maximum 800mg/dose) in 100mL normal saline over 1 hour. Pre-medication with antihistamines and antipyretics are not specifically required prior to dosing unless an indication exists. Maximum of 4 doses total over the entire course of CRS and ICANS.

10.3.2 Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS):

Neurotoxicity/ICANS has been a major complication associated with CAR T cell therapy. Confusion, aphasia, and seizures have been reported in other trials. The distinct pathophysiology of these symptoms has not been fully explained, but may be due to elevated cytokine levels or direct CAR T cell toxicity in the CNS. This toxicity has typically been reversible in previous clinical trials. Grading of ICANS will be done according to the ASTCT ICANS Consensus Grading for Adults[81] (see Tables 4 and 5). Guidelines for the management of ICANS and increased intracranial pressure (ICP) related to CAR T cells are outlined in Table 6 and 7[83]). Non-convulsive and convulsive seizures have been also been reported. All patients will be on levetiracetam from one day prior to CAR T infusion until at least day 30. Guidelines for the management of seizures associated with CAR T cells are described in Table 8[83]

Table 4: Immune Effector-Cell Associated Encephalopathy (ICE) Score Grading System[81]

Task/Question	Value 1 = appropriate 0 = inappropriate	
Year?		
Month?		
City?		
Hospital?		
Follow simple commands		
Name object 1 (point to object)		
Name object 1 (point to object)		
Name object 1 (point to object)		
Handwriting assessment		
Count backwards from 100 by 10		
	Total Score:	

ICE Score tasks may be simplified depending on patient's baseline ability (for example, a patient who cannot write a standard sentence may sign their name or draw the same figure).

Table 5: **Grading of ICANS[81]**

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE score*	7-9	3-6	0-2	0 (patient is unarousable and unable to perform ICE)
Depressed level of consciousness1	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 nonconvulsive 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 findings2	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis
Elevated ICP / cerebral edema	N/A	N/A	Focal/local edema on neuroimaging3	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 intracranial pressure/cerebral edema) not attributable to any other cause. For example, a patient with an ICE socre of 3 who has a generalized seizure is classified as having Grade 3 ICANS.

*A patient with an ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, but a patient with an ICE score of 0 may be classified as grade 4 ICANS if unarousable.

[1]Depressed level of consciousness should be attributable to no other cause (e.g., no sedating medication).

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

[3]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[86].

Table 6: Recommended guidelines for management of ICANS [83]

ICANS Grade	Sign or Symptom	Management Guidelines		
		Diagnostic Work-up	Supportive Care	Anti-IEC Therapies
Grade 1	Encephalopathy and/or depressed level of consciousness	• MRI imaging of the brain with and without contrast; CT of brain without contrast may be performed if MRI is not feasible; MRI spine if focal deficits are noted • Neurology consultation • Increase ICE score assessment to every 6 hours or more frequently if clinically indicated • EEG • Consider diagnostic lumbar puncture if other causes of encephalopathy are suspected (such as infections, autoimmune, leptomeningeal disease)	• Vigilant supportive care; aspiration precautions; IV hydration • Withhold oral intake of food/medications/fluids and assess swallowing; convert all oral medications and/or nutrition to IV if swallowing is impaired • Avoid medications that cause central nervous system depression • Low doses of lorazepam after EEG is performed (0.25-0.5mg IV q8h) or haloperidol (0.5mg IV q6h) may be used with careful monitoring for agitated patients • If no seizures on EEG, continue prophylactic levetiracetam • If EEG shows focal of generalized convulsive or non-convulsive seizure or convulsive status epilepticus, refer to further management as in Grade 3 or 4 ICANS	• Dexamethasone 10mg IV for 1 dose (or methylprednisolone equivalent) and reassess in 6 hours or earlier if clinically indicated • If associated with concurrent CRS, add tocilizumab*
Grade 2	Encephalopathy and/or depressed level of consciousness	• Neurological work-up as in Grade 1 ICANS	• Supportive care as in Grade 1 ICANS	• Dexamethasone 10mg IV every 12 hours (or methylprednisolone equivalent)

ICANS Grade	Sign or Symptom	Management Guidelines		
		Diagnostic Work-up	Supportive Care	Anti-IEC Therapies
				• If associated with concurrent CRS, add tocilizumab* • Once ICANS improves to Grade 1 or less, taper and/or stop corticosteroids depending on clinical situation
Grade 3	Encephalopathy and/or depressed level of consciousness	• Neurological work-up as in Grade 1 ICANS • Consider repeat neuro-imaging (CT or MRI) every 2-3 days for persistent ≥ Grade 3 encephalopathy • Consider diagnostic lumbar puncture if Grade 3 encephalopathy persists ≥ 2 days or earlier if other causes are suspected (e.g. infections, autoimmune, leptomeningeal disease)	• Supportive care as in Grade 1 ICANS • Transfer to ICU per institutional protocol • If there are new abnormal findings on brain imaging not related to primary malignancy, control hypertension with the goal of maintaining mean arterial pressure (MAP) within 20-25mmHg of baseline MAP; correct any uremia (dialysis if needed) and/or coagulopathy (transfuse to keep platelets >20-50 K/microliter, fibrinogen >200mg/dL and INR <1.5)	• Dexamethasone 10mg IV every 6 hours (or methylprednisolone equivalent) • If associated with concurrent CRS, add tocilizumab* • If Grade 3 encephalopathy is persistent for >24 hours, increase dexamethasone to 20mg IV every 6 hours (or methylprednisolone equivalent) • Once ICANS improves to Grade 1 or less, taper and/or stop corticosteroids depending on clinical situation
	Seizure	• Neurological work-up as in Grade 1 ICANS • EEG if clinically indicated (e.g. ongoing seizures,	• Transfer to ICU per institutional protocol • Supportive care as in Grade 1 ICANS • For focal or generalized convulsive seizures, or non-convulsive seizures,	• Dexamethasone 20mg IV every 6 hours (or methylprednisolone equivalent) • If associated with concurrent CRS, add tocilizumab*

