INDIANA UNIVERSITY
MELVIN AND BREN SIMON
COMPREHENSIVE CANCER CENTER

CD4CAR T CELL THERAPY FOR CMML

Protocol Number:
CTO-IUSCCC-0840

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Support Provided By:
Brown Center/ LLS

Protocol Version Date:
02/09/2026

IND#: 17945

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PROTOCOL SIGNATURE PAGE

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VERSION DATE: 0*2/09/2026*

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

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Investigator Name (printed)

_____

Investigator Title

_____

Name of Facility

_____

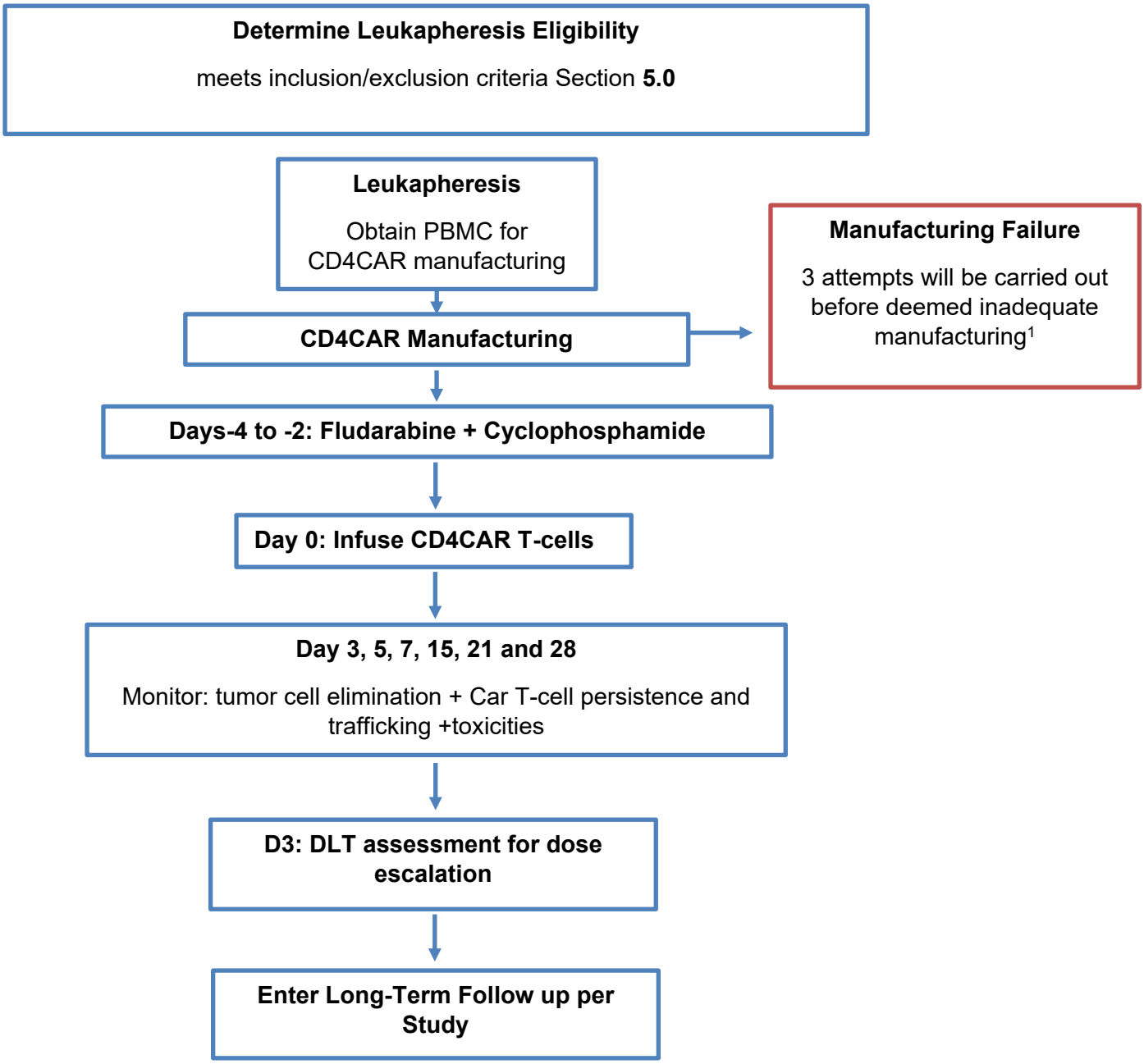
Location of Facility (City and State)

_____ □ Not Submitting to IRB

Expected IRB Submission Date

COMPLETE AND EMAIL COPY TO INDIANA UNIVERSITY SIMON COMPREHENSIVE CANCER CENTER CLINICAL TRIALS OFFICE

1. SCHEMA



1. If there is a manufacturing failure, the number of attempts, and reason for failure will be documented. Any additional manufacturing attempts will be made by using product stored from original apheresis collection.

2. BACKGROUND & RATIONALE

CD4, a T-lymphocyte membrane glycoprotein that interacts with major histocompatibility complex class II antigens, is expressed on all monocytes. CD4 is not expressed on hematopoietic stem or progenitor cells, nor is it is expressed on non-hematopoietic cells. A recent preprint showed that monocytic leukemia arises from a fundamentally different type of leukemia stem cell (LSC), designated as monocytic LSC (m-LSC), that is developmentally and clinically distinct from the more well-described primitive LSC (p-LSC) [1]. This novel m-LSC is distinguished by a unique immunophenotype (CD34-, CD4+, CD11b-, CD14-, CD36-), unique transcriptional machinery, and reliance on purine/pyrimidine metabolism. m-LSC and p-LSC subtypes could co-exist in the same leukemia patient, thereby contributing to overall tumor complexity, and predisposing to resistance to important agents such as the BCL2-inhibitor venetoclax. CD4 is expressed on T regulatory cells, and we have data that CD4 is also expressed on myeloid derived suppressor cells (MDSCs), both granulocytic and monocytic (un-published data).

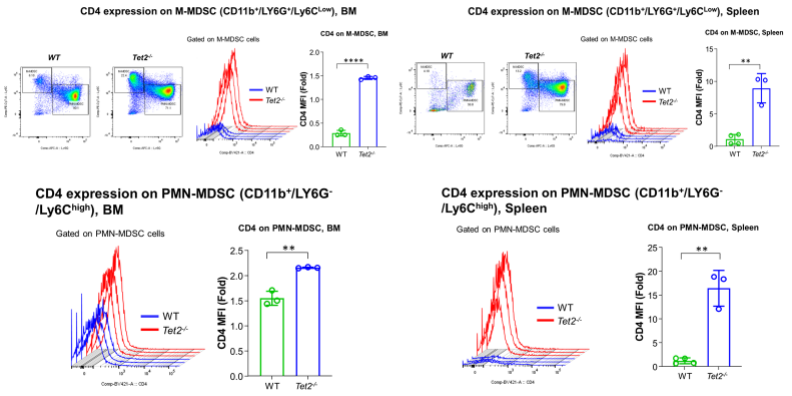


Figure 1. Both of M.MDSCs and Gr.MDSCs express CD4 in CMML in murine marrow and spleen.

Tregs and MDSCs are immunosuppressive and protumor cells and targeting them as well is novel and much needed for the success of immunotherapeutic approaches in general. These properties make CD4 an attractive target for novel therapies.

