STUDY PROTOCOL

Protocol Title:	Phase I/II Study of Autologous T Cells Engineered Using the *Sleeping Beauty System* to Express T-Cell Receptors (TCRs) Reactive Against Cancer-specific Mutations in Subjects with Solid Tumors
Protocol Number:	TCR001-201
Phase:	I/II
Date of Protocol:	**Amendment 4: 24OCT2022** Amendment 3: 14DEC2021 Amendment 2: 02SEP2021 Amendment 1: 18MAR2021 Original: 13OCT2020
Sponsor:	Alaunos Therapeutics, Inc. [redacted]
Medical Monitor:	[redacted]
Safety Reporting:	[redacted]
Email Correspondence:	[redacted]

1. CLINICAL PROTOCOL SYNOPSIS

Protocol Number: TCR001-201	**Phase:** I/II	**Country:** USA

Title of Study:

Phase I/II Study of Autologous T Cells Engineered using the *Sleeping Beauty System* to Express T-Cell Receptors (TCRs) Reactive Against Cancer-specific Mutations in Subjects with Solid Tumors

Study Center(s): Approximately 5 sites

Name of Investigational Product:

Autologous T Cells Engineered to Express T-Cell Receptors Reactive Against Mutated Neoantigens Using the *Sleeping Beauty* [redacted] System [redacted]

Name of Active Ingredient:

Neoantigen-specific TCR-T cells are the drug product defined as autologous patient-specific peripheral blood-derived T lymphocytes transposed utilizing *Sleeping Beauty* [redacted] system [redacted]

Studied Period (years):

Date first subject enrolled: April 2022

Estimated date last subject completed: March 2028

Study Objectives

Phase I Objectives:

Primary Objectives

- To define the incidence of dose limiting toxicity (DLT) and the maximum tolerated dose (MTD) or recommended Phase II dose (RP2D) of T-Cell Receptor T cells (herein referred to as TCR-T cell drug product administered [redacted]

  Arm A:

  To define the incidence of DLT and the MTD of TCR-T cell drug product delivered as a single administration.

  [redacted]

Secondary Objectives

- To evaluate the feasibility of TCR-T cell drug product manufacturing.
- To investigate translational hypotheses related to TCR-T cell persistence [redacted].

[redacted]

Study Design

This is a Phase I/II study of autologous T cells engineered using the *Sleeping Beauty* [redacted] system [redacted]

Subjects eligible for enrollment on this protocol are those for whom a TCR matching the subject's somatic mutation(s) and HLA type restriction combination is available in Alaunos Therapeutics Inc.'s [Alaunos] Clinical TCR library and have progressive or recurrent disease following standard chemotherapy or standard systemic therapy or were intolerant to previous treatment will be eligible for enrollment on this Investigational Product Protocol. Subjects will be enrolled into subgroups according to the following tumor types:

Table 1: Tumor Type Subgroups

Subgroup	Tumor Type
1a	Ovarian
1b	Endometrial
2	Colorectal
3	Pancreatic
4	Non-small cell lung cancer
5	Cholangiocarcinoma

Subjects will initially go through pre-screening to determine if there is a TCR matching the subject's somatic mutation(s) and HLA type restriction combination in Alaunos's Clinical TCR library. If subjects do not have a TCR identified, they will be discontinued and will not move forward with further screening. Upon confirming screening and apheresis eligibility, subjects will undergo an apheresis procedure to collect the cells needed to manufacture the TCR-T cell drug product.

[redacted] with *Sleeping Beauty* [redacted] Subjects will then be lymphodepleted starting 7 days prior to TCR-T cell drug product infusion. NOTE: If subjects receive a bridging therapy, lymphodepletion may not be needed.

Phase I: Dose Escalation

The Phase I part of this study is a prospective, open-label, dose-escalation study of TCR-T cell drug product in patients with progressive or recurrent solid tumors who have failed standard therapy (refer to inclusion criteria #2). This study utilizes a Bayesian optimal interval design (BOIN) with an accelerated dose escalation to determine the MTD/RP2D of TCR-T cell drug product. Subjects who fulfill the eligibility criteria will receive a single infusion of TCR-T cell drug product at the assigned dose level on Day 0. Safety and pharmacokinetic (PK) profiles, and the preliminary efficacy will be examined for each dose level.

The study starts with pre-screening to determine if there is a TCR match for the subject based on their somatic mutation(s) and HLA typing. If there is at least one TCR match for the subject, then they will begin screening assessments to determine initial eligibilityfor treatment with TCR-T cell drug product (Arm A) at Dose Level 1 (DL1) as shown in Figure 2. This dose level will follow an accelerated dose escalation and is planned to treat one subject at dose level 1 in the absence of a DLT. After the subject completes the DLT period (Day 0 to Day 28), the subject will be evaluated for toxicities. If the first subject experiences a DLT, 3 additional subjects will be enrolled and treated at DL1 before escalating to dose level 2 (DL2). If the first subject does not have a DLT(s), the next subject will be enrolled and treated at the next higher dose level (DL2). After DL1, the study will follow the pre-planned dose escalation decision rules detailed in Table 7 and defined in Appendix H. At least 2-3 subjects will be enrolled at DL2 to enable evaluation of TCR-T cell persistence data by the Safety Review Committee (SRC).

The starting dose level will be DL1. If the manufacturing for the first patient meets the criteria for DL-1 (dose level minus 1) the current BOIN design (which accommodates 4 dose levels; refer to Appendix H) accelerated will be initiated at DL-1, subsequently patients will be enrolled, and dose levels will be escalated per the BOIN design. If there is the inability to manufacture at the current

open dose level and while the dose escalation is continuing to explore higher doses, additional patients can be enrolled at the declared safe dose levels in a "dose extension" assigned by the manufactured cell count. The decision to enroll patients in these dose extensions will be made after discussion between the SRC and the Sponsor considering the available safety and efficacy data. The purpose of the additional subjects in dose level cohorts with declared safe dose levels will be to expand the available safety database at pharmacologically relevant doses.

- The SRC will review all available safety information (during the DLT period and long-term toxicities) by dose level including DL-1 (if required to be enrolled by safety or manufacturing issues) and TCR-T cell persistence data [redacted]. If TCR-T cell persistence is deemed sufficient and the dose level is considered safe in the initial subjects who have been treated at DL2, [redacted] he study will advance with the treatment of subjects at Dose Level 3 (DL3) according to the DLT probability rules. [redacted]

If the TCR-T cell persistence is deemed insufficient at DL2 [redacted], at the discretion of the SRC, Arm A will be halted. [redacted]

Subjects who withdraw from the study for reasons not related to treatment-emergent toxicities during the DLT period may be replaced. This would include subjects who withdraw due to progression and receive a subsequent therapy prior to day 28.

For the initial two subjects of Arms A [redacted] there will be a dose staggering interval of 28 days. Dose administration for the subsequent subjects will not occur until the initial two subjects have completed their safety evaluation for dose-limiting toxicities.

Subjects may be screened during this 4-week period so that apheresis material can be collected for manufacturing.

There are two dose escalation stopping rules in this BOIN study.

- Stop the trial if the lowest dose is eliminated due to overt toxicities*;
- Stop the dose escalation and estimate the MTD/RP2D if the number of new subjects treated at the current dose level reaches 9.

* Overt Toxicities defined as:

1. The occurrence of 2 or more dose limiting toxicities.
2. Consideration will also be given to longer term toxicities (e.g. > 30 days post infusion: Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS), cardiotoxicity, pulmonary toxicity,

metabolic complications, secondary macrophage-activation syndrome, prolonged cytopenia unresponsive to colony stimulating factors, grade 3 and 4 infections and subsequent malignancies)

The detailed statistical methods for dose escalation and de-escalation and estimation of MTD/RP2D are described in Section 15.1.1 and Section 15.1.2.

[redacted]

[redacted]

Statistical Methods:

This section summarizes the key, planned reporting and statistical analytic methods of the study data. A forthcoming Statistical Analysis Plan (SAP) will develop these concepts in detail.

In general, continuous variables, including baseline characteristics, will be summarized by reporting the number of observations, mean, standard deviation (SD), median, minimum and maximum and categorical/discrete variables will be summarized using frequency tables showing the number and percentage of patients within a category.

Time-to-event data will be summarized using Kaplan-Meier or Nelson-Aalen statistical techniques, as appropriate, with parameter estimates displayed at pre-defined time points along with the number of censored observations. A 2-sided 95% confidence interval for median time to event estimates will be produced in addition to the event 25th and 75th percentile summary statistic.

Baseline is defined as the last available observation prior to the first administration of study treatment.

In addition, analyses may be performed separately for each individual TCR.

Any deviations from the SAP will be reported in the clinical study report.

Refer to Section 15 for additional details on statistical analyses and endpoints.

Number of subjects (planned):

Phase I: Up to 18 subjects will be treated across all dose levels. [redacted]

Subject Inclusion Criteria

This study will enroll subjects based on histologically confirmed tumor types:

- Subgroup 1a Gynecologic cancer - Ovarian
- Subgroup 1b Gynecologic cancer - Endometrial
- Subgroup 2 Colorectal
- Subgroup 3 Pancreatic
- Subgroup 4 Non-small cell lung cancer (NSCLC). NSCLC includes but is not limited to squamous cell carcinoma, adenosquamous carcinoma or adenocarcinoma.
- Subgroup 5 Cholangiocarcinoma

Pre-screening Eligibility Criteria:

1. Patients with previous tumor genomic testing performed at a CLIA-certified or CLIA-accredited laboratory must be willing to disclose results. Patients that do not have previous tumor genomic testing must have a planned biopsy or resection (or archived tumor tissue available for genomic testing) or must provide a blood sample for circulating tumor DNA (liquid biopsy) testing.
2. Patients with previous high resolution, human leukocyte antigen (HLA) class I and II typing from a CLIA-certified or CLIA-accredited laboratory must be willing to disclose results.

Patients that do not have previous typing results must be willing to provide a blood sample for this testing.

3. Patients who have previously received at least one line of standard systemic therapy for their advanced/metastatic cancer and have either progressed, recurred, or were intolerant to the previous treatment for one of the following cancer types.
    - Gynecologic cancers (i.e., ovarian or endometrial)
    - Colorectal Cancer
    - Pancreatic Cancer
    - Non-small cell lung cancer (NSCLC;) NSCLC includes but is not limited to squamous cell carcinoma, adenosquamous carcinoma or adenocarcinomas
    - Cholangiocarcinoma
4. Patients must be able to provide written informed consent.
5. Patients must be age ≥ 18 years.
6. Clinical Performance Status of Eastern Cooperative Group (ECOG) 0 or 1.
7. Patients must not have any form of primary immunodeficiency (such as severe combined immunodeficiency disease) or uncontrolled autoimmune hemolytic anemia or idiopathic thrombocytopenic purpura, such as those patients with a declining hemoglobin level or platelet count secondary to autoimmune destruction within the 4 weeks prior to first dose of study drug, or the need for daily prednisone ≥10 mg daily (or corticosteroid equivalent) to control the autoimmune disease [redacted]
8. Patients who have received any type of organ transplant in the past 12 months are excluded.
9. Patients who have undergone xenotransplantation at any time are excluded.
10. Patients must not have any history of severe immediate hypersensitivity reaction to cyclophosphamide, fludarabine, [redacted] bendamustine (for subjects if required for lymphodepletion), or dimethyl sulfoxide (DMSO).
11. Patients with a prior history or concurrent malignancy whose natural history or treatment does not have the potential to interfere with either the safety or efficacy assessment of the investigational regimen may be included after discussion with Sponsor, Alaunos Medical Monitor, or designee
12. Patients must not have any active unstable or clinically significant medical condition that would, in the opinion of the Principal Investigator (PI) in consultation as warranted with medical monitor, result in risks to safety of a subject and/or their compliance with the protocol. Examples include, but are not limited to, a history of myocarditis or congestive heart failure (New York Heart Association functional Class III or IV), unstable angina, serious uncontrolled cardiac arrythmia, myocardial infarction within 6 months of screening, active

interstitial lung disease (ILD)/pneumonitis or a history of ILD/pneumonitis requiring chronic treatment with systemic steroids, uncontrolled asthma, or colitis.

Screening Eligibility Criteria:

Inclusion Criteria:

1. Subjects with at least one TCR matching a combination of their somatic mutations and HLA type is available in Alaunos's Clinical TCR library (refer to Appendix G) as evidenced by molecular testing results performed at a CLIA-certified or CLIA-accredited laboratory.

2. Patients who have previously received at least one line of standard systemic therapy for their advanced/metastatic cancer and have either progressed, recurred, or were intolerant to the previous treatment. Specifically:
    - Subgroup 1. Gynecologic cancers (i.e., ovarian or endometrial):
        a. Ovarian cancer: Subjects who are platinum-resistant, as defined by progression on a platinum-containing regimen or within 6 months of a previous platinum dose.
        b. Endometrial cancer: Subjects who have received at least 1 prior line of therapy for advanced/recurrent disease (including adjuvant chemotherapy and adjuvant chemo-radiation therapy) prior to apheresis. Prior hormonal therapy will not count towards prior lines of therapy. Note that 2 prior lines of treatment are required prior to lymphodepletion.
    - Subgroup 2. Colorectal cancer:
        - Patient has received systemic treatment for advanced unresectable or metastatic disease prior to apheresis
        - Must include an irinotecan or oxaliplatin-based therapy and if eligible, a targeted antibody therapy.
            - Subjects with deficient DNA mismatch repair (dMMR) or microsatellite instability-high (MSI-H) colorectal cancer should have received a checkpoint inhibitor such as pembrolizumab or nivolumab. Note that 2 prior lines of treatment are required prior to lymphodepletion.
    - Subgroup 3. Pancreatic cancer: Subjects who have progressive disease after receiving initial treatment with FOLFOX, (modified) FOLFIRINOX, and/or a gemcitabine-based therapy.
    - Subgroup 4. Non-small cell lung cancer (NSCLC):
        - Subjects with recurrent and/or metastatic disease who have disease progression or intolerance to treatment with a PD-1/PD-L1 inhibitor either as a single-agent or in combination with other checkpoint inhibitors (e.g., CTLA-4 inhibitors) and/or platinum-containing chemotherapy.
        - Subjects with targetable oncogene alterations (e.g., EGFR, ALK, ROS1, RET, MET, NTRK1-3, BRAF) must have had disease progression or

intolerance on at least one prior line of targeted therapy.