ICANS Grade	Sign or Symptom	Management Guidelines		
		Diagnostic Work-up	Supportive Care	Anti-IEC Therapies
		depressed level of consciousness) • Rule out other potential causes of seizure (i.e., beta-lactams, etc)	treat as per Table 8	• Once ICANS improves to Grade 1 or less, taper and/or stop corticosteroids depending on clinical situation
	Focal cerebral edema	• Neurological work-up as in Grade 1 ICANS • Consider repeat neuro-imaging (CT or MRI) every 24 hours until edema resolves or more frequently if clinically indicated	• Transfer to ICU per institutional protocol • Supportive care is in Grade 1 ICANS	• If focal edema is in brain stem or thalamus, methylprednisolone 1000mg/day in divided doses IV for 3 days followed by taper depending on clinical situation (if associated with concurrent CRS, add tocilizumab*) • If focal edema is in other areas of brain, methylprednisolone 1000mg/day in divided doses IV for 1 day; assess daily and continue or taper depending on clinical situation (if associated with concurrent CRS, add tocilizumab*)
Grade 4	Encephalopathy and/or depressed level of consciousness	• Neurological work-up as in Grade 1 ICANS • Repeat neuro-imaging and lumbar puncture as in Grade 3 ICANS	• Transfer to ICU per institutional protocol • Supportive care as in Grade 1 ICANS • Consider mechanical ventilation for airway protection • If there are new abnormal findings on brain imaging	•Methylprednisolone 1000mg/day in divided doses IV for 3 days followed by taper as clinically indicated; if associated with concurrent CRS, add tocilizumab* • Continue

ICANS Grade	Sign or Symptom	Management Guidelines		
		Diagnostic Work-up	Supportive Care	Anti-IEC Therapies
			not related to primary malignancy, control hypertension with the goal of maintaining MAP within 20-25mmHg of baseline MAP; correct any uremia (dialysis if needed) and/or coagulopathy (transfuse to keep platelets >20-50 K/microliter, fibrinogen >200mg/dL and INR<1.5)	corticosteroids until improvement to less than or equal to Grade 1 ICANS and then taper and stop corticosteroids depending on clinical situation • If Grade 4 ICANS is refractory for >24 hours or if patient is deteriorating rapidly, consider additional therapies including activation of safety switches if applicable (address with PI)
	Seizure	• Neurological work-up as in Grade 1 ICANS • Rule out other potential causes of seizure (i.e., beta-lactams, etc)	• Transfer to ICU per institutional protocol • Supportive care as in Grade 1 ICANS • For focal or generalized convulsive or non-convulsive seizure or convulsive status epilepticus, treat as in Table 8 • For convulsive status epilepticus, treat as in Table 8	•Methylprednisolone 1000mg/day in divided doses IV for 3 days followed by taper as clinically indicated; if associated with concurrent CRS, add tocilizumab* • If Grade 4 ICANS is refractory for >24 hours or if patient is deteriorating rapidly, consider additional therapies including activation of safety switches if applicable (address with PI)
	Motor Weakness	• Neurological work-up as in Grade 1 ICANS • MRI with and without contrast of the spine	• Transfer to ICU per institutional protocol • Supportive care as in Grade 1 ICANS	•Methylprednisolone 1000mg/day in divided doses IV for 3 days followed by taper as clinically indicated; if associated with

ICANS Grade	Sign or Symptom	Management Guidelines		
		Diagnostic Work-up	Supportive Care	Anti-IEC Therapies
				concurrent CRS, add tocilizumab* • If Grade 4 ICANS is refractory for >24 hours or if patient is deteriorating rapidly, consider additional therapies including activation of safety switches if applicable (address with PI)
	Diffuse cerebral edema or raised intracranial pressure	• Neurological work-up as in Grade 1 ICANS • Consider repeat neuroimaging as in focal cerebral edema from Grade 3 ICANS	• Transfer to ICU per institutional protocol • Supportive care as in Grade 1 ICANS • For diffuse cerebral edema or signs of raised intracranial pressure, treat as in Table 7	•Methylprednisolone 1000mg/day in divided doses IV for 3 days followed by taper as clinically indicated; if associated with concurrent CRS, add tocilizumab* • If Grade 4 ICANS is refractory for >24 hours or if patient is deteriorating rapidly, consider additional therapies including activation of safety switches if applicable (address with PI)

*When tocilizumab is required for treatment of CRS, approved doses will be used. All doses will be capped at 800mg per dose. If clinical improvement does not occur, the same dosage may be repeated for up to 3 additional doses in a 24-hour period, separated by at least 8 hours. Tocilizumab dosing: Patients <30kg: Give 12mg/kg x 1 dose (maximum 800mg/dose) in 50mL normal saline over 1 hour. Patients ≥30kg: Give 8mg/kg x 1 dose (maximum 800mg/dose) in 100mL normal saline over 1 hour. Pre-medication with antihistamines and antipyretics are not specifically required prior to dosing unless an indication exists. Maximum of 4 doses total over the entire course of CRS and ICANS.

Table 7: Recommended guidelines for management of Diffuse Cerebral Edema and/or Raised ICP related to CAR T cell therapy [83]

For papilledema without diffuse cerebral edema or other signs of raised intracranial pressure	• Acetazolamide 1000mg IV followed by 250-1000mg IV every 12 hours (monitor renal function and acid/base balance once or twice daily and adjust dose accordingly)