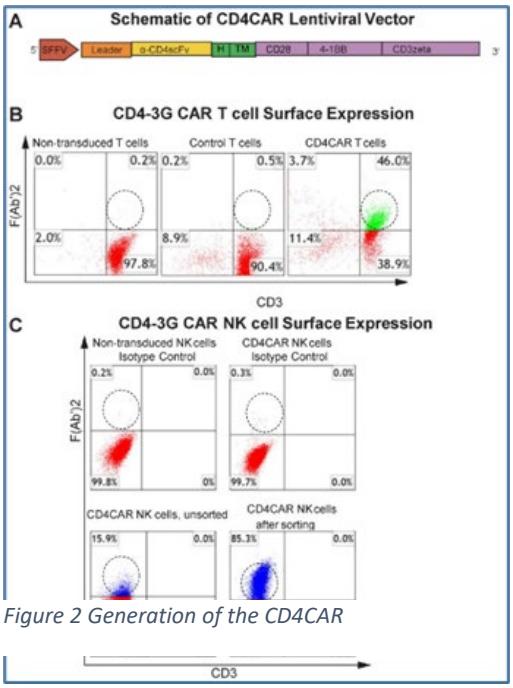


Figure 2 Generation of the CD4CAR

Our group has developed a Chimeric Antigen Receptor T and NK cells (CAR-T and CAR-NK cells, respectively) redirected to target CD4 for the treatment of myeloid malignancies expressing CD4 and for CD4 positive T cell malignancies [2] [3] . Our CD4CAR is already in clinical trial for T cell malignancies (NCT number: NCT03829540). Preliminary results from this ongoing trial have shown promising clinical responses and manageable toxicities.

The Investigational agent Fig 2: The investigational agent in this protocol is a CD4-specific chimeric antigen receptor engineered T-cell (CD4CAR T-cell) 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) [3]. To effectively bind CD4, this is paired with an antigen receptor possessing an intracellular tyrosine-based activation motif from the TCR by lentiviral transduction of a CD4CAR gene. In addition to CD4scFv, the cells will be transduced to express an intracellular signaling molecule comprised of a tandem signaling domains of CD28, 4-1BB and TCRζ. The scFv is derived from a mouse monoclonal antibody and the signaling domains are entirely of the native human sequences.

Our preclinical data for this approach in myeloid neoplasms showed that treatment with CD4CAR eliminated CD4-expressing monocytic myeloid cell lines in vitro, Fig 3 and exhibited a potent anti-leukemic effect in a systemic myeloid murine model in vivo, Fig 4. NK CAR similarly and robustly showed similar results [2]. Similar to our data in T cell neoplasms that has successfully led to a clinical trial, these preclinical data in CD4 + myeloid neoplasms with monocytic differentiation provide strong rationale to target CD4 positive CMML either as a standalone treatment or as a bridge to stem transplantation. We therefore propose to develop a clinical trial to use CD4CAR for CD4 positive CMML and aim at eliminating m-LSC.

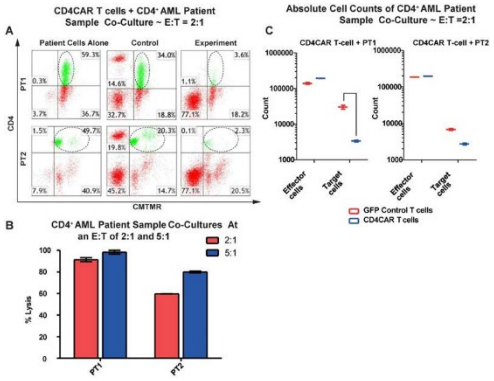


Figure 3 Anti-leukemic effects of CD4CAR in vitro

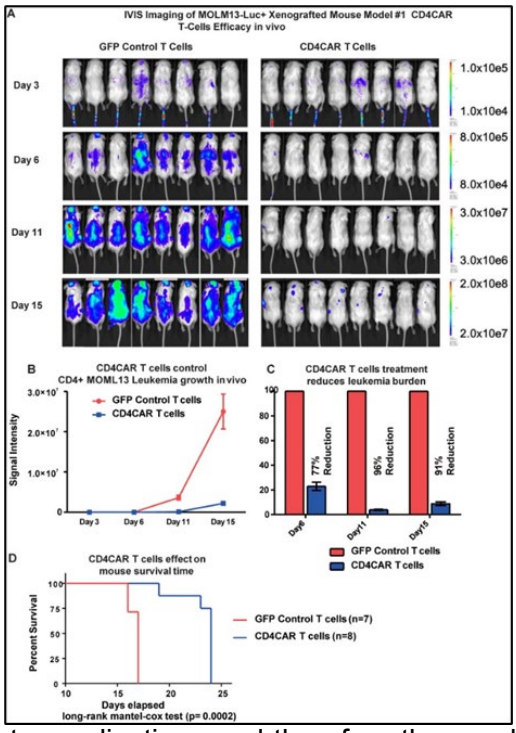


Figure 4 Anti-leukemic effects of CD4CAR in vivo

Impact. This study will inform on the safety profile, expansion, persistence, and efficacy of the CD4CAR in CMML. It will also inform on the concurrent targeting of both of Tregs and MDSCs. This will be the first cellular therapy trial in this disease in need for novel approaches.

Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic stem cell neoplasm defined by relative (≥ 10%) and absolute (≥ 1 × 10^9/L) monocytosis, the presence of dysplasia in hematopoietic precursor cells, and an inherent risk for transformation to acute myeloid leukemia (AML) [4]. The overall incidence of CMML is estimated to be around 4 cases per 100,000 persons/year; it usually affects elderly patients with a median age at diagnosis of 71–73 years, a male to female ratio of ~2.5:1, and a highly variable survival time ranging from 1 to more than 8 years [4, 5] [6] [7]. Allogeneic hematopoietic stem cell transplant (HSCT) is the only curative option and remains the treatment of choice for younger patients with higher-risk disease. However, HSCT is not without complications and therefore the need for other less aggressive therapeutic options remains valid. Hypomethylating agents including azacitidine, decitabine, and oral decitabine/cedazuridine are currently approved by the US Food and Drug Administration for the management of CMML, but are not considered curative options as they do not eradicate underlying mutations and therefore eventual progression to acute myeloid leukemia occurs, despite restoring hematopoiesis through epigenetic modulation in a subset of patients. Thus, targeted therapies exploiting specific genetic lesions and biologic cellular therapies are attractive options that need to be further investigated in the setting of CMML. CAR T-cell therapy represents a major breakthrough in cancer care since the approval of tisagenlecleucel by the Food and Drug Administration in 2017 for the treatment of pediatric and young adult patients with relapsed or refractory acute lymphocytic leukemia. As of April 2023, six CAR T cell therapies have been approved, demonstrating unprecedented efficacy in patients with B-cell malignancies and multiple myeloma. However, adverse events such as cytokine release syndrome and immune effector cell-associated neurotoxicity pose significant challenges to CAR T cell therapy [8].

3. OBJECTIVES

3.1 Primary Objectives

- Evaluate the dose limiting toxicities of a single CD4CAR dose in dose finding studies.
- Identify the maximally tolerated single CD4CAR dose for phase II investigation.
- Describe anti-tumor activity of CD4CAR in CMML.

3.2 Secondary Objectives

- Assess the *in vivo* persistence of a single dose of the CD4CAR in patients with CMML.
- Characterize the *in vivo* behavior of the CD4CAR, such as phenotype, subsets of CD4CAR T cells (Tcm, Tem, and Tregs) and associated chemokines pattern, when feasible.
- Effect of the CD4CAR on both the tumor microenvironment (TME) suppressive cells Tregs and MDSCs.