- Subgroup 5. Cholangiocarcinoma: Subjects must have histologically confirmed diagnosis of cholangiocarcinoma Stage II, III, or IV (intra-hepatic, extra-hepatic and perihilar) that is not eligible for curative resection, transplantation, or ablative therapies. Subjects who have progressed after receiving at least one line of standard therapy.

3. Patients must have measurable disease per RECIST 1.1 as assessed by the local site investigator/radiology.
4. Patient must agree to provide archival tissue (core or excisional biopsy) from a locally recurrent or inoperable or metastatic tumor lesion or new tissue must be obtained from a non-target lesion for translational research assessments
5. Patients must be able to provide written informed consent.
6. Clinical Performance Status of Eastern Cooperative Group (ECOG) 0 or 1.
7. Patient must provide written informed consent for the long-term follow-up protocol (TCR001-202) for up to 15 years post TCR-T Cell drug product infusion per FDA requirements.
8. Adequate bone marrow reserves as assessed by the following hematology laboratory criteria:
   a. ANC $\geq$1,000/mm^3 (filgrastim not within 7 days or pegfilgrastim not within 14 days)
   b. WBC $\geq$ 3,000/mm^3
   c. Platelet count $\geq$ 50,000/mm^3
   d. Hemoglobin > 8.0 g/dL. (Subjects may be administered red blood cell transfusions to achieve this cut-off value.)
9. Adequate major organ system functions as determined by the following criteria:
   a. Cardiac
      i) Left ventricular ejection fraction $\geq$50% on multiple gated acquisition (MUGA) scan and/or by echocardiography at the discretion of the investigator.

      Note: No evidence of clinically significant pericardial effusion as determined by an ECHO.
   b. Pulmonary
      i) Adequate pulmonary function, defined as grade $\leq$ 2 dyspnea and SaO2 $\geq$ 90% on room air.
   c. Renal
      i) Serum creatinine within institutional limits; OR
      ii) Calculated creatinine clearance (ClCr) (e.g., Cockcroft-Gault equation) $\geq$ 45.0 mL/min and not dialysis dependent
   d. Liver
      i) Serum ALT/AST $\leq$ 3 x ULN or $\leq$ 5 x ULN if documented liver metastases
      ii) Total bilirubin $\leq$ 2 mg/dL, except in patients with Gilbert's Syndrome, who must have a total bilirubin < 3.0 mg/dL
10. Patients may have undergone minor surgical procedures or limited-field radiotherapy provided any major organ toxicities have recovered to $\leq$ Grade 1.
11. Patients must not be pregnant or breastfeeding.
12. Female patients of non-childbearing potential must meet at least 1 of the following criteria:

- Achieved postmenopausal status, defined as follows: cessation of regular menses for at least 12 consecutive months with no alternative pathological or physiological cause; if there is uncertainty as to whether the patient has a postmenopausal status, it will be confirmed with a serum follicle stimulating hormone (FSH) level confirming the postmenopausal state;
- Have undergone a documented hysterectomy and/or bilateral oophorectomy;
- Have medically confirmed ovarian failure.

13. Patients of childbearing potential, including women with tubal ligations, must commit to using 2 highly effective forms of birth control (defined as the use of an intrauterine device, a barrier method with spermicide, condoms, or any form of hormonal contraceptives) for the duration of the study.
14. Male patients must be sterile (biologically or surgically), or their partner(s) of childbearing potential must commit to the use of highly effective method of birth control for the duration of the study.
15. Patients must be fully vaccinated against COVID-19 prior to lymphodepletion. This criterion includes a booster shot if at least 5 months have passed after receiving the second shot of a 2-dose series or 2 months after a single-dose vaccine. Exceptions must be approved by the Alaunos Medical Monitor.

Exclusion Criteria:

1. Patients who have received any type of organ transplant in the past 12 months.
2. Patients who have undergone xenotransplantation at any time.
3. Patients with known active CNS metastases; Patients with prior CNS metastatic disease that has been effectively treated and have recovered from the acute effects of radiation therapy or surgery prior to study entry, have discontinued supra physiologic doses of corticosteroid treatment for these metastases (unless required for other than the treatment of brain metastases) for at least 2 weeks and are neurologically stable for 3 months (requires magnetic resonance imaging [MRI] confirmation) may be enrolled, stability for less than 3 months may be allowed after discussion with the Alaunos Medical Monitor. Any question pertaining to this criterion should be discussed with the Alaunos Medical Monitor.
4. Positive beta-HCG in female of child-bearing potential defined as not post-menopausal for 12 months or no previous surgical sterilization or lactating females.
5. Concurrent systemic steroid therapy at a dose of $\geq$10 mg prednisone daily or equivalent is excluded. Physiologic dosing of steroids to treat adrenal insufficiency is permitted. Topical or inhaled steroids are permitted.
6. Any form of primary immunodeficiency (such as severe combined immunodeficiency disease) or uncontrolled autoimmune hemolytic anemia or idiopathic thrombocytopenic purpura, such as those with a declining hemoglobin level or platelet count secondary to autoimmune destruction within the 4 weeks prior to first dose of study drug, or the need for daily prednisone ≥10 mg daily (or corticosteroid equivalent) to control the autoimmune disease (Note: the experimental treatment being evaluated in this protocol depends on an

intact immune system. Patients who have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities.)

7. History of severe immediate hypersensitivity reaction to cyclophosphamide, fludarabine, [redacted] bendamustine (for subjects if required for lymphodepletion), or dimethyl sulfoxide (DMSO).
8. Severe chronic respiratory condition as determined by the following:

   Symptoms of severe respiratory dysfunction or pleural effusion other than a malignant pleural effusion treatable by drainage with or without pleurodesis.

9. History of a bleeding disorder or unexplained major bleeding diathesis. Note: Subjects receiving anticoagulation must have been on therapy for at least 4 weeks from the most recent diagnosis of VTE (e.g. DVT, PE) at time of lymphodepletion or TCR-T cell infusion.

   Note: Patients must be deemed clinically stable to have anticoagulant therapy held for the institutional standard (determined by individual patient characteristics) prior to and after apheresis.

   Note: Subjects that are receiving maintenance anticoagulation therapy that are unable to be transitioned to a low molecular weight heparin (LMWHs) therapy (e.g., dalteparin, enoxaparin, etc.) prior to lymphodepletion and remain on LMWH through 30 days post-TCR-T infusion, will be excluded.

10. [redacted]
11. Any major bronchial occlusion or pulmonary bleeding not amenable to palliation. More than two weeks must have elapsed since any prior palliation for major bronchial occlusion or bleeding and enrollment.
12. Patients with psychiatric illness/social situations at the time of treatment that would limit compliance with study requirements.
13. Participants with known active, uncontrolled bacterial, fungal, or viral infection, including hepatitis B virus (HBV), hepatitis C virus (HCV), known human immunodeficiency virus (HIV), Herpesvirus (HHV), cytomegalovirus (CMV) or acquired immunodeficiency syndrome (AIDS) related illness. In equivocal cases, subjects whose viral load is negative, may be eligible. HIV seropositive subjects who are healthy and low risk for AIDS related outcomes could be considered eligible. Eligibility criteria for HIV positive subjects should be evaluated and discussed with Alaunos Medical Monitor and will be based on current and past CD4 and T cell counts, history (if any) of AIDS defining conditions (e.g., opportunistic infections), and status of HIV treatment. Also, the potential for drug-drug interactions will be taken into consideration.
14. Patients with a prior history or concurrent malignancy whose natural history or treatment does not have the potential to interfere with either the safety or efficacy assessment of the investigational regimen may be included after discussion with Sponsor, Alaunos Medical Monitor or designee.
15. Active unstable or clinically significant medical condition that would, in the opinion of the Principal Investigator (PI) in consultation as warranted with medical monitor, result in risks to safety of a subject and/or their compliance with the protocol. Examples include, but are not

limited to, active interstitial lung disease (ILD)/pneumonitis or a history of ILD/pneumonitis requiring chronic treatment with systemic steroids, uncontrolled asthma, or colitis.

16. History of any one of the following cardiovascular conditions within the past 6 months: Class III or IV heart failure as defined by the NYHA (Refer to Appendix A), cardiac angioplasty or stenting, myocardial infarction, unstable angina, or other clinically significant cardiac disease.

Eligibility Criteria for Apheresis:

These criteria should be confirmed prior to the subject undergoing apheresis (see SOA for collection windows).

1. Adequate bone marrow reserves as assessed by the following hematology laboratory criteria. This requirement may be satisfied by the initial screening in Section 6.1.1 if apheresis is scheduled within 7 days of the initial screening:
   a. ANC $\geq$ 1,000/mm^3 (filgrastim not within 7 days or pegfilgrastim not within 14 days)
   b. WBC $\geq$ 3,000/mm^3
   c. Platelet count $\geq$ 50,000/mm^3
   d. Hemoglobin > 8.0 g/dL. (Subjects may be administered red blood cell transfusions to achieve this cut-off value.)
2. Adequate major organ system functions as determined by the following criteria. This requirement may be satisfied by the initial screening in section 6.1.1 if apheresis is scheduled within 28 days of the initial screening:
   a. Cardiac
      i. Left ventricular ejection fraction $\geq$ 50% on multiple gated acquisition (MUGA) scan and/or by echocardiography at the discretion of the investigator.

         Note: No evidence of clinically significant pericardial effusion as determined by an ECHO.
   b. Pulmonary
      i. Adequate pulmonary function, defined as grade $\leq$ 2 dyspnea and SaO2 $\geq$ 90% on room air.
   c. Renal
      i. Serum creatinine within institutional limits; OR
      ii. Calculated creatinine clearance (ClCr) (e.g., Cockcroft-Gault equation) $\geq$ 45.0 mL/min and not dialysis dependent
   d. Liver
      i. Serum ALT/AST $\leq$ 3 x ULN or $\leq$ 5 x ULN if documented liver metastases
      ii. Total bilirubin $\leq$ 2 mg/dL, except in patients with Gilbert's Syndrome, who must have a total bilirubin < 3.0 mg/dL
3. A washout period must have elapsed since completion of any prior systemic therapy, with guidelines as follows (windows other than what is listed below should be allowed only after consultation with the Medical Monitor); subjects' non-hematologic toxicities from any prior

systemic therapy must have recovered to ≤ Grade 1 (with the exception of neuropathy and alopecia) or baseline prior to starting apheresis.

a. Nitrosoureas: 6 weeks

b. Other cytotoxic agents: 4 weeks

c. Anti-angiogenic agents: 4 weeks

d. Targeted agents, including small molecule tyrosine kinase inhibitors: 5 half-lives or 2 weeks, whichever is shorter

e. Immune checkpoint inhibitor agents: 4 weeks and must have recovered to ≤ Grade 1 or baseline grade severity prior to checkpoint inhibitor administration from immune-related adverse events (IrAEs), other than endocrine side effects or those affecting nonessential organs or functions

f. CAR-T, other T-cell product or investigational cancer vaccine: 6 weeks

g. Systemic steroids of greater than 10 mg prednisone equivalence: 1 week

4. Subjects must not be pregnant or breastfeeding.

Eligibility Criteria for Lymphodepletion:

The inclusion criteria below should be confirmed before entering the lymphodepletion phase of the study.

1. Patients who have previously received at least one line of standard systemic therapy for their advanced/metastatic cancer and have either progressed, recurred, suboptimal response or were intolerant to the previous treatment. Specifically:

    - Subgroup 1. Gynecologic cancers (i.e., ovarian or endometrial):
        a. Ovarian cancer: Subjects who are platinum-resistant, as defined by progression on a platinum-containing regimen or within 6 months of a previous platinum dose.
        b. Endometrial cancer: Subjects must have received at least 2 prior lines of therapy for advanced/recurrent disease (including adjuvant chemotherapy and adjuvant chemo-radiation therapy) prior to lymphodepletion. Prior hormonal therapy will not count towards prior lines of therapy.

    - Subgroup 2. Colorectal cancer:
        - Subjects must have received at least 2 prior lines of systemic treatment for advanced unresectable or metastatic disease prior to lymphodepletion, which must include an irinotecan or oxaliplatin-based therapy and if eligible, a targeted antibody therapy. Subjects with deficient DNA mismatch repair (dMMR) or microsatellite instability-high (MSI-H) colorectal cancer should have received a checkpoint inhibitor such as pembrolizumab or nivolumab.

    - Subgroup 3. Pancreatic cancer: Subjects who have progressive disease after receiving initial treatment with FOLFOX, (modified) FOLFIRINOX, and/or a gemcitabine-based therapy.

- Subgroup 4. Non-small cell lung cancer (NSCLC):
    - Subjects with recurrent and/or metastatic disease who have disease progression or intolerance to treatment with a PD-1/PD-L1 inhibitor either as a single-agent or in combination with other checkpoint inhibitors (e.g., CTLA-4 inhibitors) and/or platinum-containing chemotherapy.
    - Subjects with targetable oncogene alterations (e.g., EGFR, ALK, ROS1, RET, MET, NTRK1-3, BRAF) must have had disease progression or intolerance on at least one prior line of targeted therapy.
- Subgroup 5. Cholangiocarcinoma: Subjects must have histologically confirmed diagnosis of cholangiocarcinoma Stage II, III, or IV (intra-hepatic, extra-hepatic and perihilar) that is not eligible for curative resection, transplantation, or ablative therapies. Subjects who have progressed after receiving at least one line of standard therapy.