	• Dexamethasone 20mg IV every 6 hours (or methylprednisolone equivalent) and start taper after resolution of papilledema
For diffuse cerebral edema on neuroimaging or signs of raised intracranial pressure such as decerebrate or decorticate posturing, cranial nerve VI palsy, or Cushing's triad	• Methylprednisolone 1000mg/day in divided doses IV for 3 days followed by taper as clinically indicated • Elevate head end of patient's bed to an angle of 30 degrees • Hyperventilation to achieve target $PaCO_2$ of 28-30mmHg, but maintained for no longer than 24 hours • Hyperosmolar therapy with either mannitol (20g/dL solution) OR hypertonic saline (3% or 23.4% as detailed below) o Mannitol: initial dose 0.5-1g/kg IV; maintenance dose 0.25-1g/kg IV every 6 hours while monitoring metabolic profile and serum osmolality every 6 hours; and withhold mannitol if serum osmolality is ≥320 mOsm/kg or osmolality gap is ≥40 o Hypertonic 3% saline: initial dose 250mL IV over 15 minutes, maintenance dose of 50-75 mL/hour IV while monitoring electrolytes every 4 hours; withhold infusion if serum sodium levels reach ≥155 mEq/L o Hypertonic 23.4% saline (for patients with imminent hernation): dose to be administered by physician per institutional protocol; initial dose of 30 mL IV; repeat after 15 minutes, if needed • If patient has ommaya reservoir, drain CSF to target OP <20mmHg • Control hypertension with the goal of maintaining mean arterial pressure (MAP) within 20-25mmHg of baseline MAP; correct any uremia (dialysis if needed) and/or coagulopathy (transfuse to keep platelets >20-50 K/microliter, fibrinogen >200 mg/dL and INR <1.5) • Consider neurosurgery consultation and IV anesthetics for burst-suppression pattern on EEG; transfuse to keep platelets ≥100 K/microliter if possible and correct coagulopathy in case of surgical intervention • Consider additional therapies including activation of safety switches if applicable (address with PI) • Metabolic profile every 6 hours and daily CT scans of head without contrast, with adjustments in usage of aforementioned medications to prevent rebound cerebral

	edema, renal failure, electrolyte abnormalities, hypovolemia and hypotension

Table 8: Recommended guidelines for management of: focal or generalized convulsive or non-convulsive seizures; or convulsive status epilepticus after CAR T cell therapy [83]

Recommended Management of Focal or Generalized Convulsive or Non-Convulsive Seizures
• Assess CAB / consider airway protection / check blood glucose • Consult Neurology • For focal and generalized convulsive seizures, lorazepam 1-2mg IV and repeat as needed (to a maximum cumulative dose of 4 mg) • For electrographical seizures, including non-convulsive status epilepticus, lorazepam 0.5mg IV and repeat every 5 minutes as needed (to a maximum cumulative dose of 2mg) • Levetiracetam 500-1500mg IV bolus (in addition to maintenance dose) • Replete with magnesium as needed to maintain magnesium level >2mg/dL • Thiamine 100mg IV every 8 hours for 5 days • If non-convulsive seizures persist, transfer to ICU (per institutional protocol) and add phenobarbital loading dose of 60mg IV (monitor for respiratory depression, bradycardia and hypotension) Maintenance doses after resolution of non-convulsive status epilepticus: Lorazepam 0.5 mg IV every 8 hours for 3 doses Levetiracetam 1000-1500mg IV every 12 hours Phenobarbital 30mg IV every 12 hours (about 0.5mg/kg every 12 hours) Monitor for respiratory depression, bradycardia and hypotension Assess for drug-drug interactions (i.e. may induce metabolism of azole antifungals or other CYP3A4 substrates) and consider alternative therapy if drug interactions are significant Target serum trough levels 15-40mcg/mL
Recommended Management of Convulsive Status Epilepticus
Assess CAB / consider airway protection / check blood glucose Transfer to ICU per institutional protocol Consult Neurology Lorazepam 0.1mg/kg (maximum 4mg/dose) given at a maximum rate of 2mg/minute; may repeat in 5 to 10 minutes Levetiracetam 500-1500mg IV bolus (in addition to maintenance dose) Replete with magnesium as needed to maintain magnesium >2mg/dL

Thiamine 100mg IV every 8 hours for 5 days
If seizures persist, add phenobarbital loading dose of 15mg/kg IV (monitor for respiratory depression, bradycardia and hypotension)
If refractory, consider additional therapies including activation of safety switches if applicable (address with PI)
Maintenance doses after resolution of convulsive status epilepticus
Levetiracetam 1000-1500mg IV every 12 hours
Phenobarbital 0.5mg/kg IV every 12 hours
Monitor for respiratory depression, bradycardia and hypotension
Assess for drug-drug interactions (i.e. may induce metabolism of azole antifungals or other CYP3A4 substrates) and consider alternative therapy if drug interactions are significant
Target serum trough levels 15-40mcg/mL
Continuous EEG monitoring if seizures are refractory to treatment

10.3.3 Haemophagocytic lymphohistiocytosis (HLH)/ macrophage-activation syndrome (MAS):

Although extremely rare, HLH/MAS has been described in other CAR T cell trials[75, 84] This syndrome is a hyperactivation of multiple cell lines and proinflammatory cytokines that may lead to significant multi-organ failure[85]HLH/MAS has a very similar presentation to that of CRS; however, the distinction may be problematic to make. True HLH/MAS may not respond to measures used in the treatment of CRS and may lead to increased mortality.

Due to the difficulty in distinction, Neelapu et al (2017) has proposed that the diagnosis of CAR T cell related HLH/MAS be made given: peak ferritin levels of >10,000 ng/ml during the CRS phase (typically within the first 5 days after cell infusion), and has developed any two of the following: grade ≥3 organ toxicities involving the liver, kidney, or lung, or haemophagocytosis in the bone marrow or other organs (see Figure 8). Patients with suspected HLH/MAS should be treated according to CRS treatment guidelines found in Table 3. If there is no improvement in symptoms or laboratory markers within 48 hours, the addition of system (e.g. etoposide) and intrathecal (e.g. cytarabine) chemotherapy can be considered, although the role of chemotherapy in CAR T cell HLH/MAS has yet to be defined[78].