4. OUTCOME MEASURES

4.1 Primary Outcome Measures

1. Dose limiting toxicities (DLT) defined based on Common Toxicity Criteria (CTCAE v5.0)
2. Patterns of CMML response and resistance to CD4CAR as measured by:
    a. Serial marrow sampling for CMML clonal cells estimation
    b. Peripheral count improvement level/ normalization.
    c. Time to response
    d. Others in combination as described in table 1.

4.2 Secondary Outcome Measures

1. CD4CAR persistence in CMML by Day 28 of CAR infusion.
2. Efficacy of the CD4CAR to eliminate both of Tregs and MDSCs from the CMML marrow post treatment.

5. ELIGIBILITY CRITERIA

5.1 Inclusion Criteria

1. ≥ 18 years old at the time of informed consent
2. Ability to provide written informed consent and HIPAA authorization
3. Diagnosis of CMML that is CD4+ and is recurrent or refractory to first line standard of care treatment.
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%. This will not have to be repeated if within 45 days of initial assessment
9. Adequate venous access for apheresis and no other contraindications for leukapheresis

5.2 Exclusion Criteria

1. CD4 negative CMML
2. 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
3. Uncontrolled active infection necessitating systemic therapy
4. 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
5. Concurrent use of systemic glucocorticoids in greater than replacement doses 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, pseudo rheumatism, emotional disturbances, etc) precipitated

by the temporary stoppage unless tapering can occur safely without compromising the underlying disease, the withdrawal tolerance can happen in a timeframe appropriate to enroll in this trial without safety concerns

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

6. Any uncontrolled active medical disorder that would preclude participation as outlined in the opinion of the treating investigator and/or Principal Investigator
7. HIV infection
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) during the last year
   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 CMML 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. CMML patients who transformed into AML and who were treated back into CMML status **are** eligible. CMML patients who transformed into AML and appropriate AML treatment was unsuccessful in reverting their disease back to CMML status will be treated as AML patients and are **not** eligible for the CMML study.
12. Treatment with any investigational cell/gene therapy within the past 6 months
13. Treatment with any investigational anticancer agent within the last 14 days of study entry or 5 half-lives (whichever is shorter)

5.3 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)

5.4 Eligibility for CD4CAR infusion:

5.4.1 Inclusion

1. Afebrile and not receiving antipyretics, and no evidence of active infection. If fever is attributed to underlying disease, it will not disqualify.
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 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

5.4.2 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.

5.5 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. STUDY DESIGN

This study is designed as a single arm open label traditional Phase I, 3+3, study of CD4-directed chimeric antigen receptor engineered T-cells (CD4CAR) in patients with relapsed or refractory CMML Fig 5. This study may be conducted at several academic cancer centers if need be.

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.

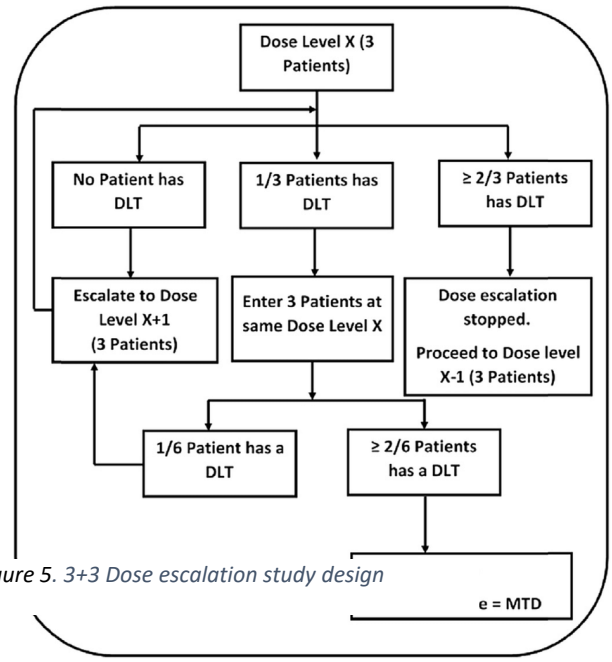


Figure 5. 3+3 Dose escalation study design

7. PATIENT REGISTRATION

All patients will be registered with the Indiana University Simon Comprehensive Cancer Center (IUSCCC) Clinical Trials Office (CTO). Regulatory files will be maintained by the IUSCCC CTO and the participating sites as applicable. 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 CTO 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.

8. TREATMENT PLAN

8.1 Screening and eligibility check:

At entry, subjects will be screened for eligibility and exclusion criteria. All screening tests and procedures must be done after consent, unless otherwise indicated in the protocol.

8.2 Treatment Regimen

The starting dose for this trial will be based on the dose level for the currently approved trial (CTO-IUSCCC-ICG122-101). Dose level 2 is the anticipated starting dose, but ultimately may be dependent on when this trial is open for enrollment.

Dose Level	Transfected Cell dose/Kg
1	8.0 x10^4 /kg
2 DL1	1.2 x10^5 /kg
3	4.0 x10^5 /kg
4	1.0 x10^6 /kg
5	2.0 x10^6 /kg

8.3 Leukapheresis:

If eligible, subjects will be leukapheresed. 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 if deemed adequate by the PI. 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).