2. No active infection requiring systemic therapy or causing fever (temperature > 38.1˚C (100.6 ˚F)) or subjects with unexplained fever (temperature > 38.1˚C (100.6 ˚F)) within 7 days prior to the day of investigational product administration. Participants with known active, uncontrolled bacterial, fungal, or viral infection, are excluded. In equivocal cases, subjects whose viral load is negative, may be eligible after discussion with the Alaunos Medical Monitor.
3. Any contraindication to fludarabine or cyclophosphamide should be discussed with the Medical Monitor prior to lymphodepletion.
4. Patients who have received any type of organ transplant in the past 12 months are excluded.
5. Patients who have undergone xenotransplantation at any time are excluded.
6. Absence of active autoimmune disease requiring ongoing systemic immunosuppressive therapy. Patients must not have any form of primary immunodeficiency (such as severe combined immunodeficiency disease) or uncontrolled autoimmune hemolytic anemia or idiopathic thrombocytopenic purpura, such as those patients with a declining hemoglobin level or platelet count secondary to autoimmune destruction within the 4 weeks prior to first dose of study drug, or the need for daily prednisone ≥10 mg daily (or corticosteroid equivalent) to control the autoimmune disease (Note: the experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities.)
7. Negative serum pregnancy test within 7 days before lymphodepletion chemotherapy for women of childbearing potential, defined as those who have not been surgically sterilized or who have not been free of menses for at least 1 year. Patients also must not be breastfeeding.
8. Clinical Performance Status of ECOG 0 or 1.
9. Patients must have measurable disease per RECIST 1.1 as assessed by the local site investigator/radiology.

10. No treatment with any investigational agent on a different clinical trial between meeting the screening eligibility criteria and enrollment (date eligibility is confirmed for lymphodepleting chemotherapy).
11. Serum creatinine within institutional limits; OR Calculated creatinine clearance (ClCr) (e.g., Cockcroft-Gault equation) ≥ 45.0 mL/min and not dialysis dependent
12. Liver
    i) Serum ALT/AST ≤ 3 x ULN or ≤ 5 x ULN if documented liver metastases
    ii) Total bilirubin ≤ 2 mg/dL, except in patients with Gilbert's Syndrome, who must have a total bilirubin < 3.0 mg/dL
13. ANC ≥ 1,000/mm^3 (≥1.0×10^9/L) within 1 week of lymphodepletion.
14. Adequate pulmonary function, defined as grade ≤ 2 dyspnea and SaO2 ≥ 90% on room air.
15. Adequate cardiac function, defined as left ventricular ejection fraction (LVEF) of ≥50% as assessed by echocardiogram or MUGA scan, or LVEF of 45-49% and clearance by a cardiologist; if patient receives cardiotoxic chemotherapy after enrollment, repeat echocardiogram or MUGA is required to reestablish eligible LVEF.
16. Patients must be fully vaccinated against COVID-19 prior to lymphodepletion. This criterion includes a booster shot if at least 5 months have passed after receiving the second shot of a 2-dose series or 2 months after a single-dose vaccine. Exceptions must be approved by the Alaunos Medical Monitor.
17. Patients may have undergone minor surgical procedures or limited-field radiotherapy provided any major organ toxicities have recovered to ≤ Grade 1.
18. Subjects must not require corticosteroid therapy or dose ≥ 10 mg per day of prednisone or the equivalent. Pulsed corticosteroid dose for disease control is acceptable until the day before the start of lymphodepletion.
19. Must not have received any live vaccines within 30 days prior to enrollment.
20. Patients with known active CNS metastases are excluded; Patients with prior CNS metastatic disease that has been effectively treated and have recovered from the acute effects of radiation therapy or surgery prior to study entry, have discontinued supra physiologic doses of corticosteroid treatment for these metastases (unless required for other than the treatment of brain metastases) for at least 2 weeks and are neurologically stable for 3 months (requires magnetic resonance imaging [MRI] confirmation) may be enrolled, stability for less than 3 months may be allowed after discussion with the Alaunos Medical Monitor. Any question pertaining to this criterion should be discussed with the Alaunos Medical Monitor.
21. Patients must not have concurrent systemic steroid therapy at a dose of >10 mg prednisone daily or equivalent. Physiologic dosing of steroids to treat adrenal insufficiency is permitted. Topical or inhaled steroids are permitted.
22. Patients must not have a history of severe immediate hypersensitivity reaction to cyclophosphamide, fludarabine, [redacted] bendamustine (for subjects if required for lymphodepletion), or dimethyl sulfoxide (DMSO).
23. Patients must not have a severe chronic respiratory condition as determined by the following:

Symptoms of severe respiratory dysfunction or pleural effusion other than a malignant pleural effusion treatable by drainage with or without pleurodesis.

24. Patients must not have a history of a bleeding disorder or unexplained major bleeding diathesis. Note: Subjects receiving anticoagulation must have been on therapy for at least 4 weeks from the most recent diagnosis of VTE (e.g. DVT, PE) at time of lymphodepletion or TCR-T cell infusion.

    Note: Patients must be deemed clinically stable to have anticoagulant therapy held for the institutional standard (determined by individual patient characteristics) prior to and after apheresis.

    Note: Subjects that are receiving maintenance anti-coagulation therapy that are unable to be transitioned to a low molecular weight heparin (LMWHs) therapy (e.g., dalteparin, enoxaparin, etc.) prior to lymphodepletion and remain on LMWH through 30 days post TCR-T infusion, will be excluded.

25. [redacted]

26. Patients must not have any major bronchial occlusion or pulmonary bleeding not amenable to palliation. More than two weeks must have elapsed between any prior palliation for major bronchial occlusion or bleeding and enrollment.

27. Patients with psychiatric illness/social situations at the time of treatment that would limit compliance with study requirements are excluded.

28. Patients with a prior history or concurrent malignancy whose natural history or treatment does not have the potential to interfere with either the safety or efficacy assessment of the investigational regimen may be included after discussion with Sponsor, Alaunos Medical Monitor, or designee

29. Patients must not have a history of any one of the following cardiovascular conditions within the past 6 months: Class III or IV heart failure as defined by the NYHA (Refer to Appendix A), cardiac angioplasty or stenting, myocardial infarction, unstable angina, or other clinically significant cardiac disease.

30. Patients must not have any active unstable or clinically significant medical condition that would, in the opinion of the Principal Investigator (PI) in consultation as warranted with medical monitor, result in risks to safety of a subject and/or their compliance with the protocol. Examples include, but are not limited to, active interstitial lung disease (ILD)/pneumonitis or a history of ILD/pneumonitis requiring chronic treatment with systemic steroids, uncontrolled asthma, or colitis.

Eligibility Criteria for TCR-T Cell Drug Product Infusion:

Prior to administration of TCR-T cell drug product, subjects will have to meet the following criteria (within approximately 24 hours):

1. CRP and ferritin results within normal institutional limits. Abnormal results to be discussed with medical monitor prior to TCR-T cell administration.
2. Clinical Performance Status of ECOG 0 or 1.
3. No active infection requiring systemic therapy or causing fever (temperature > 38.1˚C (100.6 ˚F)) or subjects with unexplained fever (temperature > 38.1˚C (100.6 ˚F)) within 7 days prior to the day of investigational product administration.

4. Neurotoxicity, if present following fludarabine administration, must be resolved to grade ≤ 1. If >grade 1, approval from medical monitor is required.
5. Serum creatinine within institutional limits; OR Calculated creatinine clearance (ClCr) (e.g., Cockcroft-Gault equation) ≥ 45.0 mL/min and not dialysis dependent
6. Adequate pulmonary function, defined as grade ≤2 dyspnea and SaO2 ≥ 90% on room air.
7. ANC < 1,000/mm^3 (< 1.0×10^9/L) within 72 hours prior to dosing of TCR-T cell administration. Subjects with ANC ≥1,000/mm^3 (< 1.0×10^9/L) must be discussed with the medical monitor prior to TCR-T cell drug product administration.
8. Must not have received any live vaccines within 30 days prior to enrollment.

Duration of Study:

The start of the study is defined as the date when the first subject is consented into the Investigational Product study and the study stop date is the date of the last subject's last visit.

The duration of this study from the time of initiating screening until the completion of survival follow-up is anticipated to be approximately 25 months. After subjects complete the follow-up period or discontinue from this study, they will be followed in the long-term follow-up protocol (TCR001-202) for up to 15 years post-TCR-T cell drug product infusion.

Study Treatment, Dosage and Mode of Administration:

Lymphodepletion Chemotherapy:

The standard lymphodepleting regimen for most subjects is as follows:

Cyclophosphamide: 60 mg/kg x 2 days IV in 250 mL D5W infused simultaneously with mesna according to institutional procedure.

Fludarabine: 25 mg/m^2/day IVPB daily over 30 minutes for 5 days. Fludarabine will be started approximately 1-2 hours after the cyclophosphamide and mesna on days -7 and -6. Note: if ALC is <100, remaining doses of fludarabine may be stopped at the discretion of the investigator.

At the discretion of the investigator, heavily pre-treated or irradiated subjects who are expected not to have as much bone marrow reserve may be treated with a lower dose lymphodepleting regimen:

Cyclophosphamide: 30 mg/kg x 2 days IV in 250 mL D5W infused simultaneously with mesna according to institutional procedure.

Fludarabine: 25 mg/m^2/day IVPB daily over 30 minutes for 5 days. Fludarabine will be started approximately 1-2 hours after the cyclophosphamide and mesna on days -7 and -6. Note: if ALC is <100, remaining doses of fludarabine may be stopped at the discretion of the investigator.

In the event the patient had previous intolerance to cyclophosphamide or due to supply issues, bendamustine 90 mg/m^2/day for 2 days or bendamustine 70 mg/m^2/day + fludarabine 30 mg/m^2/day for 3 days may be substituted with approval from the medical monitor.

Subjects who have sufficient lymphodepletion from bridging therapy do not need additional lymphodepletion as described in Section 8.2 prior to infusion of TCR-T cell drug product.

TCR-T cell drug product:

Neoantigen-specific TCR-T cells: The Phase I study will examine up to 4 dose levels (Table 2). Dosing will begin at Dose Level 1. Dose-limiting toxicity (DLT) monitoring will occur through 28 days after TCR-T cell infusion. The proposed doses to be evaluated in the planned Phase I/II clinical study will be:

Table 2. TCR-T Dose Levels (Intended Dose and Dose Range)

Dose Level	TCR^+ Cells	Minimum	Maximum
DL-1	$<1.0x10^9$	NA	$<1.0x10^9$
DL1	$5\ x10^9$	$1.0\ x10^9$	$<10x10^9$
DL2	$40\ x10^9$	$10x10^9$	$<70x10^9$
DL3	$100x10^9$	$70x10^9$	$150x10^9$

Depending on cell concentration in the infusion bag(s) and the intended dose, subjects will receive an infusion of cells from one or more bags via non-filtered tubing. A maximum volume of 400 mL will be intravenously infused over a duration that may last up to 180 minutes or as clinically determined by an investigator for patient safety; bags will be gently agitated during infusion to prevent cell clumping. Dose splitting may be considered in certain situations (e.g. subject experiences cytokine release syndrome [CRS] or infusion-related reaction [IRR]) following consultation with the Alaunos Medical Monitor.

Patients obtaining any response except progressive disease are potentially eligible for a repeat treatment consisting of lymphodepleting chemotherapy followed by an infusion of TCR-T cell drug product. Retreatment will be at least 2 months after the original treatment. The dose of TCR-T cells administered during repeat treatments will be at the dose level currently enrolling patients receiving initial TCR-T cell drug product infusions. The repeat treatments will include the same apheresis collection, if needed, lymphodepleting chemotherapy, and manufacturing schedule as the initial treatment, if residual TCR-T cell drug product is not available. If patients were initially matched to multiple TCRs a discussion between the investigator and Sponsor should occur to determine which TCR will be utilized for re-treatment.

Patients must not have experienced a DLT with their first treatment and must meet the same eligibility requirements listed in Section 6.1. The patients must undergo screening evaluation as listed in Table 12 except infectious disease serology, and brain MRI are not required to be repeated unless clinically indicated. Follow-up testing for retreatment will be the same as for the first treatment. In the case where the planned infusion is with the same library TCR as the first infusion, patients must have molecular testing to confirm matching epitope (i.e., genomic testing and HLA typing of tumor tissue collected after initial TCR-T treatment).

The patient maybe re-enrolled on the core/treatment study as a new patient to allow this re-treatment and these patients will be considered in the total sample size for this study. A maximum of 2 total treatments can be administered to any one patient, and at least 2 months must elapse between the first treatment and the second treatment.

DLTs that occur in the cohort of patients receiving a repeat treatment will not affect the dose escalation of patients receiving an initial treatment. However, excessive DLTs among retreatment subjects will result in a discontinuation of this practice. Specifically, if 2 of the first 3 patients re-treated, 3 or more of the first 6 re-treated patients, 4 or more of the first 9 retreated

patients, or greater than 1/3 of the total patients receiving a repeat treatment experience a DLT during repeat treatment, then repeat treatments will be discontinued altogether.

The proposed dose levels are based on previous experience at the NCI (National Cancer Institute) demonstrating that doses of TCR-T cell drug product below $1x10^9$ cells are largely ineffective and up to $1.5x10^{11}$ cells can be given safely and result in clinical regressions of metastatic disease (Lu et al., 2017). Doses up to $1.5x10^{11}$ cells are also administered in an ongoing NCI study with autologous PBL transduced with retroviral constructs encoding alpha/beta chains of tumor specific TCRs (ClinicalTrials.gov Identifier: NCT04102436).

[redacted]

Criteria for evaluation:

Safety:

The SRC will conduct continuous reviews of data for safety and/or efficacy:

- SRC will conduct a comprehensive review of all reported adverse events (for screening, treatment and follow up periods) at least monthly (modified by enrollment) in subjects treated on the trial and will determine if enrollment should continue. If an event meets a criterion for pausing the study, the SRC will convene to evaluate the safety event(s) and to make a recommendation and decision on the enrollment of additional subjects at the same dose level.

Due to potential institutional conflict of interest, the protocol will be monitored by an external DSMB that will review safety and efficacy annually from the date of Western Institutional Review Board (WIRB) approval.