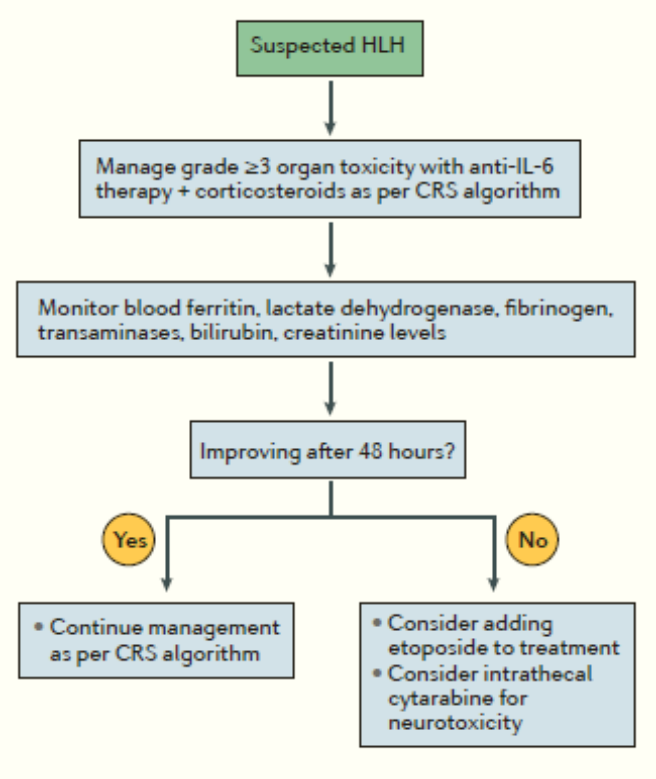
Figure 8 Proposed diagnostic criteria for CAR T cell related HLH/MAS[78]

A patient might have HLH/MAS if he/she had a peak serum ferritin level of >10,000 ng/ml during the cytokine-release syndrome phase of CAR-T-cell therapy (typically the first 5 days after cell infusion) and subsequently developed any two of the following:

- Grade ≥3 increase in serum bilirubin, aspartate aminotransferase, or alanine aminotransferase levels*
- Grade ≥3 oliguria or increase in serum creatinine levels*
- Grade ≥3 pulmonary oedema*
- Presence of haemophagocytosis in bone marrow or organs based on histopathological assessment of cell morphology and/or CD68 immunohistochemistry

CAR, chimeric antigen receptor; HLH, haemophagocytic lymphohistiocytosis; MAS, macrophage-activation syndrome. *Grading as per Common Terminology Criteria for Adverse Events, version 4.03 (REF. 43)

Figure 9: Proposed management of CAR T cell related HLH/MAS [78]



*Recommended dose of etoposide: 75-100mg/m2 IV every 4-7 days; consider cytarabine (100mg) intrathecally with or without hydrocortisone (50-100mg intrathecally for ICANS)83.

10.3.4 CD4+ cell depletion:

It is possible that T cell depletion will occur temporarily[80]. Antimicrobial prophylaxis consistent with guidelines of prophylaxis followed for patients treated for AIDS (NIH guidelines) will be utilized until T cell recovery.

10.3.5 Grading System Data Collection

While grading for clinical management purposes follows the ASTCT Consensus Grading for CRS and neurotoxicity[81] (see Tables 2 and 5), CRS grading data will also be collected using the Lee (2014) criteria (see Table 9), to improve understanding of differences in grading within a study.

Table 9: Lee (2014) CRS Grading System[70]

Grade 1	Symptoms are not life-threatening and require symptomatic treatment only (e.g. fever, nausea, fatigue, headache, myalgias, malaise)
Grade 2	Symptoms require and respond to moderate intervention: • Oxygen requirement <40% FiO_2 OR • Hypotension responsive to IV fluids or low dose of one vasopressor OR • Grade 2 organ toxicity[1]
Grade 3	Symptoms require and respond to aggressive intervention: • Oxygen requirement ≥40% FiO_2 OR • Hypotension requiring high-dose or multiple vasopressors OR • Grade 3 organ toxicity[1] or grade 4 transaminitis
Grade 4	Life-threatening symptoms: • Requirement for ventilator support OR • Grade 4 organ toxicity[1] (excluding transaminitis)
Grade 5	Death

[1]As per CTCAE v5.0

11. EARLY WITHDRAWAL OF SUBJECTS

Subjects who do not complete the study protocol will be considered to have prematurely discontinued the study. The reasons for premature discontinuation (for example, voluntary withdrawal, toxicity, death) must be recorded on the case report form. Final study evaluations will be completed at the time of discontinuation. Potential reasons for premature discontinuation include:

- The subject is lost to follow-up.
- The judgment of the principal investigator that the patient is too ill to continue.
- Patient noncompliance with study therapy and/or clinic appointments.
- Pregnancy
- Voluntary withdrawal: a patient may remove himself/herself from the study at any time without prejudice. A patient may withdraw from the study at any time they wish to withdraw consent.
- Significant and rapid progression of malignancy, including development of CNS metastases, requiring additional routine or experimental anticancer therapy, including medical, radiation or surgical intervention (with the exclusion of hematopoietic stem cells transplant). In this case all subjects, regardless of treatment received, should undergo long-term follow-up for secondary malignancy and replication-competent lentivirus until 15 years after infusion of CD4CAR.
- Toxicity or a serious adverse event that requires the subject being withdrawn from the trial.
- Termination of the study by the principal investigator, the institution, the IRB, or the Food and Drug Administration after appropriate team's discussion.

11.1 Criteria for discontinuing a subject's participation in the study:

If a subject develops a condition that precludes CD4CAR infusion after enrollment but before infusion, the subject will be prematurely discontinued. This will be done at the judgment of the PI, and could include for example, the occurrence of an intercurrent illness requiring the institution of systemic immunosuppression.

11.2 Data Collection and Follow-up for Withdrawn Subjects

Follow-up data collection after cell therapy clinical trials can be indefinite. As long as patients have detectable cells transduced with the lentiviral vector, they should be followed for toxicity, immune reactions, and any long-term adverse events. Many patients who respond to cell therapy may also have prolonged DFS but are also at risk for late relapse. It is our practice to follow all patients treated with cellular therapy indefinitely at least until the time alternative treatment is required for their disease, and/or they are no longer at risk for toxicity from the infused cells (i.e. loss of engraftment). Therefore, we plan to continue data collection regarding 1) engraftment as long as patients are at risk (until evidence of loss of detectable transduced T cells); 2) DFS until there is disease progression; 3) survival until the time of death or 4) until the patient withdraws consent for clinical data collection.