Manufacturing of the CD4CAR: Process details in Fig 6

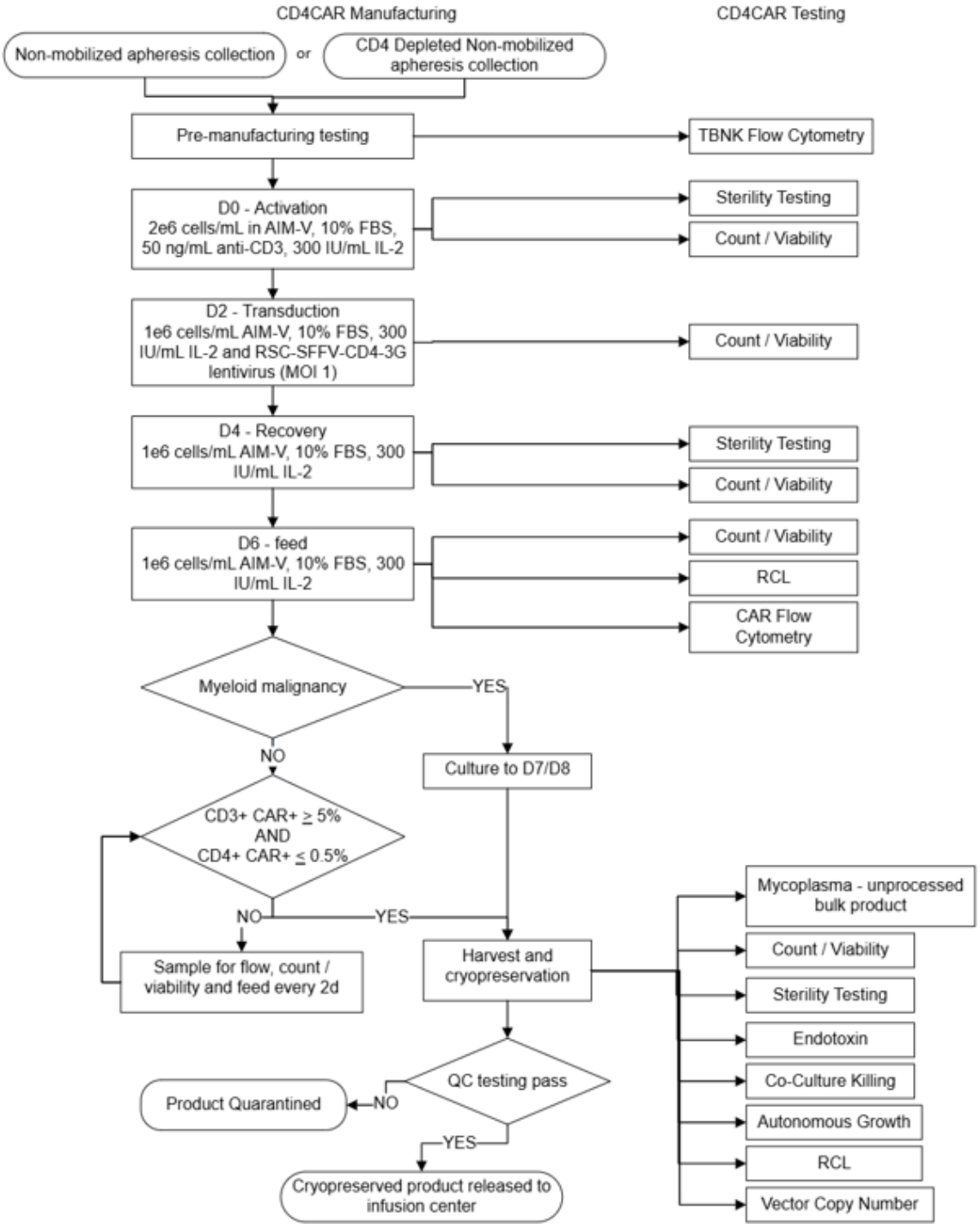


Figure 6: CD4CAR manufacturing steps

The CAR construct that is used in this study was developed by iCellGene 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.

8.4 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 level) 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 can be created from an additional manufacturing run. If needed, they will be shipped to the site per above.

8.5 Packaging and shipment

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.

The manufactured CD4CAR cellular product will be shipped according to the manufacturing site procedure in a qualified dry shipper, either within the institution or outside of the institution. The cryoshipper is used to transport cellular therapy products to and from the stem cell lab, either within the institution or outside of the institution. The cryoshipper must be filled/charged with liquid nitrogen at least 24 hours in advance of shipment of the cryoshipper.

8.6 Receiving and Storage

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 cells. A chain of custody document will be maintained to document movement of the cells in the facility.

Any damaged or unusable study drug (active drug or comparator) will be documented in the study files.

8.7 Thawing

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

8.8 Dispensing of Study Drug

After logging the cells in the cell therapy lab, bags/vials (20 to 100 ml capacity) containing CART-4-transduced T cells will be stored in a monitored freezer at or below -150°C. Infusion bags will be stored in the freezer until needed.

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 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 Cell Immunotherapy and Transduction Facility (CIT) as specified below.

8.9 Return or Destruction of Study Drug if needed

CD4CAR T cells may require return to the CIT for a variety of reasons, including but not limited to:

1) Mislabeled product

2) Condition of patient prohibits infusion/injection

3) Subject refuses infusion/injection; any unused frozen product will be returned to the CIT for disposal or storage.

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.

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.

8.10 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 5 days before infusion so that CD4CAR cells may be given 2-3 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.

8.11 CD4CAR Infusion

Infusion begins 2 to 3 days after completion of conditioning chemotherapy unless toxicities related to conditioning chemotherapy of ≥ grade 3 are present. 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.

- 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.

- The thawed cells will be given over approximately 5-15 and up to 30 minutes, depending on the product volume as dictated by the dose level.

- Subjects' **vital signs** will be assessed, and pulse oximetry will be done immediately prior to infusion (+/- 5 minutes), every 15 minutes (+/- 5 minutes) during infusion, at the end of the infusion (+/- 5 minutes), and every 15 minutes thereafter (+/- 5 minutes) for 1 hour and until these are stable and satisfactory.

8.12 Post-infusion laboratory testing to assess disease response, progression, CD4CAR engraftment and persistence

- A blood sample for baseline CD4CAR expression level will be collected on day -4 prior to conditioning chemotherapy. Then according to the schedule of events.
- Cytokine levels will be evaluated per schedule of events except during a cytokine release syndrome (CRS) or Immune effector cell-Associated Neurotoxicity Syndrome (ICANS)event in which it will be collected as needed approximately every 8 (+/- 2) hours as feasible, in consideration of subject availability, and clinical/research staff operations, and until resolution.
- Follow up bone marrow sampling will be done for standard of care (SOC) disease follow-up and for research purposes as per the schedule of events, but also as deemed necessary by the treating team elsewise. Other disease site biopsies if feasible and applicable, will be collected to analyze CAR T-cell trafficking and engraftment as needed, as determined by the treating physician.
- Optional blood samples for future unspecified research will also be collected at the time of estimated maximum engraftment, and the time of clinical response, or disease progression or both, if the patient responds and then progresses after the initial response, per the discretion of the treating physician.
- Patients will return to the research site per schedule of events or more often as clinically indicated for scheduled evaluations or as needed evaluations.

8.13 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).

8.13.1 *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.

8.13.2 *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 oximetry) will be done and documented just prior to CAR T cell infusion (+/- 5 minutes). During CAR T cell infusion, monitor and document vital signs every 15 minutes (+/- 5 minutes) for the duration of the infusion, then immediately following infusion (+/- 5 minutes), and every 15 minutes

for at least one hour (+/- 5 minutes) 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].

8.13.3 *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.

8.13.4 *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 Appendix IV).

8.13.5 *Infectious disease*

Preferred prophylactic antimicrobials are trimethoprim-sulfamethoxazole (TMP-SMX) DS tablet three times weekly for pneumocystis jirovecii 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.

8.13.6 *Stable subjects:*

Subjects' vital signs (temperature, heart rate, respiratory rate, pulse oximetry and blood pressure) will be assessed and continuous pulse oximetry 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.

8.14 Follow-up

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.

9. TOXICITIES TO BE MONITORED/DOSAGE MODIFICATIONS

9.1 Grading AEs and Defining Dose Limiting Toxicity (DLT) determination

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 in 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 Appendix III)
3. Grade 4 CRS of any duration (graded according to ASTCT CRS: See Appendix III)
4. Grade ≥ 3 immune effector cell-associated neurotoxicity syndrome (ICANS) of any duration (graded according to ASTCT ICANS Consensus grading for Adults: See Appendix IV)
5. Any Grade 4 hematologic toxicity that does not resolve to Grade ≤ 2 within 28 days (4 weeks) from CD4CAR infusion except for the following:
    a. Lymphopenia, 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 HSCT 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.

9.2 Grading of CRS:

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 [9] [10] [11].

The ASTCT CRS Consensus Grading criteria will be used [12]. 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. 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. 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.

9.3 Grading of Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS):

ICANS may manifest as delirium, encephalopathy, aphasia, lethargy, difficulty concentrating, agitation, tremor, seizures, and, rarely, cerebral edema. In addition, headache is very common and might not represent neurotoxicity per se. Previously considered in aggregate with CRS, neurotoxicity is now treated as a separate entity owing to its distinct timing and response to intervention. Neurologic symptoms may occur during or more commonly after CRS symptoms (but rarely before CRS), vary among patients, and have an unclear pathophysiology, distinct from CRS. One challenge has been to identify the symptoms most relevant to neurotoxicity [12].