Safety will be evaluated based on frequency and severity of adverse events (AEs), serious adverse events (SAEs), laboratory abnormalities, electrocardiograms (ECGs), vital signs and physical/neurologic examination findings by study period and dose level. The severity of AEs will be graded using National Cancer Institute (NCI) CTCAE v5.0. Reporting from the time of informed consent until lymphodepletion will only include AEs and SAEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw). AEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw) will be recorded as adverse events in the CRF as outlined in Section 13.6. SAEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw) will be recorded in the CRF as outlined in Section 13.6 and reported to Sponsor as defined in Section 13.8. AEs and SAEs considered not protocol-related should be considered medical history.

The reporting period of all adverse events and serious adverse events will begin from the start of lymphodepletion through end of study regardless of relationship to study treatment. Refer to Section 13 for more details.

Stopping and Pausing Rules:

The study will be paused and further investigation initiated if:

- Occurrence of Grade 4 DLTs in 2 subjects at any time during the conduct of the study trial;
- One treatment related death occurs after the administration of TCR-T cell drug product;
- Death not related to disease progression;
- Development of a secondary malignancy, Epstein-Barr virus (EBV) lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject. Note: accrual of EBV positive subjects will be halted if this is observed.

There are two dose escalation stopping rules in this BOIN study:

- Stop the trial if the lowest dose is eliminated due to overt toxicities*;
- Stop the dose escalation and estimate the MTD/RP2D if the number of new subjects treated at the current dose level reaches 9.

* Overt Toxicities defined as:

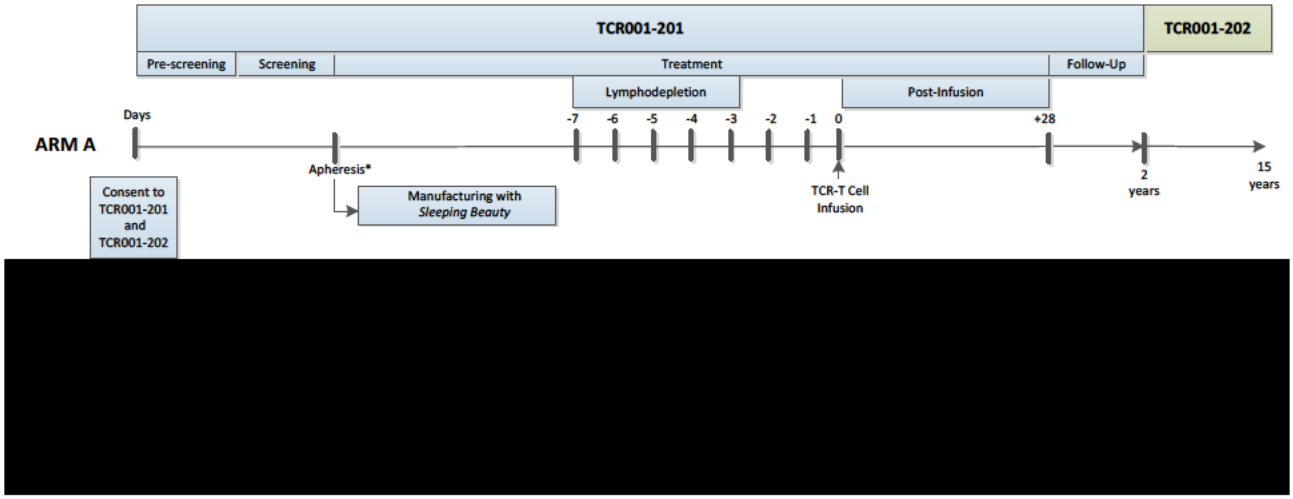
1. The occurrence of 2 or more dose limiting toxicities.

2. Consideration will also be given to longer term toxicities (e.g. > 30 days post infusion: Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS), cardiotoxicity, pulmonary toxicity, metabolic complications, secondary macrophage-activation syndrome, prolonged cytopenia unresponsive to colony stimulating factors, grade 3 and 4 infections and subsequent malignancies)

If any of the above events occur, enrollment of new subjects will be paused, pending review by the SRC. The SRC will recommend if changes to the enrollment of additional subjects are required, including but not limited to amending the protocol prior to enrollment of additional subjects, or discontinuing enrollment in the study.

In the event that any stopping rule is met, the FDA and Institutional Review Board will be informed in a timely manner. Alaunos will discuss the investigation and the data with the FDA to identify next steps, which may include stopping the study.

Figure 1. Treatment Arms for TCR-T Cell Drug Product



NOTE: TCR001-201 is the Phase I/II study discussed in this protocol. [redacted] TCR001-202 is the long-term follow-up study. Subjects will transition to TCR001-202 following completion of the 2-year follow-up period of TCR001-201 or upon disease progression (whichever comes first).

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2. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 3. Abbreviations and Specialist Terms

Abbreviation or Specialist Term	Explanation
AE	Adverse event
APC	Antigen-presenting cells
AESI	Adverse event of special interest
AUC_{D0-D28}	Area under the concentration over time curve from Day 0 to Day 28
CBC	Complete Blood Count
Cmax	Maximum concentration observed
CoA	Certificate of Analysis
CR	Complete Response
CRC	Colorectal Cancer
CRS	Cytokine Release Syndrome
CTCAE	Common Terminology Criteria for Adverse Events
DL	Dose Level
DLT	Dose-Limiting Toxicity
dMMR	Deficient in DNA mismatch repair
DP	Drug Product
DSMB	Data Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EDC	Electronic data capture
FAS	Full Analysis Set
GCP	Good Clinical Practice
GM-CSF	Granulocyte macrophage colony stimulating factor
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human Leukocyte Antigen
HLH	Hemophagocytic Lymphohistiocytosis
ICANS	Immune Effector Cell-Associate Neurotoxicity Syndrome

Abbreviation or Specialist Term	Explanation
ICE	Immune Effector Cell-Associated Encephalopathy
ICF	Informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IFN-γ	Interferon gamma
[redacted]	[redacted]
IL-6	Interleukin-6
IrAE	Immune-related Adverse Event
IRB	Institutional Review Board
IRF	Independent Review Facility
IRR	Infusion Related Reaction
ISR	Injection Site Reaction
LMWH	Low molecular weight heparin
LOQ	Limit of Quantification
LVEF	Left Ventricular Ejection Fraction
KM	Kaplan-Meier
MAS	Macrophage Activation Syndrome
MSI-H	Microsatellite-instability high
MTD	Maximum Tolerated Dose
MUGA	Multiple-gated Acquisition Scan
NCI	National Cancer Institute
NSCLC	Non-small Cell Lung Cancer
OAE	Other Significant Adverse Event
ORR	Objective Response Rate
OS	Overall Survival
PBL	Peripheral Blood Lymphocyte
[redacted]	[redacted]
PCR	Polymerase Chain Reaction
PD	Progressive Disease
PFS	Progression-Free Survival

Abbreviation or Specialist Term	**Explanation**
PI	Principal Investigator The investigator who leads the study conduct at an individual study center. Every study center has a principal investigator.
PPP	Per Protocol Population
PR	Partial Response
REP	Rapid Expansion Protocol
RP2D	Recommended Phase II Dose
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Stable Disease
SOP	Standard Operating Procedure
SRC	Safety Review Committee
TBV	Total Blood Volume
TCR	T-Cell Receptor
TCR-T	T-cell receptor engineered T cell
TIL	Tumor Infiltrating Lymphocyte
TLS	Tumor Lysis Syndrome
Tmax	Time to Cmax
[redacted]	[redacted]
WBC	White Blood Cell

3. INTRODUCTION

3.1. Background and Rationale

Adoptive T-cell therapy has emerged as a promising approach to treat cancer (Gattinoni, Powell, Rosenberg, & Restifo, 2006; Rohaan, Wilgenhof, & Haanen, 2019; Rosenberg & Restifo, 2015). Tumor-derived neoantigens (cancer-specific non-synonymous mutations) appear to be an ideal immunotherapy target because they are distinguished from germline and could be recognized as non-self by the host immune system. Neoantigens can be specifically recognized by neoantigen-specific T-cell receptors (TCRs) in the context of HLA. Recognition of the neoantigen is driven by T cells interrogating the peptide-human leukocyte antigen (HLA) complex on the cell surface. When T cells identify a foreign peptide bonded to the HLA of the target cell through their TCR, the T cell will kill the cancer cell expressing the neoantigen. Most neoantigens are unique to each patient's tumor-requiring unique sets of TCRs to recognize these private mutations which are discovered in "real time" on a patient-by-patient basis (Rosenberg & Restifo, 2015). These TCRs are autologous as they are derived from and for the patient. Some neoantigens recur in hotspots (*e.g.*, TP53, RAS, EGFR) and are shared between tumors and between patients, making possible the *a priori* identification of TCRs. These TCRs maybe allogeneic derived from 3rd party unrelated donors and belonging to a "library" pre-identified for specific mutations, which are likely shared neoantigens (e.g., occurring in "hotspots"), expressed by the autologous tumor. High resolution typing for human leukocyte antigen (HLA) class I and II is required to ensure that the transposed TCR-T cell product is specifically designed for recognition of and binding to the cognate neoantigens presented by tumor cells in the context of appropriate HLA.

Alaunos Therapeutics Inc. (Alaunos) plans to develop autologous, [redacted] T-cells genetically modified utilizing the *Sleeping Beauty* system, [redacted] n subjects with solid tumors. *Sleeping Beauty* transposition was originally developed from fish undergoing their evolutionary maturation and has been adapted for genetic transfer into human cells (Ivics, Hackett, Plasterk, & Izsvak, 1997). Co-transfer of two *Sleeping Beauty* DNA plasmids leads to stable transgene expression originating from the transposon (Harjeet Singh et al., 2007). The *Sleeping Beauty* transposase plasmid transiently expresses SB11 transposase enzyme that digests the second plasmid, the *Sleeping Beauty* transposon, at inverted/direct repeats and ligates the transposon cassette containing the gene of interest, *i.e.,* TCR, preferably into TA dinucleotide repeats within the genome. Neoantigen-reactive TCRs are chimeras of human variable chains and mouse constant chains and are therefore termed mTCR. The rationale for the use of mouse constant chains is to minimize the risk for mispairing between the introduced and endogenous TCRs. In previous studies at the NCI Surgery Branch, mouse/human chimeric TCRs (ClinicalTrials.gov Identifier: NCT02111850, NCT00393029, NCT01273181) and fully murine TCRs (ClinicalTrials.gov Identifier: NCT01967823) were infused and a retrospective study revealed that only 23% of patients had a humoral antibody response to the murine TCR (Davis et al., 2010). Analysis of the putative murine TCR epitopes targeted by the human antibodies demonstrated reactivity to the variable regions and not the constant regions. Antibodies were detected against the introduced TCR ranging from 3-6 months after transfer, suggesting that there was no impairment of acute anti-tumor effects by the infused T-cells. Anti-mouse TCR antibodies were detected in both responding and nonresponding patients indicating that these

endogenous humoral responses were not limiting in the ability to achieve meaningful objective clinical response. Based on these findings, the impact of neutralizing humoral response to the murine constant chains of transposed TCRs poses a negligible risk to the patients.

High frequencies of $mTCR^+$ cells can be generated through enrichment by anti-mouse TCRβ constant chain antibody (clone H57) and expansion with pan-T cell (CD3-specific) activating antibody OKT3 in a rapid expansion protocol (D.C. Deniger et al., 2016). *Sleeping Beauty* DNA plasmids have been approved for use in clinical trials evaluating the ability of T-cells modified with chimeric antigen receptors to treat B-cell malignancies (ClinicalTrials.gov Identifier: NCT00968760, NCT01497184, NCT01362452, NCT01653717, NCT02194374, NCT02529813) (Field et al., 2013; Hackett, Largaespada, & Cooper, 2010; Jena, Dotti, & Cooper, 2010; Kebriaei et al., 2012; Peng et al., 2009; H. Singh, Huls, Kebriaei, & Cooper, 2014). Recently, the *Sleeping Beauty* system was adapted for expression of neoantigen-specific TCRs in patient peripheral, blood-derived T cells (D.C. Deniger et al., 2016). A Phase II clinical trial sponsored by National Cancer Institute (NCI) is underway, evaluating the adopted transfer of T-cells genetically modified with neoantigen-reactive, mTCRs using the *Sleeping Beauty* transposon/transposase system (ClinicalTrials.gov Identifier: NCT04102436).

Development of this TCR-T cell therapy requires identification of the TCRs from the neoantigen-reactive T-cell culture and transfer of one or more of the TCRs into a younger repertoire of T cells from the peripheral circulation such that a high expression of the neoantigen-reactive TCRs occurs at clinically meaningful cell doses. Thus, it is important to evaluate the ability of T cells to target neoantigens expressed by the autologous tumor. The process that led to the identification of these TCRs is extensively described in the literature (Cafri et al., 2019; D. C. Deniger et al., 2018; Malekzadeh et al., 2019; Tran, Robbins, & Rosenberg, 2017; Wang et al., 2019). Briefly, next-generation sequencing is performed; the mutations expressed by the tumor cells are identified using bioinformatics tools. Next, reagents that mimic the mutated amino-acid sequence (tandem minigenes) as well as the mutated protein (long peptides) can be synthesized and introduced into antigen-presenting cells (dendritic cells) for presentation in the context of the patient's own HLA molecules. T cells derived from tumors (tumor infiltrating lymphocytes) or from the blood are co-cultured with the dendritic cells expressing the mutated neoantigens. T-cell reactivity is evaluated by cytokine release assays and expression of the T-cell activation molecules tested by flow cytometry. Once the reactive T cells are sorted, the TCR sequence is evaluated by PCR and reconstructed *in silico*. Then the mutated TCR sequence is synthesized and can be cloned into the *Sleeping Beauty* vector for the generation of T cells expressing the specific mutated TCR and can be expanded exponentially as modified from (D.C. Deniger et al., 2016).