Patients who are followed at other institutions or practices, because of preference or geographical concerns will have follow-up via notes from their local physician and/or phone interviews with periodic study assessments done at the clinical research site. An example would be a patient referred from out of state but cared for at another center. These patients are encouraged to stay within 60-90 minutes of driving distance (or approximately 30 miles) of study sites for at least 6 weeks post infusion and until deemed stable. Thereafter, we will obtain toxicity and other clinical assessments from the treating physician per schedule of events or as possible. Every effort will be made to contact subjects who appear to be lost to follow-up in order to at least obtain survival data. In the event a subject fails to complete the follow-up requirements, documentation of all attempts to contact the subject includes at least 3 telephone contacts (on different days and at different times of the day), and a certified letter.

Subjects will be withdrawn from DFS assessments if 1) there is evidence for lack of response, relapse or progressive disease after 6 months of follow-up or 2) at any time they require new treatment for their disease (i.e. conventional chemotherapy) but not a preplanned transplant. Subjects will be withdrawn from survival assessments at the time of death.

12. STATISTICAL PLAN

12.1Sample Size

Up to 30 subjects will be treated with CD4CAR cells in this study.

12.2Subject Population(s) for Analysis

The subject population to be analyzed for primary and secondary endpoints will include all patients infused with CD4CAR cells.

A second population of patients will include all patients enrolled on study but who do not receive CD4CAR cells. Reasons patients do not receive cell infusions are likely to include 1) ineffective transduction of autologous T cells; 2) rapid progression, clinical deterioration, and/or death between the time of enrollment and infusion; 3) subject withdrawal.

The number of patients enrolled versus the number of patients infused will be described and is a measure of the feasibility of this therapy for patients with various malignancies to be treated. We anticipate recruiting 20 subjects in order to have 15 who receive the CD4CAR cell infusion.

12.3Subject Demographics/Other Baseline Characteristics

Demographic and other baseline data will be summarized descriptively for all subjects. Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, minimum, and maximum will be presented.

12.4Treatments (Study Drug, Concomitant Therapies, Compliance)

Descriptive statistics will be used to describe the number of subjects using the study drug at the different dose levels. Compliance will be presented as the percentage of subjects who took the treatment at each dose level. Concomitant medications and significant non-drug therapies prior to and after the start of the study drug will be summarized.

12.5Primary Endpoints: Safety

Adverse events will be summarized by NCI CTC grade and by relationship to dosage. Other safety data such as laboratory toxicities, clinical events and other findings will also be tabulated. All data will be included in the data listings.

12.6Secondary Endpoint: Efficacy

Anti-tumor activity will be assessed as a secondary trial endpoint. Anti-tumor efficacy of CD4CAR cells will be determined in a follow-on study. Frequency and percentage of partial response and complete response, as well as MRD will be described for all subjects. Overall survival and progression-free survival will be estimated using the Kaplan-Meier curves. Cause of death will be tabulated.

Another exploratory endpoint is to test the relative engraftment of CD4CAR positive cells . The change in the ratio of CD4CAR positive cells over time will be compared using a Wilcoxon signed-rank test for paired data. This nonparametric test is very efficient (>95%) compared to the t-test if the underlying data are normally distributed.

13. SAFETY AND ADVERSE EVENTS

Safety will be assessed by monitoring and recording potential adverse effects of the treatment, using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse events (CTCAE) version 5.0 at each study visit. Subjects will be monitored by medical histories, physical examinations, and blood studies to detect potential toxicities from the treatment.

13.1Definitions:

13.1.1 Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.) given (a) the research procedures are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being study;
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures

involved in the research)

- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

Only a small subset of adverse events occurring in human subjects participating in research will meet these three criteria for an unanticipated problem. Furthermore, there are other types of incidents, experiences, and outcomes that occur during the conduct of human subjects research that represent unanticipated problems but are not considered adverse events. For example, some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased risk of harm, but no harm occurs.

13.1.2 Adverse Event (AE)

An adverse event is an unplanned, unwanted event which occurs to a study participant and which is possibly, but not necessarily, related to the use of protocol therapy. While some events may not initially appear to be associated with the use of the study treatment, a relationship may not emerge until sufficient numbers of reports accumulate from participating sites. An AE may also include a newly occurring event or a previous condition that has increased in severity or frequency since the administration of the investigational product. Assessment of the occurrence of an AE will be based on changes in the subject's physical examination, laboratory results and/or signs and symptoms. AEs will be monitored until they are resolved or are clearly determined to be due to a subject's stable or chronic condition or intercurrent illness. Medical care will be provided, as defined in the informed consent document, for any AE related to participation in this clinical trialAbnormal results of diagnostic procedures
Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- Is associated with a serious adverse event
- Is associated with clinical signs or symptoms
- Leads to additional treatment or to further diagnostic tests
- Is considered by the investigator to be of clinical significance

13.1.3 Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management;
- Is considered by the investigator to be of clinical significance

13.1.4 Serious Adverse Event (SAE)

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that:

1. Death
2. A life-threatening adverse event. Life-threatening is defined as an adverse event or suspected adverse reaction that places the subject at immediate risk of death. It does not include an adverse event or

suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

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

13.1.5 Determining Attribution

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

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

13.1.6 Unexpected Adverse Event

An adverse event, of which the specificity or severity is not listed in the study protocol, product inserts, investigator brochure or informed consent document.

13.2Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

13.2.1 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

13.2.2 Post-study Adverse Event

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s). Investigators at secondary study sites are responsible for notifying the Principal Investigator of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The

Principal Investigator should also be notified if an investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

13.2.3 Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event. As was noted above, events related to the cytotoxic chemotherapy will be excluded from adverse event reporting.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.

13.3Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome.

13.4Adverse Event Reporting Requirements

13.4.1 Adverse Event Reporting Period

Documentation of adverse events will begin at the time of apheresis and will continue until subjects complete or withdraw from the study.