The ASTCT ICANS Consensus Grading for Adults [12], as well as the Immune Effector-Cell Associated Encephalopathy (ICE) will be used. 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 score 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. Depressed level of consciousness should be attributable to no other cause (e.g., no sedating medication). Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading. Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v5.0.

9.4 CD4+ cell depletion:

It is possible that T cell depletion will occur temporarily [13]. Antimicrobial prophylaxis consistent with guidelines of prophylaxis followed for patients treated for AIDS (NIH guidelines) will be utilized until T cell recovery.

9.5 HLH and MAS:

For assessment and management of Hemophagocytic lymphohistiocytosis (HLH)/ macrophage-activation syndrome (MAS): Although extremely rare, HLH/MAS has been described in other CAR T cell trials. This syndrome is a hyperactivation of multiple cell lines and proinflammatory cytokines that may lead to significant multi-organ failure. 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) [14] 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 hemophagocytosis in the

bone marrow or other organs. Patients with suspected HLH/MAS should be treated according to CRS treatment guidelines. 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.

Other CAR T cell therapy related toxicities to be reported and general management guidelines for considerations:

9.6 Replication-competent lentivirus (RCL)

RCR testing results from production lots and patient monitoring should be documented in amendments to the IND file. Positive results from patient monitoring should be reported immediately as an adverse experience in the form of an IND safety report (21 CFR 312.32). Negative results should be reported by way of the IND annual report (21 CFR 312.33) or Development Safety Update Report (DSUR), if used in lieu of reporting in annual reports. In addition, to enhance the accumulation of data on RCR testing assays, CBER encourages members of the gene therapy community to publish data and/or discuss data publicly regarding their experience with different vector producer cell lines, patient monitoring, and safety.

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.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

9.7 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.

9.8 Management guidelines of CAR T cell related Toxicities:

For CRS, ICANS, HLH, please see detailed appendixes, III, IV and V

Note: ***Management guidelines for events that are not listed in the appendices are unexpected events and will be treated per Institutional Guidelines for standard of care.***

10. SCHEDULE OF EVENTS

10.1 Screening To CD4CAR Administration

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.

	Screening**	Leukapheresis**	Second Screening (See section 5.3) *,**	Conditioning Chemotherapy**	Third Screening (See Section 5.4)	CD4CAR Infusion
	-42 to -7	~ Day -35	~Day -7 to Day -5	~Day -4 to -2	~Day -1	Day 0
REQUIRED ASSESSMENTS						
Informed Consent Form	X					
Demographics and Medical history	X		X	X	X	
Leukapheresis[1]		X[1,14]				
Conditioning Chemotherapy				X		
Hospital Stay				X		
Cytokine release syndrome evaluations[12]						X
Neurological Examination (ICANS/ICE)[13]						X
Physical examination	X			X	X	X[8]
Psychosocial Assessment[11]	X					
ECOG performance status	X			X	X	
EKG (12 lead)	X[1]				X	
Chest X-ray	X[AN]			X		
Cardiac function assessment[20,23]	X[1]		X[AN]			
Adverse Events		X				
Concomitant Medications[19]	X	X				

Survival		X

	Screening**	Leukapheresis**	Second Screening (See section 5.3) *,**	Conditioning Chemotherapy**	Third Screening (see section 5.4)	CD4CAR Infusion
	-42 to -7	~ Day -35	~Day -7 to Day -5	~Day -4 to -2	~Day -1	Day 0
SOC LABORATORY ASSESSMENTS						
CBC with diff[4,20]	X[1]		X	X	X[6]	X[6]
CMP (chemistry)[4,20]	X[1]		X	X	X	X
Creatinine clearance Assessment	X[1]			X[AN]		X[AN]
PT, PTT	X[1]				X	X
CRP[3] and Ferritin Level	X[1]				X	X
Syphilis[18, 20]	X[1]					
Pregnancy Test[2]	X		X			
Infectious Disease Markers[14]	X[1]					
Viral and Fungal Markers[15]	X					X
Spinal Tap and CSF analysis[5]			X[AN]			
Tissue Sample[5]			X[AN]			
Disease assessment marrow[5, 20]	X		X[AN]			
Urinalysis (routine)[16]				X		
disease assessment blood(clinical flow)[5, 20]	X[1]				X[6]	X[6]
ABO & Rh Screen[20]	X[1]					
Bone Marrow Aspirate/Biopsy[21]	X[AN]					

RESPONSE ASSESSMENT						
Blood response[22]	X					
Marrow response[22]	X			X[AN]		
Cytogenetic response[22]	X					
Clinical benefit[22]	X					
TREATMENT						
Administer CD4CAR						X[6]

	Screening**	Leukapheresis**	Conditioning Chemotherapy**	CD4CAR Infusion
	-42 to -7	~ Day -35	~Day -4 to -2	Day 0
CORRELATIVE STUDIES				
Research Flow Cytometry[17]			X	
Transgene copy number (TCN)[10]			X	
HAMA[7]			X	
Replication competent lentivirus (RCL)[10]			X	
Cytokine Panel[3]			X	
Optional blood samples for future research[9]			X	

Footnotes:

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

Note: 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.

* Procedures completed during second screening only need completed once during Day -7 to Day-5.

** 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.

1. The following screening tests must be completed, and subject is confirmed eligible: CMP, CBC, Cardiac function assessment, creatinine clearance, disease assessment blood (clinical flow), Infectious disease markers, Syphilis, PT, PTT, ABO & rh screen, EKG, and ferritin level.

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

3. 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 participant 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.

4. 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.

5. Screening disease assessments should be completed within 7 days of Leukapheresis. Can perform as clinically indicated, as deemed necessary by physician at other times pre-infusion. Submission of tissue samples to the sponsor is not required. Tumor assessments may be performed by Imaging (any technique), Physical Exam, Bone Marrow Biopsy, or Spinal Tap/cerebrospinal fluid analysis.

   A bone marrow biopsy for disease assessment (disease assessment marrow) for **must** occur within **14 days of Day 0**. Also, a disease assessment blood (clinical flow) (leukemia assay **and** lymphocyte subset quantitation, including CD4, CD8, and CD3 cell determination [t-cell subsets]) must be collected **within 24 hours** prior to CD4CAR infusion. The clinical flow may be repeated more frequently as necessary at investigator discretion.

6. To be obtained within 24 hours prior to CD4CAR infusion: CBC with diff, assessment of CD3, CD4, and CD8 counts.

7. HAMA to be collected on Day -4 prior to start of conditioning chemotherapy.

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

9. Optional blood samples: to be obtained on Day -4 prior to start of conditioning chemotherapy. 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.

10. RCL and TCN to be performed on Day -4 prior to start of conditioning chemotherapy. RCL testing will be performed by the Indiana University Gene Therapy Testing Laboratory.

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

12. 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 Appendix III for more details.

13. The nursing staff will conduct focused neurologic examinations approximately 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 ICANS using the American Society for Transplantation and Cellular Therapy's Immune Effector Cell-Associated Encephalopathy (ICE) score grading scale. See Appendix IV for more details.

14. Infectious disease markers (IDMs) 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

    Note: If IDM results are not available by day and time of leukapheresis, **do not** proceed with leukapheresis. The subject must be rescheduled after results are available.

15. Viral and Fungal Disease markers include Aspergillus Galactomannan Ag, CMV qPCR, EBV qPCR Fungitell-D, HHV-6 qPCR to be performed at least once weekly while inpatient.