3.2. Benefit/Risk Assessment

3.2.1. Toxicity of Adoptive Cell Therapies

Cytokine-release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) can be fatal if not recognized early. Therefore, patients who undergo adoptive cell therapy need to be managed by specialized teams including physicians with expertise in these toxicities, intensive care specialists and neurologists. Management of patients becomes more complicated when the therapy is administered in the outpatient setting. In those cases, patients

should be hospitalized as soon as they develop a symptom or sign of toxicity, and caregivers must be taught to recognize symptoms of ICANS.

The first clinical manifestation of adoptive cell toxicity is CRS. It usually starts with fever that can exceed 40°C, and includes other symptoms such as malaise, headache, myalgias, and tachycardia. Possible manifestations include organ dysfunctions, cytopenias, and coagulopathy. In severe cases, patients can develop life-threatening capillary leakage with hypoxia and hypotension. Rarely, hemophagocytic lymphohistiocytosis can arise. CRS usually occurs in the first week after adoptive cell infusion, although delayed CRS is possible. Time to resolution is generally 7 to 8 days, but some patients may need more than 30 days to recover.

The standard of care for CRS is Actemra® (tocilizumab), an anti-IL-6 receptor antagonist. If the patient does not respond to Actemra® (tocilizumab), corticosteroids can be effective in reversing CRS. Some data suggest that the anti-IL-6 monoclonal antibody siltuximab or the IL-1 receptor antagonist anakinra may have clinical efficacy. In CRS, patients can require vasopressors to correct hypotension and oxygen supply or intubation for hypoxia.

The severity of CRS has been correlated with the peak of *in vivo* adoptive cell proliferation and disease burden. A faster T cell expansion can be promoted by higher cell dose, heavily pretreated bone marrow disease, and also by some kinds of pre-conditioning, such as fludarabine-containing regimens. The risk of severe CRS is also increased in patients with comorbidities and in those who develop the syndrome within 3 days of infusion (Lee et al., 2019).

ICANS may occur as an adoptive cell-related encephalopathy syndrome. The clinical manifestations of ICANS are very wide ranging, as toxicity does not affect a specific region of the central nervous system. They include encephalopathy (confusion or delirium), expressive aphasia or language disturbance, motor weakness, myoclonus or tremor, headache, seizures, and a depressed level of consciousness. In rare cases patients can rapidly develop diffuse cerebral oedema. Expressive aphasia seems to be a typical symptom. ICANS onset can range from a few hours to three to four weeks after adoptive cell infusion. It can occur almost simultaneously with CRS or even after CRS has resolved. ICANS is usually self-limiting, and most symptoms reverse in three to four weeks, with persistent abnormalities being uncommon. For ICANS, the American Society for Transplantation and Cellular Therapy (ASTCT) consensus grading is based on 5 elements: the 10-point immune effector cell-associated encephalopathy (ICE) score, depressed level of consciousness, seizure, motor findings, and elevated intracranial pressure/cerebral edema (Appendix E). Severity can range from grade 1 to grade 4, with the grade determined by the most severe event. The ICE score is a tool that measures alterations in speech, orientation, handwriting, and concentration (Appendix C). Severe ICANS develops almost only in patients who have experienced CRS, with severity being influenced by disease type, disease burden, patient's age, and treatment history. For grading and management, refer to Appendix B.

3.2.2. Risk Assessment

Potential risks associated with the study intervention and study procedures and measures to control the risks are summarized in Table 4 and Table 5. Detailed information about the potential risks and reasonably expected AEs of adoptive cell therapies in general, and potentially of

TCR-T cell drug product in particular, are found in the Investigator's Brochure. Mitigation strategies are discussed in the concomitant medication section and the appendices.

The Sponsor will immediately notify the Principal Investigator if any additional safety or toxicology information becomes available during the study. This study will be performed in compliance with the protocol, International Conference on Harmonisation (ICH), Good Clinical Practice (GCP) and applicable regulatory requirements. Aspects of the study concerned with the investigational product(s) will meet the requirements of US FDA – Good Manufacturing Practice (US GMP).

Table 4. Risk Assessment for TCR-T Cell Drug Product

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study Intervention: TCR-T CELL DRUG PRODUCT		
Cytokine Release Syndrome (CRS)	CRS is commonly observed following immune-based biotherapeutics such as TCR-T cells and CD3-engaging bispecific antibodies. Patients can present with fever, rigor, malaise, headache, nausea/vomiting, or more severe, life-threatening symptoms of hypoxia, pulmonary edema, tachycardia, hypotension, aphasia, confusion, or seizures.	Premedication for CRS prophylaxis with tocilizumab in all subjects will be administered on Day -5 as part of the subject's pre-conditioning regimen (Section 8).
Hemophagocytic lymphohistiocytosis (HLH)/macrophage activation syndrome (MAS)	HLH and MAS may occur in the setting of CRS. Clinical and laboratory features of MAS include fever, increased ferritin levels, pancytopenia, hemophagocytosis in bone marrow or lymph nodes, fibrinolytic coagulopathy, and liver dysfunction.	Subjects may receive treatment including Actemra® (tocilizumab) and corticosteroids given intravenously. Canakinumab may also be used (Section 10.1.10).
Infections and febrile neutropenia	The occurrence of infections and febrile neutropenia is common following infusion with the use of lymphodepletion and adoptive cell therapies.	Subjects will be monitored for signs and symptoms of infection and treated appropriately. Subjects, at the discretion of the Investigator, will be given anti-infective agents and G-CSF as needed. Infection prophylaxis: Subjects, at the discretion of the Investigator, will be given anti-infective agents as needed Section 10.1.1. Pneumocystis: Subjects can receive regimens of doses of sulfamethoxazole + trimethoprim as fixed combination beginning at the start of lymphodepletion Section 10.1.1. Fungal infection: Fluconazole can be initiated and given either orally or as an IV dose (Section 10.1.1).

Table 4. Risk Assessment for TCR-T Cell Drug Product (Continued)

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Neurologic adverse events (AEs) and immune effector cell associated neurologic syndrome (ICANS)	Neurologic AEs occur very commonly following infusion with CAR -T products and ICANS occurs frequently with adoptive cell therapy and like CRS, is associated with rapid TCR-T cell expansion and high tumor burden as well as other factors.	Subjects will receive aggressive treatment including corticosteroids. Actemra® (tocilizumab) may be administered if neurotoxicity is accompanied by CRS (Section 10.1.9 and Appendix C).
Prolonged cytopenia	Grade 3 or higher prolonged cytopenia following lymphodepletion and adoptive cell therapy infusion occur and included thrombocytopenia, neutropenia, and anemia, and should be carefully monitored.	Blood counts should be monitored following TCR-T cell product infusion, as prolonged neutropenia is associated with increased risk of infection.
Tumor lysis syndrome (TLS)	TLS occurs when tumor cells release their contents into the blood stream, either spontaneously or due to treatment, leading to metabolic disturbances including hyperuricemia, hyperkalemia, hypophosphatemia, and hypocalcemia.	Management of cardiac and neuromuscular abnormalities and preservation of kidney function are most important. Allopurinol can be used to reduce the hyperuricemia and rasburicase can be used to preserve or improve renal function Section 9.4.4.
Viral reactivation	Viral infections are very commonly associated with lymphodepletion and adoptive cell therapy infusion.	Herpes or Epstein Barr virus: Subjects can receive valacyclovir orally or acyclovir intravenously if the patient cannot take oral antiviral medications after lymphodepletion Section 10.1.1.
Hypersensitivity reaction (Type 1 and Type 3)	Type 1 hypersensitivity or allergic reactions are theoretically possible in response to an injected protein and may be manifested by shortness of breath, rash, anaphylaxis, or angioedema. Immune complex-mediated Type 3 hypersensitivity reactions are similar to Type 1 reactions but are likely to be delayed and may include, in addition to the aforementioned symptoms, polyarthritis, myalgia, fever, or glomerulonephritis.	Subjects will be treated symptomatically with supportive care, antihistamines, or corticosteroids, and continued monitoring (Section 10.1.6).
Nausea and diarrhea	A general risk with cell therapies.	Prophylactics can be used at the Investigator's discretion with Sponsor approval (Section 10.1.12).
Pain	A general risk with cell therapies.	Anti-inflammatory and opioid drugs can be administered as needed during the study as long as there is no known or expected drug-drug interaction (Section 10.1.13).

Table 4. Risk Assessment for TCR-T Cell Drug Product (Continued)

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Anemia Thrombocytopenia	A general risk with cell therapies.	Primary transfusion support may be permitted as indicated by the current American Society of Clinical Oncology (ASCO) and American Association of Blood Banks (AABB) guidelines (Section 10.1.15).
Unknown risks to an embryo, fetus or nursing infant	There are no studies with TCR-T Cell drug product in pregnant or lactating women.	Women who are pregnant or breastfeeding are not eligible to participate in this study. Women of childbearing potential and men must use highly effective contraception methods as specified in Section 11.3.15.1.
Infusion-related reactions (IRR)	Infusion reactions may occur with TCR-T Cell drug product. IRR is typically characterized by fever, chills (rigor), and less commonly, hypotension.	If such symptoms are observed in the study, prophylactic medication will be administered to reduce the symptoms for in subsequent patients (Section 10.1.4).

[redacted]

3.2.3. Benefit Assessment

Since this is a first-in-human (FIH) study, the benefit of the treatment of TCR-T cell drug product has not yet been determined; however, there may be an opportunity, at one or more dose levels, to provide clinical benefit to individual participant(s) based on previous experience with adoptive cell therapy.

3.2.4. Overall Benefit: Risk Conclusion

The success of this investigational therapy cannot be predicted at this time, but because the subjects will have progressed or recurred following standard chemotherapy or standard systemic therapy or were intolerant to previous treatment and have a short life expectancy, the potential benefit may outweigh the risks.

4. STUDY OBJECTIVES

4.1. Phase I Objectives

4.1.1. Primary Objectives

- To define the incidence of dose limiting toxicity (DLT) and the maximum tolerated dose (MTD) or recommended phase II dose (RP2D) of T-Cell Receptor T cells (herein referred to as TCR-T cell drug product) administered without IL-2 (Arm A) [redacted].

  Arm A:
  To define the incidence of DLT and the MTD of TCR-T cell drug product delivered as a single administration.

  [redacted]

4.1.2. Secondary Objectives

- To evaluate the feasibility of TCR-T cell drug product manufacturing.
- To investigate translational hypotheses related to TCR-T cell persistence without IL-2 (Arm A) [redacted]

[redacted]

5. STUDY DESIGN

5.1. Overall Study Design

This is a Phase I/II study of autologous T cells engineered using the *Sleeping Beauty* transposon/transposase system to express T-cell receptors (TCR) reactive against neoantigens in subjects with solid tumors.

Subjects eligible for enrollment on this protocol are those for whom a TCR matching the subject's somatic mutation(s) and HLA type restriction combination is available in Alaunos' Clinical TCR library and have progressive or recurrent disease following standard chemotherapy or standard systemic therapy or were intolerant to previous treatment. This study will enroll subjects with the following tumor type subgroups:

Table 6. Tumor Type Subgroups

Subgroup	Tumor Type
1a	Ovarian
1b	Endometrial
2	Colorectal
3	Pancreatic
4	Non-small cell lung cancer
5	Cholangiocarcinoma

This study includes Pre-Screening, Screening, Pre-Treatment, Treatment and Follow-up Periods. The study starts with pre-screening to determine if there is a TCR match for the subject based on their somatic mutation(s) and HLA typing. If there is at least one TCR match for the subject, then they will begin screening for treatment with TCR-T cell drug product. The screening period is from the time the subject has a TCR identified until the subject is deemed eligible by the Medical Monitor of the screening eligibility criteria. The Pre-Treatment Period starts when the Screening period ends. Subjects are considered to be enrolled, when they meet the criteria for lymphodepletion.

During the Pre-Treatment Period, subjects will undergo an apheresis [redacted] ridging therapy after apheresis will be allowed following consultation with the Alaunos Medical Monitor once the apheresis product has been accepted at the manufacturing site. To receive bridging therapy, the patient must meet all labeled requirements for such therapy. Bridging therapy will be customized to the patient's tumor and disease burden. All prior treatments will be recorded, including documentation of whether any bridging therapy was for maintenance purposes or to induce remission of rapidly progressing disease. The type and duration of bridging therapy will be left to the discretion of the physician after discussion with the medical monitor. Subjects who have sufficient lymphodepletion from bridging therapy may not need additional lymphodepletion as described in Section 8.2 prior to infusion of TCR-T cell drug product.

During the Treatment Period, lymphodepletion (Section 8.2), if needed, will proceed after confirmation of inclusion criteria for lymphodepletion (enrollment). Subjects will start lymphodepletion 7 days (Day 7) prior to TCR-T cell drug product infusion. Subjects will receive prophylactic tocilizumab treatment on Day -5 (single, intravenous dose, 600mg). After the lymphodepleting regimen, TCR-T cell drug product will be administered to the subject by infusion on Day 0 at the subject's assigned dose level. Dose splitting may be considered in certain situations (e.g. subject experiences CRS or IRR) following consultation with the Alaunos Medical Monitor. [redacted]

The Follow-up Period will begin after the subject completes their Day 28 visit. In the Follow-up Period, clinical and radiologic response will be evaluated at 6 and 12 weeks after TCR-T cell drug product infusion and every 12 weeks thereafter until they complete the 2-year follow-up period or discontinue from this study (e.g., due to disease progression, initiation of new anti-cancer therapy, withdraw consent, etc.), whichever occurs first. Enrollment into the long-term follow-up study (below) will occur after completion or discontinuation from this study.

Note: All subjects will be followed in the long-term follow-up protocol (TCR001-202) for up to 15 years post-TCR-T cell drug product infusion.

5.1.1. Description of Dose Escalation Phase I

The Phase I part of this study is a prospective, open-label, dose-escalation study of TCR-T cell drug product in patients with progressive or recurrent solid tumors who have failed standard therapy (refer to inclusion criteria). This study utilizes a Bayesian optimal interval design (BOIN) with an accelerated dose escalation to determine the MTD/RP2D of TCR-T cell drug product. Subjects who fulfill the eligibility criteria will receive a single infusion of TCR-T cell drug product at the assigned dose level on Day 0. Safety and pharmacokinetic (PK) profiles, and the preliminary efficacy will be examined for each dose level.