> **Note:** Events occurring as a direct result of any cytotoxic chemotherapy administered during the screening period will be excluded from the adverse event-reporting period.

If CD4CAR product is undetectable (both research flow cytometry and transgene copy number testing must be negative for two consecutive follow-up time points), documentation of adverse events may be limited to SAEs, AEs ≥ Grade 3, infections requiring intervention (e.g. intravenous immunoglobulin administration), RCL, and secondary carcinogenesis, starting 6 months after CD4CAR infusion.

Follow-up procedures for participants who receive additional routine or experimental anticancer therapy, including medical, radiation or surgical intervention (with the exclusion of hematopoietic stem cells transplant) will be limited to documentation of secondary malignancy and replication-competent lentivirus, which will continue for up to 15 years after infusion of CD4CAR.

13.4.2 Infection Reporting

Rates of serious infections that exceed institutional approximate rates associated with immunocompromising

therapies such as allogeneic transplants will be reported to the FDA. The coordinating institution rates are summarized in the table below:

Bacterial	Bacteremia	~20%
Bacterial	Pneumonia	~35%
Fungal	Probable/Proven	~40%
Viral	Serious Viral infection (disease)	~15%

13.4.3 Participating Site Reporting Responsibilities

13.4.3.1 Reporting to the IU Simon Comprehensive Cancer Center

Any serious adverse event or unanticipated problem occurring must be reported to the IU Simon Comprehensive Cancer Center within **1 business day** of notification or discovery of the incident, using the SAE Reporting Form provided in the study procedure manual (SPM). This form must be accompanied by a cover letter which: identifies the event, is signed by the local principal investigator or treating physician, includes the applicable study number and title, and contains the following:

- Site assessment of the event attribution to investigational product or study procedure
- Site assessment of event expectedness (expected vs. unexpected)
- Assessment of whether or not the research places subjects at a greater risk of harm than was previously known or recognized
- Assessment of the event's effect on the risk to benefit ratio
- Statement as to whether the informed consent statement should reflect changes in the potential risks involved
- Statement as to whether the event has been reported previously, and if so, whether the frequency is considered unusually high

Send to: IUSCCC Clinical Trials Office
ATTN: Multicenter Project Manager/ CTO-IUSCCC-ICG122-101
E-mail: IUSCCSAE@iu.edu

The Multicenter Project Manager, or designee, will distribute the reports to all participating sites, the FDA and to the Funder as per section 13.4.5 below. Copies of all serious adverse event reports or unanticipated problems reports will be kept on file in the IU Simon Comprehensive Cancer Center Clinical Trials Office.

13.4.3.2 Reporting to the IRB

Each participating site will report adverse events and unanticipated problems to their IRB per local guidelines. Any event that requires expedited reporting to the local IRB will also be submitted to the IU Simon Comprehensive Cancer Center for distribution to the Funder.

13.4.4 Coordinating Center Reporting Responsibilities

In addition to the responsibilities above, the Coordinating Center will also be responsible for reporting events

to the Funder and the FDA.

13.4.4.1 Reporting to the FDA

Per CFR 312.32 (c), the sponsor-investigator of the IND *(Dr. Salman)* must notify the Food and Drug Administration (FDA) and all participating investigators in a written IND safety report of any adverse experience that meets the criteria outlined below. There are two types of reports to the FDA: 7-day and 15-day reports.

7-Day Reports:

The sponsor-investigator of the IND must notify the FDA and all participating sites in a written IND safety report of any adverse reaction:

- **fatal or life-threatening adverse reaction** that is ***both***
- **suspected to be associated with use of the drug *and***
- **unexpected (not in IB)**

The FDA will be notified as soon as possible but no later than **7 calendar** days after the sponsor-investigator's initial receipt of the information.

15-Day IND Reports:

The sponsor-investigator of the IND must notify the FDA and all participating sites in a written IND safety report of any adverse reaction:

- **suspected to be associated with use of the drug** that is ***both***
- **serious *and***
- **unexpected (not in IB)**

The FDA will be notified as soon as possible but no later than **15 calendar** days after the sponsor-investigator's initial receipt of the information.

Report Content:

Each written notification may be submitted on FDA Form 3500A or in a narrative format and must bear prominent identification of its contents, i.e., "IND Safety Report". For purposes of this protocol, the **MedWatch Report Form (FDA 3500A mandatory reporting), along with FDA Form 1571, and a cover letter** submitted to the appropriate FDA division, will serve as the written IND safety report. Follow-up information to a safety report should be submitted as soon as the relevant information is available. Submit to the FDA:

- MedWatch Report Form (FDA 3500A)
- FDA Form 1571
- Cover Letter

Notify the FDA via phone or fax using MedWatch 3500A (mandatory reporting form)

- Phone: 1-888-463-6332, option #1
- Fax: 1-800-FDA-0178

The IUSCCC Protocol Development Coordinator should be contacted to assist with all FDA submissions and will be provided with a copy of all events that are reported to the FDA. All IND submissions will be maintained in a master file in the Clinical Trials Office of the IU Simon Comprehensive Cancer

Center.

13.4.4.2 Additional FDA Reporting:

1. Any finding from tests in laboratory animals that: suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

2. Additional IND holders (sponsors) are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports. Per FDA recommendations, we plan to submit the results to the IND as an amendment for FDA review after completion of the first dose cohort

13.4.5 Reporting to Participating Sites

The Multicenter Project Manager, or designee, will distribute reports which are serious, unexpected and suspected to be associated with the study intervention (possibly, probably or definitely related) to all participating sites in the form of an Expedited Safety Report (external safety/IND report) within 15 calendar days from determination that the suspected adverse reaction qualifies for reporting. Copies of these Expedited Safety Reports will be kept on file in the IU Simon Comprehensive Cancer Center Clinical Trials Office.