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

17. Research Flow Cytometry will evaluate the presence of CD4CAR expression to be performed on Day -4 prior to start of conditioning chemotherapy.

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

19. 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.

20. These tests do not need to be repeated if completed after consent and within 28 days of Day -7: disease assessments (blood and marrow), CMP, CBC, Cardiac function assessment, Syphilis, and ABO & Rh screen.

21. 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.

22. Baseline response assessments will be completed via documentation and assessment of SOC CBC results, SOC clinical flow, SOC next generation sequencing (NGS) testing results (when applicable), SOC Bone marrow results; as well as physical exam and completion of the Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS).

23. Cardiac function assessment may include ECHO or MUGA.

10.2 Short- And Long-Term Follow-Up

If any tests/procedures diverge from those listed on the Schedule of Events below due to Institutional Policies, those policies will be followed with approval of the Sponsor Investigator. These will <u>not</u> constitute a deviation.

	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 through Discharge	Day 15	Day 21	Day 28	Monthly from months 2-6	Quarterly from month 6 to year 2	Bi-annually year 2-15
							+/-3 days	+/-3 days	+/-3 days	+/-7 days	+/-30 days	+/-30 days
REQUIRED ASSESSMENTS												
Demographics and Medical history[11]												X
Hospital Stay			X									
Cytokine release syndrome evaluations[12]			X									
Neurological Examination (ICANS/ICE)[13]			X									
Physical examination						X						X^{AN}
EKG (12 lead)[6]	X^{AN}								X			
ECOG performance status								X				
Cardiac function assessment[18]			X^{AN}									
Adverse Events[11]						X						X

Concomitant Medications[14]	X											X
Follow-Up Questionnaire												X
Survival	X											
	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 through Discharge	Day 15	Day 21	Day 28	Monthly from months 2-6	Quarterly from month 6 to year 2	Bi-annually year 2-15
							+/-3 days	+/-3 days	+/-3 days	+/-7 days	+/-30 days	+/-30 days
SOC LABORATORY ASSESSMENTS												
CBC with diff[3]	X											
CMP (chemistry)[3]	X											
PT,PTT[7]		X				X	X					
CRP[2,8] and Ferritin Level	X						X					
Viral and Fungal Markers[9]	X											
Spinal Tap and CSF analysis[3]	X[AN]											
Tissue Sample[3]	X[AN]											
Disease assessment marrow[4]							X		X			
Urinalysis (routine)							X[AN]					
disease assessment						X	X		X	X	X	

blood(clinical flow)[4,15]												
Bone Marrow Aspirate/Biopsy[4,16]			X[AN]				X[AN]					
	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7 through Discharge[1]	Day 15	Day 21	Day 28	Monthly from months 2-6	Quarterly from month 6 to year 2	Bi-annually year 2-15
							+/-3 days	+/-3 days	+/-3 days	+/-7 days	+/-30 days	+/-30 days
RESPONSE ASSESSMENT												
Blood response[17]									X	X		
Marrow response[17]									X	X		
Cytogenetic response[17]									X	X		
Clinical benefit[17]									X	X		
CORRELATIVE STUDIES												
Research Flow Cytometry[8]		X		X		X	X	X	X	X	X	
Transgene copy number (TCN)[8]		X		X		X	X	X	X	X	X	
HAMA[8]						X	X	X	X	X	X	
Replication competent lentivirus (RCL)[10]										X	X	X[AN]
Cytokine Panel[2,8]		X		X		X	X	X	X	X	X	

Optional blood samples for future research[5]						X			X	X		

Footnotes:

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

Note: 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.

1. Repeat procedures listed under Day 7 until subject is discharged. 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 in order to evaluate the presence of CD4CAR cells on day 3, 5, 7, 15, 21, and 28 post infusion, or as clinically indicated. **HAMA sample will be drawn a single time on day 7 if subject is inpatient past day 7**. Once discharged, subjects will undergo approx. once a week thereafter for four weeks (or until Day 28) the following: physical exam, documentation of adverse events and blood draws for hematology, chemistry, viral and fungal markers, engraftment, and research labs.

2. 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.

3. 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.

4. Perform disease assessments 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 Days 15 and 28 post CD4CAR infusion for marrow and at days 7, 15, 28, and monthly (months 2-6) for blood; and 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 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 may be performed by Imaging (any technique), Physical Exam, Bone Marrow Biopsy, peripheral blood test, or Spinal Tap/cerebrospinal fluid analysis.

5. Optional blood samples: approximately on Day 7, Day 28 (+/-3 days), and 3 months (+/- 7 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.

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

7. Follow up PT/PTT test will be performed at a follow up visit occurring approximately on Day 15 post CD4CAR infusion, or more often if clinically indicated.

8. These tests are performed until CD4CAR is undetected by two consecutives TCN or flow cytometry tests and at principal investigator and treating physician discretion, and additionally as needed per PI discretion. TCN is only positive if greater than 5.

9. 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.

10. RCL to be performed at 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.

11. 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)

12. 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 Appendix III.

13. The nursing staff will conduct focused neurologic examinations approximately 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 ICANS using the American Society for Transplantation and Cellular Therapy's Immune Effector Cell-Associated Encephalopathy (ICE) score grading scale. See Appendix IV for more details.

14. All prescription and nonprescription medications, vitamins, herbal and nutritional supplements, taken by the subject will be recorded 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.

15. Collection of the disease assessment blood (clinical flow) will coincide with disease assessment collection timepoints (see footnote 4). The clinical flow will include lymphocyte subset quantitation, including CD4, CD8, and CD3 cell determination (t-cell subsets) **and** A leukemia/lymphoma assay. The clinical flow may be repeated more frequently as necessary at investigator discretion.

16. 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.

17. Response assessments will be completed via documentation and assessment of SOC CBC results, SOC clinical flow, SOC next generation sequencing (NGS) testing results (when applicable), SOC Bone marrow results (Day 28 (+/-3 days) and 90 (+/-10 days) only); as well as physical exams and completion of the Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score (MPN-SAF TSS) on Day 28, and monthly from months 2-6.

18. Cardiac function assessment may include ECHO or MUGA.

11. ADVERSE EVENTS DEFINITIONS AND REPORTING

Safety will be assessed by monitoring and recording potential adverse effects (AEs) 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.

11.1 Adverse Event (AE)

An adverse event is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a drug, without any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose. Adverse events will be graded according to the NCI Common Toxicity Criteria, Version *5*.0 (Appendix II).

11.2 Suspected Adverse Reaction (SAR)

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

Examples of types of evidence that would suggest a causal relationship between the drug and the adverse event:

• A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome).

• One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture).

• An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

11.3 Adverse Reaction (AR)

An adverse reaction is any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions where there is reason to conclude that the drug caused the event.

11.4 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if it results in any of the following outcomes:

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. Requires inpatient hospitalization > 24 hours or prolongation of existing hospitalization
   NOTE: Hospitalizations that are not considered SAEs are:
   - Hospitalization planned prior to first study intervention
   - Hospitalization for elective treatment of a pre-existing condition unrelated to the study intervention
   - Hospitalization lasting ≤ 24 hours

11.5 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

11.6 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.

11.7 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.