The study starts with pre-screening to determine if there is a TCR match for the subject based on their somatic mutation(s) and HLA typing. If there is at least one TCR match for the subject, then they will begin screening assessments to determine initial eligibility for treatment with TCR-T cell drug product (Arm A) at Dose Level 1 (DL1) as shown in Figure 2. This dose level will follow an accelerated dose escalation and is planned to treat one subject at dose level 1 in the absence of a DLT. After the subject completes the DLT period (Day 0 to Day 28), the subject will be evaluated for toxicities. If the first subject experiences a DLT, 3 additional subjects will be enrolled and treated at DL1 before escalating to dose level 2 (DL2). If the first subject does not have a DLT(s), the next subject will be enrolled and treated at the next higher dose level (DL2). After DL1, the study will follow the pre-planned dose escalation decision rules detailed in Table 7 and defined in Appendix H. At least 2-3 subjects will be enrolled at DL2 to enable evaluation of TCR-T cell persistence data by the Safety Review Committee (SRC).

The starting dose level will be DL1. If the manufacturing for the first patient meets the criteria for DL-1 (dose level minus 1) the current BOIN design (which accommodates 4 dose levels; refer to Appendix H) accelerated will be initiated at DL-1, subsequently patients will be enrolled, and dose levels will be escalated per the BOIN design. If there is the inability to

manufacture at the current open dose level and while the dose escalation is continuing to explore higher doses, additional patients can be enrolled at the declared safe dose levels in a "dose extension" assigned by the manufactured cell count. The decision to enroll patients in these dose extensions will be made after discussion between the SRC and the Sponsor considering the available safety and efficacy data. The purpose of the additional subjects in dose level cohorts with declared safe dose levels will be to expand the available safety database at pharmacologically relevant doses.

- The SRC will review all available safety information (during the DLT period and long-term toxicities) by dose level including DL-1 (if required to be enrolled by safety or manufacturing issues) and TCR-T cell persistence data [redacted]). If TCR-T cell persistence is deemed sufficient and the dose level is considered safe in the initial subjects who have been treated at DL2, [redacted] and the study will advance with the treatment of subjects at Dose Level 3 (DL3) according to the DLT probability rules. [redacted]

Table 7. TCR-T Dose Levels (Intended Dose and Dose Range)

Dose Index for BOIN	Dose Level	TCR^+ Cells	Minimum	Maximum
1	DL-1	$<1.0x10^9$	NA	$<1.0x10^9$
2	DL1	$5\ x10^9$	$1.0\ x10^9$	$<10x10^9$
3	DL2	$40\ x10^9$	$10x10^9$	$<70x10^9$
4	DL3	$100x10^9$	$70x10^9$	$150x10^9$

If the TCR-T cell persistence is deemed insufficient at DL2 [redacted], at the discretion of the SRC, Arm A will be halted, [redacted]

Subjects who withdraw from the study for reasons not related to treatment-emergent toxicities during the DLT period may be replaced. This would include subjects who withdraw due to progression and receive a subsequent therapy prior to day 28.

For the initial two subjects of Arms A and B there will be a dose staggering interval of 28 days. Dose administration for the subsequent subjects will not occur until the initial two subjects have completed their safety evaluation for dose-limiting toxicities.

Subjects may be screened during this 4-week period so that apheresis material can be collected for manufacturing.

There are two dose escalation stopping rules in this BOIN study.

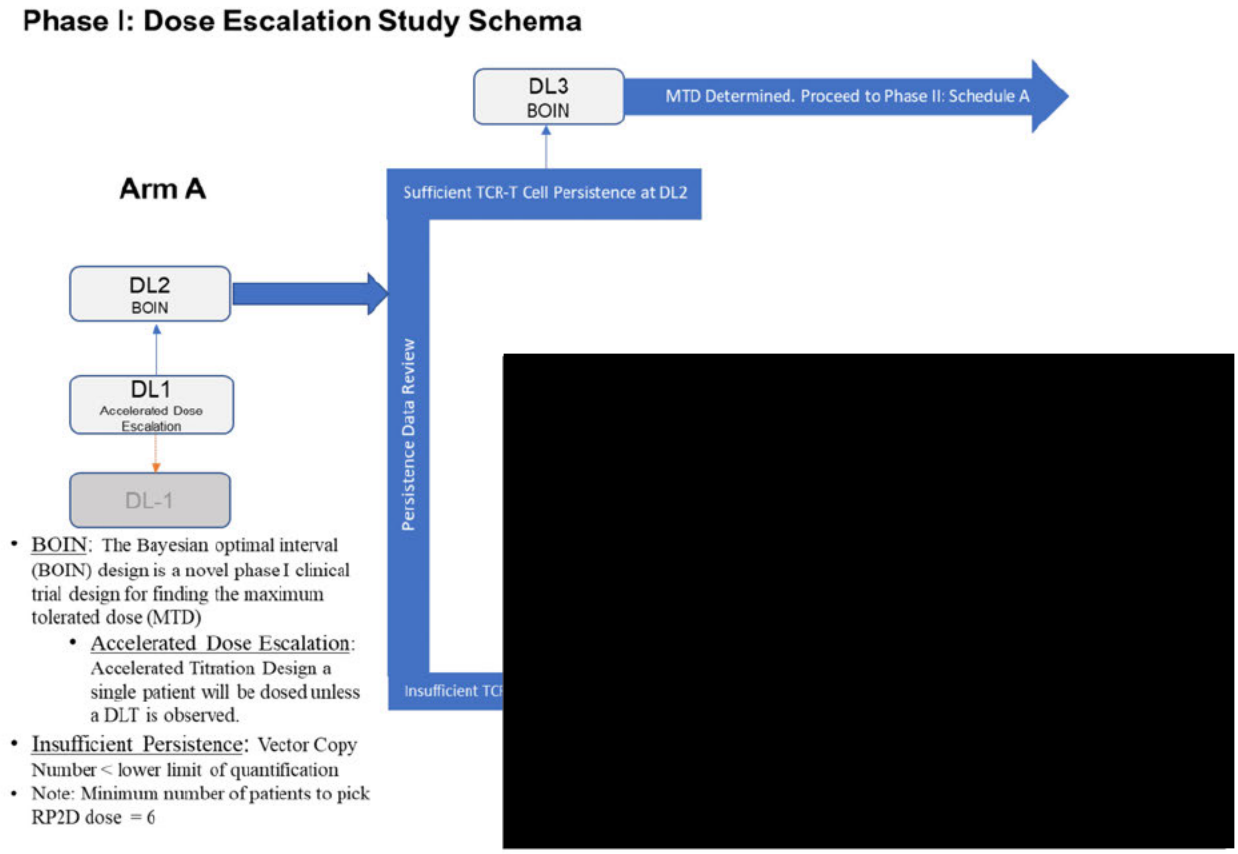
- Stop the trial if the lowest dose is eliminated due to overt toxicities*;
- Stop the dose escalation and estimate the MTD/RP2D if the number of new subjects treated at the current dose level reaches 9.

* Overt Toxicities defined as:

1. The occurrence of 2 or more dose limiting toxicities.
2. Consideration will also be given to longer term toxicities (e.g. > 30 days post infusion: Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS), cardiotoxicity, pulmonary toxicity, metabolic complications, secondary macrophage-activation syndrome, prolonged cytopenia unresponsive to colony stimulating factors, grade 3 and 4 infections and subsequent malignancies)

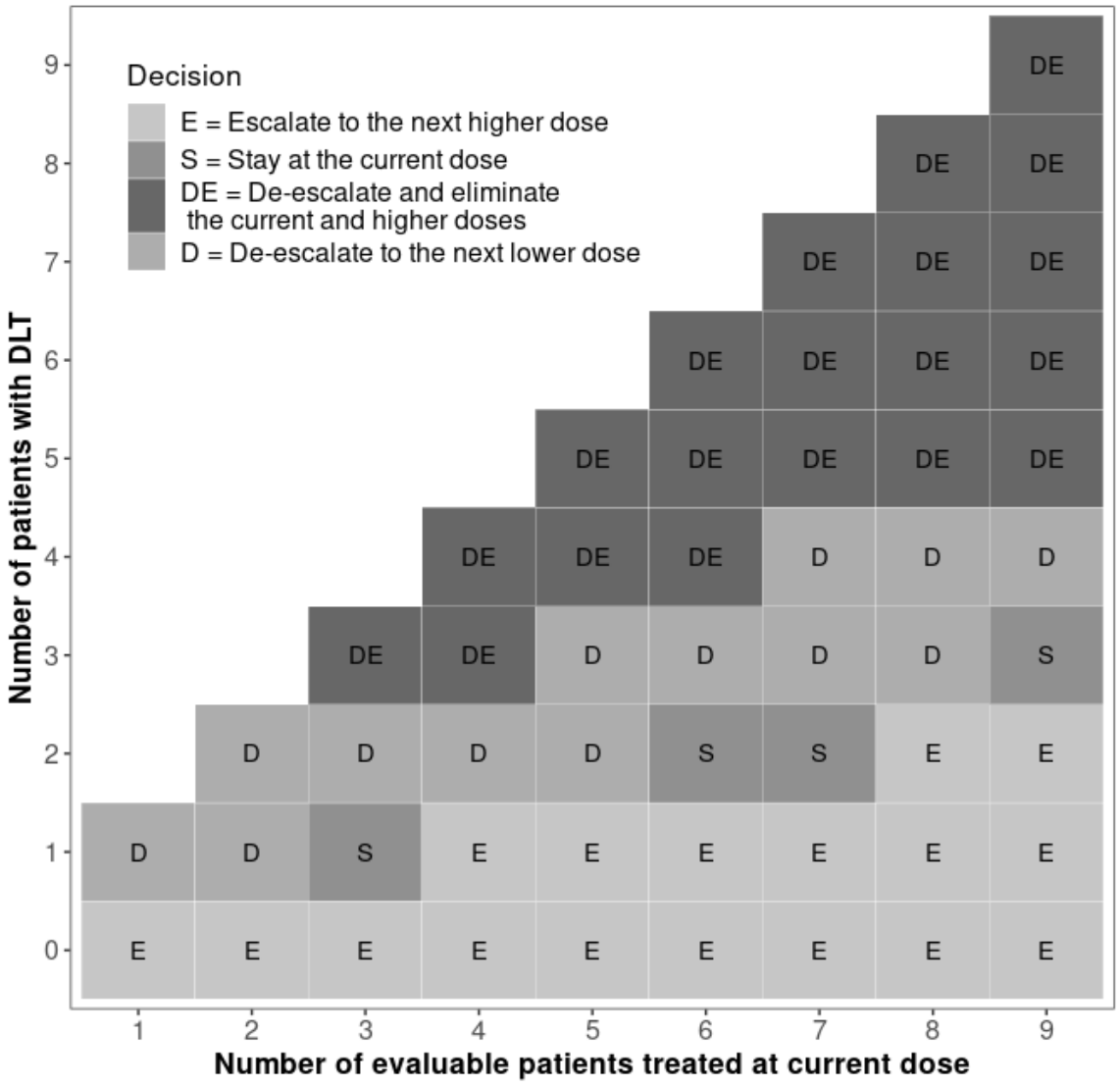
The detailed statistical methods for dose escalation and de-escalation and estimation of MTD/RP2D are described in Section 15.1.1 and Section 15.1.2.

Figure 2. Phase I Dose Escalation Study Schema



BOIN: Bayesian Optimal Interval, DL: Dose Level, DLT: Dose-limiting toxicity, [redacted] RP2D: Recommended Phase II Dose

Figure 3. BOIN Escalation and De-escalation Rules



[redacted]

For the null response rate, p_0 (H_0) for each tumor type subgroup; refer to Table 18 for additional details.