14. CRITERIA FOR STOPPING / PAUSING THE STUDY

Enrollment of subjects and treatment of consented participants on the study will be put on hold if any of the following events occur provided if deemed as study procedure related:

- 2 subjects develop a DLT within any dose level.
- Any subject develops uncontrolled T cell proliferation that does not respond to anti-T cell management.
- Diagnosis of a T cell malignancy other than the primary disease, until insertional mutagenesis is ruled out
- Progression of a subject's primary T cell malignancy after a year period of control, if evidence of insertional mutagenesis is detected
- If a subject's Vβ repertoire is found to be monoclonal or oligo clonal, and the pattern of vector insertion is found to favor a single dominant insertion site
- Any adverse event, exclusive of elective surgical procedures, requiring admission to an intensive care unit for management.
- A single death event (with the exception of death from progressive disease)

  Principal Investigator, DSMC, or any independent review board or regulatory body decides that subject safety may be compromised by continuing the study

For any event outlined above, this will trigger a pause in enrollment while the toxicity is assessed by the PI, study team, and the DSMC. The DSMC will examine all relevant data, including global assessment of the safety data, to determine if patient enrollment should resume. If the Sponsor terminates the study for safety reasons, the Sponsor will notify the investigators and the relevant regulatory authorities.

15. DATA AND SAFETY MONITORING

15.1 Data Safety Monitoring Committee

The Data Safety Monitoring Committee (DSMC) of the Indiana University Simon Comprehensive Cancer Center (IUSCCC) is responsible for patient safety and privacy protection, compliance with required reporting, and study integrity for all trials conducted at IUSCCC. Members are subject matter experts from multiple disciplines, therapeutic modalities, as well as research experts, who are appointed by the Associate Director for Clinical Research. The DSMC will provide independent oversight of the clinical trial so that study integrity is assured.

The DSMC will meet per the currently approved DSMP, led by the DSMC Chair, and will review overall trial progress, toxicity, data integrity, accrual, monitoring and auditing reports, unanticipated problems and study non-compliance events that require expedited reporting. Meeting outcomes will be maintained in the IUSCCC Clinical Trials Office (CTO).

Specifically the DSMC has the following responsibilities:

- Assessment of the adequacy of trial-specific Data Monitoring and Safety Plan (DSMP) of cancer relevant studies.
- Review safety data for investigator initiated trials including all adverse events, unanticipated problems and study non-compliance events requiring expedited reporting.
- Conduct routine study monitoring and auditing in compliance with the IUSCCC data quality control review process.

Furthermore, the DSMC conducts an administrative review of serious adverse events (SAEs), deviations, reportable events, and any other outstanding business. Major issues may require further DSMC review or action.

For any increase in frequency of grade 3 or above adverse events (above the rate reported in the Investigator Brochure or package insert), the principal investigator will notify the DSMC Chair immediately. The notification will include the incidence of study adverse events, grades, and attributions, as well as investigator statements regarding comparison with risks per the IB/ package insert.

At any time during the conduct of the trial, if it is the opinion of the investigators that the risks (or benefits) to the subject warrant early closure of the study, the DSMC Chair and Compliance Officer must be notified within 1 business day via email, and the IRB must be notified within 5 business days. Alternatively, the DSMC may initiate suspension or early closure of the study based on its review.

15.2 Data Safety Monitoring Plan

This trial will comply with the current requirements of the Data and Safety Monitoring Plan (DSMP) of the IUSCCC. The CTO of the IUSCCC will be the Coordinating Center for this multicenter phase I trial.

In accordance with the DSMP of the IUSCCC, investigators will conduct continuous review of data and patient safety. Weekly review meetings for Phase I trials are required and will include the: principal investigator, clinical research specialist, and/or research nurse data manager and/or study coordinator, and other members as per the principal investigator's discretion. In addition, conference calls with investigators and staff at participating sites will be scheduled at least weekly (and more often as needed) to discuss study progress. If the study is not open to accrual, there are no patients on treatment or in follow-up,If there are no patients on treatment or in follow-up, email communication will be used in lieu of a teleconference, or in the circumstance where a scheduling conflict does not permit phone attendance. Weekly meeting summaries will include and document review of data and patient safety by including for each dose level: the number of patients, significant toxicities as described in the protocol, dose adjustments, responses observed, eligibility of patients enrolled at each site, serious adverse events (SAEs) or unanticipated problems (UPs) (both IUSCCC and those reported from other institutions), dose adjustments, and protocol deviations. Study teams should maintain meeting minutes and attendance for submission to the DSMC upon request.

15.2.1 DSMC DLT Review

The study sponsor-investigator, principal investigator and study team are responsible for conducting continuous review of data and subject safety at their weekly Phase I meetings. Prior to making dose escalation/expansion/de-escalation decisions, the study team will officially review all toxicity events for each subject for confirming treatment-related DLT. The study statistician will assist the determination of DLT and the interpretation of the statistical rule for dose escalation. Once a decision has been reached by the investigator, the official decision and toxicity data will be submitted to the DSMC via email (IUSCC-DLT-Review-L@list.iu.edu) and copy the DSMC Chair and Compliance Officer. Treating additional subjects may not proceed until official DSMC correspondence confirms approval of dosing decisions for the next stage.

15.2.2 IND Annual Reports

For trials with an IND held locally by the IU principal investigator or university, the IND Annual Report will be prepared and submitted to the Compliance Team. This report will be reviewed by the DSMC at the time of FDA submission.

15.2.3 Study Auditing and Monitoring

All trials conducted at the IUSCCC are subject to auditing and/or monitoring per the Institutional DSMP. Reports will be reviewed by the full DSMC at the time of study review.

15.2.4 Data Management/ Oncore Reporting Requirements

The DSMC reviews data and study progress directly from Oncore; therefore, timely data entry and status updates are vital. Study data must be entered within Oncore promptly, no later than 15 business days from study visit occurrence. Subject status in Oncore will be updated in real time, as this may affect overall trial enrollment status. Global SAEs and deviations will be reviewed on a monthly basis by the DSMC Chair directly from Oncore.

15.2.5 Study Accrual Oversight

Accrual data will be entered into the IU Simon Comprehensive Cancer Center OnCore system. The Protocol Progress Committee (PPC) reviews study accrual twice per year, while the PPC coordinator reviews accrual quarterly.