For RCL testing, Testing should be done pretreatment and at three, six, and twelve months after treatment, and yearly for up to fifteen (15) years. One may provide a rationale to discontinue all active testing of patient samples for RCR in the safety monitoring section of clinical protocol. This

trial will stop testing after the 12 months if CD4CAR is undetectable, and upon the first negative CD4CAR expression testing after marrow reconstitution for subjects undergoing stem cell transplant. We recommend a yearly review of medical history be collected in lieu of patient samples, as a part of long-term follow-up. This history should be targeted towards determination of clinical outcomes suggestive of retroviral disease, such as cancer, neurologic disorders, or hematologic disorders. As part of the long-term follow-up protocol, a yearly long-term follow-up clinical report should be submitted to the IND.

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 subjects who receive additional routine or experimental anticancer therapy, including medical, radiation or surgical intervention (with the exclusion of HSCT) will be limited to documentation of secondary malignancy which will continue for up to 15 years after infusion of CD4CAR.

11.7.1 *Participating Site Reporting Responsibilities*

11.7.1.1 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 FDA Form 3500A 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: IUCARE CRO Project Manager/ CTO-IUSCCC-0840

E-mail: IUSCCSAE@iu.edu

The IUCARE CRO Project Manager, or designee, will distribute the reports to all participating sites, and the FDA as per section 11.7.2 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.

11.7.1.2 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.

11.7.2 *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.

11.7.2.1 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**

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

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.

11.7.2.2 Reporting to Participating Sites

The IUCARE CRO 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.

11.7.3 *Reporting to the IRB*

Unanticipated problems involving risks to subjects or others will be reported promptly to the IRB if they:
• are unexpected;
• are related or possibly related to participation in the research; and
• suggest that the research places subjects or others at a greater risk of harm than was previously known or recognized.

If the serious adverse event does not meet all three (3) criteria listed above, the event does not have to be promptly reported to the Indiana University IRB. However, it should be reported at the time of continuing review.

Prompt reporting of unanticipated problems to the IRB is defined as within 5 days from becoming aware of the event.

11.7.4 *Reporting to the IUSCCC DSMC*

Regardless of study sponsorship, the study team must enter all initial and follow-up SAE, expedited, and noncompliance reports into OnCore® for review by the DSMC chair and/or coordinator. Expedited reports may include IRB Prompt Report Forms, AdEERS reports, MedWatch, and additional SAE forms as required by the sponsor. When follow-up information is received, a follow-up report should also be created in OnCore®. This DSMC reporting requirement is in addition to any other regulatory bodies to be notified (i.e., IRB, FDA, pharmaceutical company, etc.). The DSMC chair and/or coordinator will review all SAE, expedited, and noncompliance reports monthly.

12. CRITERIA FOR REMOVAL FROM STUDY

12.1 Subject Discontinuation

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.

12.2 Criteria for discontinuing a subject's participation in the study prior to CD4CAR infusion:

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.

12.3 Lost to Follow-up

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.

12.4 Disease Free Survival Assessment Discontinuation

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.

12.5 Survival Assessment Discontinuation

Subjects will be withdrawn from survival assessments at the time of death.

13. STATISTICAL PLAN

Sample Size and statistical design

Up to 30 subjects will be treated with CD4CAR cells in this study, with a total of 4 dose levels. The 3+3 design will be used in the Phase I dose-finding trial to find the MTD for a future phase II study.

Subject 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.

Subject 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.

Treatments (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.

Primary 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. Summary statistics will be provided.

Secondary Endpoint: Efficacy and TME changes

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 secondary endpoint is to test the relative persistence of CD4CAR positive cells, CD4CAR differentiation/ repolarization in vivo/ and CD4CAR influence on the abundance of T regs and MDSCs. The change in the ratio of CD4CAR positive cells and subsets, and in the Tregs and MDSCs, over time will be compared using a non-parametric Wilcoxon signed-rank test for paired data without the normality data assumption.

14. EVALUATION OF RESPONSE

(On both of marrow, peripheral blood, and any other disease site that can be deemed safe to biopsy)

Morphology:

- Complete Response (CR): Absence of CMML cells in the marrow and other disease sites
- Partial Response (PR): % reduction on CMML cells compared to pre-treatment %
- Stable Disease (SD): No morphological changes are seen on the marrow
- Disease Progression (DP): worsening of the CMML cells disease estimate or transformation into acute myeloid leukemia.

Flowcytometry:

- CR: absence of CMML cell using CMML flow panel
- PR: %reduction on disease burden as evaluated by CMML flow panel
- SD: No significant reduction on disease status.

Molecular disease assessment

- Molecular CR: Disappearance of all pre-treatment detected molecular abnormalities per next-generation sequencing (NGS).

- Molecular Residual Disease (MRD): Detection of pretreatment molecular alterations at a lower level in the absence of positive flowcytometry and morphology
- Molecular progression of disease (MPD): Detection of additional molecular alteration that were not seen pre-treatment.

Table 1: Other Potential Response Criteria

Table 2. Proposed criteria for measurement of treatment response in adult MDS/MPN

CR (presence of all of the following improvements)*
Bone marrow: ≤5% myeloblasts (including monocytic blast equivalent in case of CMML) with normal maturation of all cell lines and return to normal cellularity*
Osteomyelofibrosis absent or equal to "mild reticulin fibrosis" (≤grade 1 fibrosis)†
Peripheral blood‡
WBC ≤10 × 10^9 cells/L
Hgb ≥11 g/dL
Platelets ≥100 × 10^9/L; ≤450 × 10^9/L
Neutrophils ≥1.0 × 10^9/L
Blasts 0%
Neutrophil precursors reduced to ≤ 2%
Monocytes ≤1 × 10^9/L
Extramedullary disease: Complete resolution of extramedullary disease present before therapy (eg, cutaneous disease, disease-related serous effusions), including palpable hepatosplenomegaly
Provisional category of CR with resolution of symptoms:‡ CR as described above, and complete resolution of disease-related symptoms as noted by the MPN-SAF TSS
Persistent low-level dysplasia is permitted given subjectivity of assignment of dysplasia*
Complete cytogenetic remission
Resolution of previously present chromosomal abnormality (known to be associated with myelodysplastic, syndrome myeloproliferative neoplasms, or MDS/MPN), as seen on classic karyotyping with minimal of 20 metaphases or FISH§
Partial remission
Normalization of peripheral counts and hepatosplenomegaly with bone marrow blasts (and blast equivalents) reduced by 50%, but remaining >5% of cellularity *except* in cases of MDS/MPN with ≤5% bone marrow blasts at baseline
Marrow response
Optimal marrow response: Presence of all marrow criteria necessary for CR without normalization of peripheral blood indices as presented above.
Partial marrow response: Bone marrow blasts (and blast equivalents) reduced by 50%, but remaining >5% of cellularity, *or* reduction in grading of reticulin fibrosis from baseline on at least 2 bone marrow evaluations spaced at least 2 mo apart
Clinical benefit
Requires 1 of the following in the absence of progression or CR/partial response and independent of marrow response (cord blood response must be verified at ≥8 wk) to be considered a clinical benefit
Erythroid response
Hgb increase by ≥2.0 g/dL
TI for ≥ 8 wk for patients requiring at least 4 packed red blood cell transfusions in the previous 8 wk
Only red blood cell transfusions given based on physician's judgment for a pretreatment Hgb of ≤8.5 g/dL will count in the red blood cell TI response evaluation‖
Platelet response

15. DATA FORMS AND SUBMISSION SCHEDULE

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. PATIENT CONSENT AND PEER JUDGMENT

The protocol and informed consent form for this study must be approved in writing by the appropriate Institutional Review Board (IRB) prior to any patient being registered on this study.