5.2. Study Endpoints

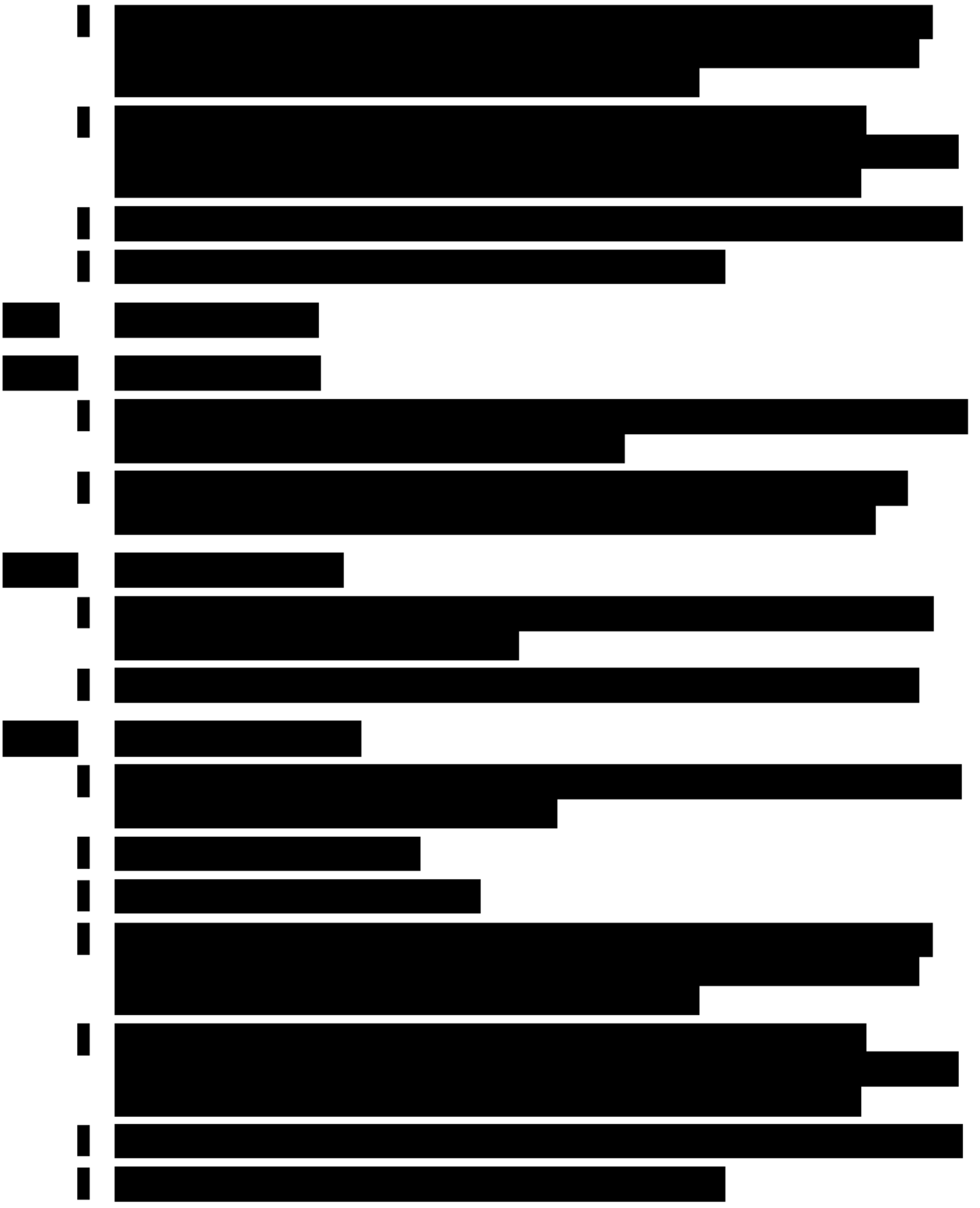
5.2.1. Phase I Endpoints

5.2.1.1. Primary Endpoints

- Number of DLTs and AEs
  - Neurotoxicity and cytokine release syndrome events will be assessed according to the ASTCT criteria (Lee et al., 2019). Refer to Appendix B, Appendix C, and Appendix E.
  - All other AEs will be as assessed by the NCI CTCAE (Version 5.0).
- Frequency, relatedness, severity, and duration of treatment emergent and treatment related AEs (NCI CTCAE Version 5.0).

5.2.1.2. Secondary Endpoints

- Final T cell count, including percent viability, for appropriate clinical dose. Additional details will be provided in the Statistical Analysis Plan (SAP).
- Duration of TCR-T cell drug product persistence



5.3. Dose Limiting Toxicity Definition

Severity of AEs will be graded according to NCI CTCAE Version 5.0. For the purpose of dose escalation, any of the AEs listed below that occur within 28 days after the TCR-T cell drug product infusion and are attributable to TCR-T cell drug product and unrelated to tumor, intercurrent illness, or concomitant medications will be classified as DLTs. Due to the requirement for lymphodepletion prior to investigational product administration, and the expected toxicities associated with lymphodepletion, hematological toxicities will not be considered DLTs unless specified below.

Clinically important or persistent toxicities (e.g., toxicities responsible for significant dose delay) that are not included in the definitions below may also be considered a DLT following review by the SRC. All DLTs need to represent a clinically significant shift from baseline.

A DLT is a study intervention related AE occurring within the DLT window (Day 0 to Day 28 days post TCR-T cell drug product infusion), defined based on NCI CTCAE Version 5.0 as the following:

Hematologic

- Grade 4 neutropenia lasting ≥ 14 days
- Grade 4 thrombocytopenia
- Grade 3 thrombocytopenia associated with clinically significant bleeding,
- ≥ Grade 3 febrile neutropenia associated with hemodynamic compromise or objective evidence of infection.

Non-hematologic

- CRS grade 3 or 4 that does not improve to grade ≤ 2 within 72 hours despite appropriate treatment.
- Grade 3 Immune-effector cell-associated neurotoxicity syndrome (ICANS) that does not return to grade ≤2 or lower within 7 days.
- Any grade 5 adverse event (not due to disease progression or to a known cause other than TCR-T (e.g., automobile accident)).
- Grade 3 hepatic toxicity that does not return to grade ≤ 2 or lower within 7 days.
- Grade 3 toxicity involving vital organs that does not improve to grade ≤ 2 or lower within 7 days of onset.
- Grade 4 toxicity not previously specified.

In addition to the related AEs mentioned above, any related grade 3 toxicity and all grade 4 toxicities will be considered DLTs except the following:

- Grade 3 anemia that is not associated with other clinically significant complications
- Expected chemotherapy toxicities due to lymphodepletion including cytopenias, fludarabine related adverse reactions, and cyclophosphamide related hemorrhagic cystitis.

5.4. Study Oversight for Safety Evaluation

A formal SRC will be comprised of the study Investigator(s), the Alaunos Medical Monitor and sponsor representatives, e.g., a member of the clinical study team, a member of the Drug Safety and Pharmacovigilance group, etc. The SRC will be initiated to review ongoing safety data and facilitate decisions for escalation and expansion. The study will proceed as planned if the SRC determines that the benefit risk assessment supports enrollment and treatment of additional subjects in the study.

The SRC will conduct continuous reviews of data for safety and/or efficacy:

- SRC will conduct a comprehensive review of all reported adverse events (for screening, treatment and follow up periods) at least twice monthly (modified by enrollment) by the subjects treated on the trial and will determine if enrollment should continue. If an event meets a criterion for pausing the study, the SRC will convene to evaluate the safety event(s) and to make a recommendation and decision on the enrollment of additional subjects at the same dose level.
- After the escalation rules are met at a dose level and the last subject treated at that dose level has completed the DLT period (Day 0 to Day 28), the SRC will review TCR-T cell persistence data (i.e., vector copy number (VCN)) through Day 28 of subjects treated at DL2. If TCR-T cell persistence is deemed sufficient in the initial subjects who have been treated at DL2, [redacted] the study will advance with the treatment of subjects at Dose Level 3 (DL3) according to the DLT probability rules. [redacted]

The protocol will also be monitored by an external DSMB that will review safety and efficacy at least annually.

Safety will be evaluated based on frequency and severity of adverse events (AEs), serious adverse events (SAEs), laboratory abnormalities, electrocardiograms (ECGs), vital signs and physical/neurologic examination findings by study period by dose level and in aggravate. The severity of AEs will be graded using National Cancer Institute (NCI) CTCAE Version 5.0. Reporting from the time of informed consent until lymphodepletion will only include AEs and SAEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw). AEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw) will be recorded as adverse events in the CRF as outlined in Section 13.6. SAEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw) will be recorded in the CRF as outlined in Section 13.6 and reported to Sponsor as defined in Section 13.8. AEs and SAEs considered not protocol-related should be considered medical history.

The reporting period of all adverse events and serious adverse events will begin from the start of lymphodepletion through end of study regardless of relationship to study treatment. Refer to Section 13 for more details.

6. SELECTION AND WITHDRAWAL OF SUBJECTS

6.1. Subject Inclusion Criteria

This study will enroll subjects based on histologically confirmed tumor types:

Subgroup 1a	Gynecologic cancer — Ovarian
Subgroup 1b	Gynecologic cancer — Endometrial
Subgroup 2	Colorectal
Subgroup 3	Pancreatic
Subgroup 4	Non-small cell lung cancer (NSCLC). NSCLC includes but is not limited to squamous cell carcinoma, adenosquamous carcinoma or adenocarcinoma.
Subgroup 5	Cholangiocarcinoma

6.1.1. Pre-screening Eligibility Criteria

1. Patients with previous tumor genomic testing performed at a CLIA-certified or CLIA-accredited laboratory must be willing to disclose results. Patients that do not have previous tumor genomic testing must have a planned biopsy or resection (or archived tumor tissue available for genomic testing) or must provide a blood sample for circulating tumor DNA (liquid biopsy) testing.
2. Patients with previous high resolution, human leukocyte antigen (HLA) class I and II typing from a CLIA-certified or CLIA-accredited laboratory must be willing to disclose results. Patients that do not have previous typing results must be willing to provide a blood sample for this testing.
3. Patients who have previously received at least one line of standard systemic therapy for their advanced/metastatic cancer and have either progressed, recurred, or were intolerant to the previous treatment for one of the following cancer types.
    - Gynecologic cancers (i.e., ovarian or endometrial)
    - Colorectal Cancer
    - Pancreatic Cancer
    - Non-small cell lung cancer (NSCLC): NSCLC includes but is not limited to squamous cell carcinoma, adenosquamous carcinoma or adenocarcinomas
    - Cholangiocarcinoma
4. Patients must be able to provide written informed consent.
5. Patients must be age ≥ 18 years.
6. Clinical Performance Status of Eastern Cooperative Group (ECOG) 0 or 1.
7. Patients must not have any form of primary immunodeficiency (such as severe combined immunodeficiency disease) or uncontrolled autoimmune hemolytic anemia or idiopathic thrombocytopenic purpura, such as those patients with a declining hemoglobin level or

platelet count secondary to autoimmune destruction within the 4 weeks prior to first dose of study drug, or the need for daily prednisone ≥10 mg daily (or corticosteroid equivalent) to control the autoimmune disease (Note: the experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities.)

8. Patients who have received any type of organ transplant in the past 12 months are excluded
9. Patients who have undergone xenotransplantation at any time are excluded.
10. Patients must not have any history of severe immediate hypersensitivity reaction to cyclophosphamide, fludarabine, [redacted] bendamustine (for subjects if required for lymphodepletion), or dimethyl sulfoxide (DMSO).
11. Patients with a prior history or concurrent malignancy whose natural history or treatment does not have the potential to interfere with either the safety or efficacy assessment of the investigational regimen may be included after discussion with Sponsor, Alaunos Medical Monitor, or designee
12. Patients must not have any active unstable or clinically significant medical condition that would, in the opinion of the Principal Investigator (PI) in consultation as warranted with medical monitor, result in risks to safety of a subject and/or their compliance with the protocol. Examples include, but are not limited to, a history of myocarditis or congestive heart failure (New York Heart Association functional Class III or IV), unstable angina, serious uncontrolled cardiac arrythmia, myocardial infarction within 6 months of screening, active interstitial lung disease (ILD)/pneumonitis or a history of ILD/pneumonitis requiring chronic treatment with systemic steroids, uncontrolled asthma, or colitis.

6.1.2. Screening Eligibility Criteria:

Inclusion Criteria:

1. Subjects with at least one TCR matching a combination of their somatic mutations and HLA type is available in Alaunos's Clinical TCR library (refer to Appendix G) as evidenced by molecular testing results performed at a CLIA-certified or CLIA-accredited laboratory.

2. Patients who have previously received at least one line of standard systemic therapy for their advanced/metastatic cancer and have either progressed, recurred, or were intolerant to the previous treatment. Specifically:
    - Subgroup 1. Gynecologic cancers (i.e., ovarian or endometrial):
        a. Ovarian cancer: Subjects who are platinum-resistant, as defined by progression on a platinum-containing regimen or within 6 months of a previous platinum dose.

b. Endometrial cancer: Subjects who have received at least 1 prior line of therapy for advanced/recurrent disease (including adjuvant chemotherapy and adjuvant chemo-radiation therapy) prior to apheresis. Prior hormonal therapy will not count towards prior lines of therapy. Note that 2 prior lines of treatment are required prior to lymphodepletion.

- Subgroup 2. Colorectal cancer:
  - Patient has received systemic treatment for advanced unresectable or metastatic disease prior to apheresis.
  - Must include an irinotecan or oxaliplatin-based therapy and if eligible, a targeted antibody therapy.
    - Subjects with deficient DNA mismatch repair (dMMR) or microsatellite instability-high (MSI-H) colorectal cancer should have received a checkpoint inhibitor such as pembrolizumab or nivolumab. Note that 2 prior lines of treatment are required prior to lymphodepletion.
- Subgroup 3. Pancreatic cancer: Subjects who have progressive disease after receiving initial treatment with FOLFOX, (modified) FOLFIRINOX, and/or a gemcitabine-based therapy.
- Subgroup 4. Non-small cell lung cancer (NSCLC):
  - Subjects with recurrent and/or metastatic disease who have disease progression or intolerance to treatment with a PD-1/PD-L1 inhibitor either as a single-agent or in combination with other checkpoint inhibitors (e.g., CTLA-4 inhibitors) and/or platinum-containing chemotherapy.
  - Subjects with targetable oncogene alterations (e.g., EGFR, ALK, ROS1, RET, MET, NTRK1-3, BRAF) must have had disease progression or intolerance on at least one prior line of targeted therapy.
- Subgroup 5. Cholangiocarcinoma: Subjects must have histologically confirmed diagnosis of cholangiocarcinoma Stage II, III, or IV (intra-hepatic, extra-hepatic and perihilar) that is not eligible for curative resection, transplantation, or ablative therapies. Subjects who have progressed after receiving at least one line of standard therapy.

3. Patients must have measurable disease per RECIST 1.1 as assessed by the local site investigator/radiology.
4. Patient must agree to provide archival tissue (core or excisional biopsy) from a locally recurrent or inoperable or metastatic tumor lesion or new tissue must be obtained from a non-target lesion for translational research assessments

5. Clinical Performance Status of Eastern Cooperative Oncology Group (ECOG) 0 or 1. .

6. Patient must provide written informed consent for the long-term follow-up protocol (TCR001-202) for up to 15 years post TCR-T Cell drug product infusion per FDA requirements.

7. Adequate bone marrow reserves as assessed by the following hematology laboratory criteria:
   a. ANC ≥ 1,000/mm^3 (filgrastim not within 7 days or pegfilgrastim not within 14 days)
   b. WBC ≥ 3,000/mm^3
   c. Platelet count ≥ 50,000/mm^3
   d. Hemoglobin > 8.0 g/dL. (Subjects may be administered red blood cell transfusions to achieve this cut-off value.)

8. Adequate major organ system functions as determined by the following criteria:
   a. Cardiac
      i) Left ventricular ejection fraction ≥50% on multiple gated acquisition (MUGA) scan and/or by echocardiography at the discretion of the investigator.