15.2.6 SAE Reporting Guidelines

All serious adverse events (SAEs) will be captured in the IUSCCC OnCore® system within 1 business day of notification. Initial SAE reporting will include as much detail as available, with follow-up to provide complete information. Attributions will be assessed to study drugs, procedures, study disease, and other alternate etiology.

15.2.7 Reporting Death

Death will be captured in the Case Report Form and reported per local IRB reporting guidelines.

15.2.8 Protocol Deviation Reporting

Protocol deviations will be entered into OnCore within 5 days of discovery and reviewed by the DSMC Chair on a monthly basis. Findings will be reported to the full DSMC at the time of study review. For serious or repetitive protocol deviations, additional action may be required by the DSMC.

15.2.9 Continuing Review

All Continuing Reviews (CR) will be reviewed annually or as dictated by the Institutional Review Board. Participating sites will submit a copy of the CR with attachments to the IUSCCC Multicenter Project Mananger, or designee.

15.2.10 Case Report Forms and Data Submission

This study will utilize electronic Case Report Form completion in the OnCore® database. A calendar of events and required forms are available in OnCore® at https://cancer.iu.edu/oncore. The OnCore® database is a comprehensive database used by the IUSCCC CTO and supported by Indiana University. Access to data through OnCore® is restricted by user accounts and assigned roles. Once logged into the OnCore® system with a user ID and password, OnCore® defines roles for each user, which limits access to appropriate data.

All source documents are to remain in the patient's clinic file. All documents should be kept according to applicable federal guidelines. Clinical trial data in OnCore® are periodically monitored by the IU Simon Comprehensive Cancer Center per the DSMC Charter.

16. DATA FORMS AND SUBMISSION

This study will utilize electronic Case Report Form completion in the OnCore® database. A calendar of events and required forms are available in OnCore®. The OnCore® database is a comprehensive, web-based, Clinical Trial Management System (CTMS) which utilizes an Oracle database. OnCore® was developed by Forte Research Systems, Inc. and is used by the IUSCCC

Clinical Trials Office and supported by the Indiana Clinical and Translational Sciences Institute (CTSI). OnCore® properly used is compliant with Title 21 CFR Part 11. The system is supported and managed by Advarra, Inc. who have developed a security program that is compliant with HIPAA and HITECH requirements. Applications and databases are housed off-site at Rackspace, Inc. which is SSAE16 SOC2, ISO 27001, PCI, and HITRUST certified.

OnCore® provides users secure access with unique IDs/passwords and restricts access by assigned roles, from any location, to record, manage, and report on data associated with the operation and conduct of clinical trials.

All source documents are to remain in the patient's clinic file. All documents should be kept according to applicable federal guidelines. Clinical trial data in OnCore® are periodically monitored by the IUSCCC Data Safety Monitoring Committee.

16.1Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the Principal Investigator. In such an instance, it is the responsibility of the Principal Investigator to inform the investigator/institution as to when these documents no longer need to be retained.

17. MULTICENTER GUIDELINES

17.1 Study Documents

Each participating site must submit regulatory documents (informed consents, 1572s, Financial Disclosures, IRB approval documents, Continuing Reviews, Amendments, patient brochures or recruitment material etc.) to the Coordinating Center. The Coordinating Center will provide each site with a comprehensive list of the required documents prior to study start-up, throughout the duration of the study and upon study close-out. It is the responsibility of the participating site to maintain copies of all documentation sent to the Coordinating Center.

17.2 Study Initiation

Before activating the clinical trial at each participating site, the IUSCCC CTO multicenter project manager, or designee, will ensure that:

- Full **Institutional Review Board (IRB) approval** has been obtained.
- Research staff at the participating site has been trained in data entry into OnCore®

- A **start-up meeting** with each institution has taken place via telephone conference. The start-up meeting will cover protocol details (including eligibility criteria, treatment plan, etc.), responsibilities of the participating investigators, and reporting procedures.

- A financial **conflict of interest statement** from each investigator has been obtained.

17.3 Patient Enrollment

After eligibility is confirmed by the participating site staff, a completed eligibility checklist, supporting source documentation, and signed consent will be sent to IUSCCC for verification. Subject ID will be assigned by OnCore® upon subject registration by the site. The Multicenter Project Manager, or designee, will return the enrollment information to the site after review. The site staff will then register the patient in OnCore®. Additional details of this process can be found in the Study Procedure Manual.

17.4 Data Monitoring

All multicenter investigator initiated trials conducted at the IUSCCC are subject to data monitoring by the Multicenter Project Manager and the IUSCCC Compliance Office, or designee. External sites will be notified of upcoming monitoring visits and will be expected to provide the Multicenter Project Manager, IUSCCC Compliance Office, or designee, with de-identified source documents for remote monitoring of patients. Queries will be issued in OnCore® and a detailed monitoring report will be provided to the participating site. The IUSCCC will also forward any monitoring and/or auditing reports to the DSMC.
When a patient enrolled on this trial, or the trial itself, is selected for local monitoring or auditing, the participating site will forward the results to the Multicenter Project Manager, or designee. In addition, if a participating site patient is selected for local auditing by the IUSCCC DSMC, the site will be responsible for sending IUSCCC de-identified source documents.

17.5 Record Retention

All documentation of adverse events, records of study drug receipt, dispensation, destruction, and all IRB correspondence will be stored in accordance with all applicable federal guidelines. Following closure of the study, each participating site will maintain a copy of all site study records in a safe and secure location. The Coordinating Center will inform the investigator at each site at such time that the records may be destroyed.

18. ETHICAL CONSIDERATIONS

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachment 1 for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal

consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject and the investigator-designated research professional obtaining the consent.

19. SUBJECT STIPENDS Or PAYMENTS

There is no subject stipend/payment for participation in this protocol.

20. STUDY DISCONTINUATION

The study may be discontinued at any time by the IRB, the Principal Investigator, the Institution, the FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

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