Changes to the protocol, as well as a change of principal investigator, must also be approved by the Board. Records of the Institutional Review Board review and approval of all documents pertaining to this study must be kept on file by the investigator (housed in the Clinical Trials Office) and are subject to inspection at any time during the study. Periodic status reports must be submitted to the Institutional Review Board as required by the board, as well as notification of completion of the study and a final report within 3 months of study completion or termination.

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

17. DATA AND SAFETY MONITORING PLAN

17.1 Data and 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.

17.2 Data Safety and 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 multi-site 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, email communication will be used in lieu of a teleconference. *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.

17.3 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.iupui.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.

17.4 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.

17.5 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.

17.6 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.

17.7 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.

17.8 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.

17.9 Reporting Death

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

17.10 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.

17.11 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 IUCARE CRO Project Manager, or designee.

17.12 Protocol Deviation Reporting

Investigators are required to submit protocol deviations to the DSMC via the OnCore® database. 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.

17.13 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.

18. IUCARE CRO GUIDELINES

18.1 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.

18.2 Study Initiation

Before activating the clinical trial at each participating site, the IUCARE CRO 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.

18.3 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. The IUCARE CRO 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.*

18.4 Data Monitoring

All multi-site investigator-initiated trials conducted at the IUSCCC are subject to data monitoring by the IUCARE CRO 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 IUCARE CRO 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 IUCARE CRO 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.

18.5 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.

19. REFERENCES

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20. APPENDICES

20.1 Appendix I. Performance Scales/Scores

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

20.2 Appendix II. NCI CTCAE Version 5.0

Due to the size of the latest version of the Common Toxicity Criteria, copies of this appendix are not included with this protocol document.

An electronic copy is available on the CTEP web site,

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

20.3 Appendix III. Cytokine Release Syndrome (CRS)

Grading of CRS (ASTCT CRS Consensus Grading)

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever*	Temperature ≥38° C	Temperature ≥38° C	Temperature ≥38° C	Temperature ≥38° C
		With		
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.

Guidelines for the management of CRS

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	• Consider tocilizumab* for 1 dose for persistent fever lasting greater than 3 days

CRS Grade	CRS Parameter	Management Guidelines		
		Diagnostic Work-Up	Supportive Care	Anti-IEC Therapies
			• 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	
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 • 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)
	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,	• 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

CRS Grade	CRS Parameter	Management Guidelines		
		Diagnostic Work-Up	Supportive Care	Anti-IEC Therapies
			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	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 • 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 • If on one vasopressor: tocilizumab as in Grade 2 CRS and dexamethasone 10mg IV every 6 hours (or 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	• Supplemental oxygen including high-flow nasal cannula, face mask, non-rebreather mask, or Venturi mask as needed	• Tocilizumab* and dexamethasone 10mg IV every 6 hours (or methylprednisolone equivalent) if not administered previously; tocilizumab may be

CRS Grade	CRS Parameter	Management Guidelines		
		Diagnostic Work-Up	Supportive Care	Anti-IEC Therapies
		cultures, and chest radiography)	• Symptomatic management of fever as in Grade 1 CRS • Symptomatic management of constitutional symptoms and organ toxicities as per standard guidelines	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 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	• Transfer patient to ICU • Positive pressure ventilation including CPAP, BiPAP, mechanical ventilation	• 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

CRS Grade	CRS Parameter	Management Guidelines		
		Diagnostic Work-Up	Supportive Care	Anti-IEC Therapies
		cultures, and chest radiography)	• Symptomatic management of fever as in Grade 1 CRS • Symptomatic management of constitutional symptoms and organ toxicities as per standard guidelines	• 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 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.

20.4 Appendix IV. Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS)

Immune Effector-Cell Associated Encephalopathy (ICE) Score Grading System

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).

Grading of ICANS

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

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
				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 score 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.

Recommended guidelines for management of ICANS

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	• 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	• 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*

ICANS Grade	Sign or Symptom	Management Guidelines		
		Diagnostic Work-up	Supportive Care	Anti-IEC Therapies
		• Consider diagnostic lumbar puncture if other causes of encephalopathy are suspected (such as infections, autoimmune, leptomeningeal disease)	• 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	
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) • 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

ICANS Grade	Sign or Symptom	Management Guidelines		
		Diagnostic Work-up	Supportive Care	Anti-IEC Therapies
	Seizure	• Neurological work-up as in Grade 1 ICANS • EEG if clinically indicated (e.g., ongoing seizures, depressed level of consciousness) • 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 seizures, or non-convulsive seizures, treat as per Appendix IV	• Dexamethasone 20mg IV every 6 hours (or methylprednisolone equivalent) • If associated with concurrent CRS, add tocilizumab* • 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 not related to primary malignancy, control	•Methylprednisolone 1000mg/day in divided doses IV for 3 days followed by taper as clinically indicated; if associated with concurrent CRS, add tocilizumab* • Continue corticosteroids until improvement to less than or equal to Grade 1 ICANS and then taper

ICANS Grade	Sign or Symptom	Management Guidelines		
		Diagnostic Work-up	Supportive Care	Anti-IEC Therapies
			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)	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 Appendix IV • For convulsive status epilepticus, treat as in Appendix IV	•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 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)

ICANS Grade	Sign or Symptom	Management Guidelines		
		Diagnostic Work-up	Supportive Care	Anti-IEC Therapies
	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 Appendix IV	•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.

Recommended guidelines for management of Diffuse Cerebral Edema and/or Raised ICP related to CAR T cell therapy

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)

	○ 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 ○ 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 ○ 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

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

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

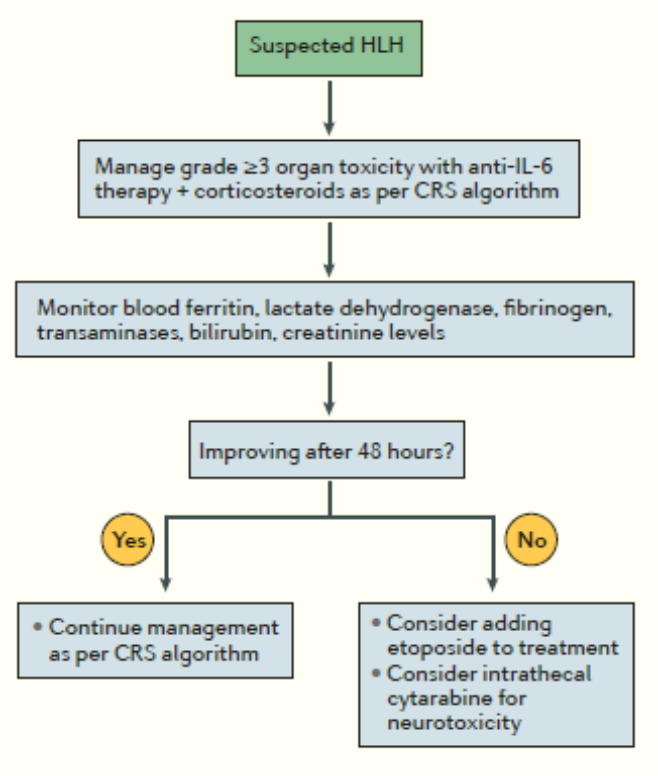
20.5 Appendix V. Haemophagocytic lymphohistiocytosis (HLH)/ macrophage-activation syndrome (MAS):

Proposed diagnostic criteria for CAR T cell related HLH/MAS

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

Proposed management of CAR T cell related HLH/MAS



*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)