      Note: No evidence of clinically significant pericardial effusion as determined by an ECHO.
   b. Pulmonary
      i) Adequate pulmonary function, defined as grade ≤ 2 dyspnea and SaO2 ≥ 90% on room air.
   c. Renal
      i) Serum creatinine within institutional limits; OR
      ii) Calculated creatinine clearance (ClCr) (e.g., Cockcroft-Gault equation) ≥ 45.0 mL/min and not dialysis dependent
   d. Liver
      i) Serum ALT/AST ≤ 3 x ULN or ≤ 5 x ULN if documented liver metastases
      ii) Total bilirubin ≤ 2 mg/dL, except in patients with Gilbert's Syndrome, who must have a total bilirubin < 3.0 mg/dL

9. Patients may have undergone minor surgical procedures or limited-field radiotherapy provided any major organ toxicities have recovered to ≤ Grade 1.

10. Patients must not be pregnant or breastfeeding.

11. Female patients of non-childbearing potential must meet at least 1 of the following criteria:

    - Achieved postmenopausal status, defined as follows: cessation of regular menses for at least 12 consecutive months with no alternative pathological or physiological cause; if there is uncertainty as to whether the patient has a postmenopausal status, it will be confirmed with a serum follicle stimulating hormone (FSH) level confirming the postmenopausal state;

- Have undergone a documented hysterectomy and/or bilateral oophorectomy;
- Have medically confirmed ovarian failure.

12. Patients of childbearing potential, including women with tubal ligations, must commit to using 2 highly effective forms of birth control (defined as the use of an intrauterine device, a barrier method with spermicide, condoms, or any form of hormonal contraceptives) for the duration of the study.
13. Male patients must be sterile (biologically or surgically), or their partner(s) of childbearing potential must commit to the use of highly effective method of birth control for the duration of the study.
14. Patients must be fully vaccinated against COVID-19 prior to lymphodepletion. This criterion includes a booster shot if at least 5 months have passed after receiving the second shot of a 2-dose series or 2 months after a single-dose vaccine. Exceptions must be approved by the Alaunos Medical Monitor.

6.1.3. Exclusion Criteria:

1. Patients who have received any type of organ transplant in the past 12 months.
2. Patients who have undergone xenotransplantation at any time.
3. Patients with known active CNS metastases; Patients with prior CNS metastatic disease that has been effectively treated and have recovered from the acute effects of radiation therapy or surgery prior to study entry, have discontinued supra physiologic doses of corticosteroid treatment for these metastases (unless required for other than the treatment of brain metastases) for at least 2 weeks and are neurologically stable for 3 months (requires magnetic resonance imaging [MRI] confirmation) may be enrolled, stability for less than 3 months may be allowed after discussion with the Alaunos Medical Monitor. Any question pertaining to this criterion should be discussed with the Alaunos Medical Monitor.
4. Positive beta-HCG in female of child-bearing potential defined as not post-menopausal for 12 months or no previous surgical sterilization or lactating females.
5. Concurrent systemic steroid therapy at a dose of ≥10 mg prednisone daily or equivalent is excluded. Physiologic dosing of steroids to treat adrenal insufficiency is permitted. Topical or inhaled steroids are permitted.
6. Any form of primary immunodeficiency (such as severe combined immunodeficiency disease) or uncontrolled autoimmune hemolytic anemia or idiopathic thrombocytopenic purpura, such as those with a declining hemoglobin level or platelet count secondary to autoimmune destruction within the 4 weeks prior to first dose of study drug, or the need for daily prednisone ≥10 mg daily (or corticosteroid equivalent) to control the autoimmune disease. Note: the experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune competence

may be less responsive to the experimental treatment and more susceptible to its toxicities.

7. History of severe immediate hypersensitivity reaction to cyclophosphamide, fludarabine, [redacted] bendamustine (for subjects if required for lymphodepletion), or dimethyl sulfoxide (DMSO).
8. Severe chronic respiratory condition as determined by the following:

   Symptoms of severe respiratory dysfunction or pleural effusion other than a malignant pleural effusion treatable by drainage with or without pleurodesis.

9. History of a bleeding disorder or unexplained major bleeding diathesis. Note: Subjects receiving anticoagulation must have been on therapy for at least 4 weeks from the most recent diagnosis of VTE (e.g. DVT, PE) at time of lymphodepletion or TCR-T cell infusion.

   Note: Patients must be deemed clinically stable to have anticoagulant therapy held for the institutional standard (determined by individual patient characteristics) prior to and after apheresis.

   Note: Subjects that are receiving maintenance anti-coagulation therapy that are unable to be transitioned to a low molecular weight heparin (LMWHs) therapy (e.g., dalteparin, enoxaparin, etc.) prior to lymphodepletion and remain on LMWH through 30 days post-TCR-T infusion, would be excluded.

10. [redacted]
11. Any major bronchial occlusion or pulmonary bleeding not amenable to palliation. More than two weeks must have elapsed since any prior palliation for major bronchial occlusion or bleeding and enrollment.
12. Patients with psychiatric illness/social situations at the time of treatment that would limit compliance with study requirements.
13. Participants with known active, uncontrolled bacterial, fungal, or viral infection, including hepatitis B virus (HBV), hepatitis C virus (HCV), known human immunodeficiency virus (HIV), Herpesvirus (HHV), cytomegalovirus (CMV) or acquired immunodeficiency syndrome (AIDS) related illness. In equivocal cases, subjects whose viral load is negative, may be eligible. HIV seropositive subjects who are healthy and low risk for AIDS related outcomes could be considered eligible. Eligibility criteria for HIV positive subjects should be evaluated and discussed with Alaunos Medical Monitor and will be based on current and past CD4 and T cell counts, history (if any) of AIDS defining conditions (e.g., opportunistic infections), and status of HIV treatment. Also, the potential for drug-drug interactions will be taken into consideration.
14. Patients with a prior history or concurrent malignancy whose natural history or treatment does not have the potential to interfere with either the safety or efficacy assessment of the

investigational regimen may be included after discussion with Sponsor, Alaunos Medical Monitor or designee

15. Active unstable or clinically significant medical condition that would, in the opinion of the Principal Investigator (PI) in consultation as warranted with medical monitor, result in risks to safety of a subject and/or their compliance with the protocol. Examples include, but are not limited to, , active interstitial lung disease (ILD)/pneumonitis or a history of ILD/pneumonitis requiring chronic treatment with systemic steroids, uncontrolled asthma, or colitis.
16. History of any one of the following cardiovascular conditions within the past 6 months: Class III or IV heart failure as defined by the NYHA (Refer to Appendix A), cardiac angioplasty or stenting, myocardial infarction, unstable angina, or other clinically significant cardiac disease.

6.1.4. Eligibility Criteria for Apheresis:

These criteria should be confirmed prior to the subject undergoing apheresis (see SOA for collection windows).

1. Adequate bone marrow reserves as assessed by the following hematology laboratory criteria. This requirement may be satisfied by the initial screening in Section 6.1.1 if apheresis is scheduled within 7 days of the initial screening:
   a. ANC ≥ 1,000/mm^3 (filgrastim not within 7 days or pegfilgrastim not within 14 days)
   b. WBC ≥ 3,000/mm^3
   c. Platelet count ≥ 50,000/mm^3
   d. Hemoglobin > 8.0 g/dL. (Subjects may be administered red blood cell transfusions to achieve this cut-off value.)
2. Adequate major organ system functions as determined by the following criteria. This requirement may be satisfied by the initial screening in section 6.1.1 if apheresis is scheduled within 28 days of the initial screening:
   a. Cardiac
      i) Left ventricular ejection fraction ≥50% on multiple gated acquisition (MUGA) scan and/or by echocardiography at the discretion of the investigator.

      Note: No evidence of clinically significant pericardial effusion as determined by an ECHO.
   b. Pulmonary
      i) Adequate pulmonary function, defined as grade ≤ 2 dyspnea and SaO2 ≥ 90% on room air.
   c. Renal
      i) Serum creatinine within institutional limits; OR
      ii) Calculated creatinine clearance (ClCr) (e.g., Cockcroft-Gault equation) ≥ 45.0 mL/min and not dialysis dependent
   d. Liver
      i) Serum ALT/AST ≤ 3 x ULN or ≤ 5 x ULN if documented liver metastases
      ii) Total bilirubin ≤ 2 mg/dL, except in patients with Gilbert's Syndrome, who must have a total bilirubin < 3.0 mg/dL

3. A washout period must have elapsed since completion of any prior systemic therapy with guidelines as follows (windows other than what is listed below should be allowed only after consultation with the Medical Monitor); subjects' non-hematologic toxicities from any prior systemic therapy must have recovered to ≤ Grade 1 (with the exception of neuropathy and alopecia) or baseline prior to starting apheresis.
    a. Nitrosoureas: 6 weeks
    b. Other cytotoxic agents: 4 weeks
    c. Anti-angiogenic agents: 4 weeks
    d. Targeted agents, including small molecule tyrosine kinase inhibitors: 5 half-lives or 2 weeks, whichever is shorter
    e. Immune checkpoint inhibitor agents: 4 weeks and must have recovered to ≤ Grade 1 or baseline grade severity prior to checkpoint inhibitor administration from immune-related adverse events (IrAEs), other than endocrine side effects or those affecting nonessential organs or functions
    f. CAR-T, other T-cell product or investigational cancer vaccine: 6 weeks
    g. Systemic steroids of greater than 10 mg prednisone equivalence: 1 week
4. Subjects must not be pregnant or breastfeeding.

6.1.5. Eligibility Criteria for Lymphodepletion:

The criteria should be confirmed before entering the lymphodepletion phase of the study.

1. Patients who have previously received at least one line of standard systemic therapy for their advanced/metastatic cancer and have either progressed, recurred, suboptimal response or were intolerant to the previous treatment. Specifically:
    - Subgroup 1. Gynecologic cancers (i.e., ovarian or endometrial):
        a. Ovarian cancer: Subjects who are platinum-resistant, as defined by progression on a platinum-containing regimen or within 6 months of a previous platinum dose.
        b. Endometrial cancer: Subjects must have received at least 2 prior lines of therapy for advanced/recurrent disease (including adjuvant chemotherapy and adjuvant chemo-radiation therapy) prior to lymphodepletion. Prior hormonal therapy will not count towards prior lines of therapy.
    - Subgroup 2. Colorectal cancer:
        - Subjects must have received at least 2 prior lines of systemic treatment for advanced unresectable or metastatic disease prior to lymphodepletion, which must include an irinotecan or oxaliplatin-based therapy and if eligible, a targeted antibody therapy. Subjects with deficient DNA mismatch repair (dMMR) or microsatellite instability-high (MSI-H) colorectal cancer should have received a checkpoint inhibitor such as pembrolizumab or nivolumab.
    - Subgroup 3. Pancreatic cancer: Subjects who have progressive disease after receiving initial treatment with FOLFOX, (modified) FOLFIRINOX, and/or a gemcitabine-based therapy.

- Subgroup 4. Non-small cell lung cancer (NSCLC):
  - Subjects with recurrent and/or metastatic disease who have disease progression or intolerance to treatment with a PD-1/PD-L1 inhibitor either as a single-agent or in combination with other checkpoint inhibitors (e.g., CTLA-4 inhibitors) and/or platinum-containing chemotherapy.
  - Subjects with targetable oncogene alterations (e.g., EGFR, ALK, ROS1, RET, MET, NTRK1-3, BRAF) must have had disease progression or intolerance on at least one prior line of targeted therapy.
- Subgroup 5. Cholangiocarcinoma: Subjects must have histologically confirmed diagnosis of cholangiocarcinoma Stage II, III, or IV (intra-hepatic, extra-hepatic and perihilar) that is not eligible for curative resection, transplantation, or ablative therapies. Subjects who have progressed after receiving at least one line of standard therapy.

2. No active infection requiring systemic therapy or causing fever (temperature > 38.1˚C (100.6 ˚F)) or subjects with unexplained fever (temperature > 38.1˚C (100.6 ˚F)) within 7 days prior to the day of investigational product administration. Participants with known active, uncontrolled bacterial, fungal, or viral infection, are excluded. In equivocal cases, subjects whose viral load is negative, may be eligible after discussion with the Alaunos Medical Monitor.
3. Any contraindication to fludarabine or cyclophosphamide should be discussed with the Medical Monitor prior to lymphodepletion.
4. Patients who have received any type of organ transplant in the past 12 months are excluded.
5. Patients who have undergone xenotransplantation at any time are excluded.
6. Absence of active autoimmune disease requiring ongoing systemic immunosuppressive therapy. Patients must not have any form of primary immunodeficiency (such as severe combined immunodeficiency disease) or uncontrolled autoimmune hemolytic anemia or idiopathic thrombocytopenic purpura, such as those patients with a declining hemoglobin level or platelet count secondary to autoimmune destruction within the 4 weeks prior to first dose of study drug, or the need for daily prednisone ≥10 mg daily (or corticosteroid equivalent) to control the autoimmune disease (Note: the experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune competence may be less responsive to the experimental treatment and more susceptible to its toxicities.)
7. Negative serum pregnancy test within 7 days before lymphodepletion chemotherapy for women of childbearing potential, defined as those who have not been surgically sterilized or who have not been free of menses for at least 1 year. Patients also must not be breastfeeding.
8. Clinical Performance Status of ECOG 0 or 1.

9. Patients must have measurable disease per RECIST 1.1 as assessed by the local site investigator/radiology.
10. No treatment with any investigational agent on a different clinical trial between meeting the screening eligibility criteria and enrollment (date eligibility is confirmed for lymphodepleting chemotherapy).
11. Serum creatinine within institutional limits; OR Calculated creatinine clearance (ClCr) (e.g., Cockcroft-Gault equation) ≥ 45.0 mL/min and not dialysis dependent
12. Liver
    i) Serum ALT/AST ≤ 3 x ULN or ≤ 5 x ULN if documented liver metastases
    ii) Total bilirubin ≤ 2 mg/dL, except in patients with Gilbert's Syndrome, who must have a total bilirubin < 3.0 mg/dL
13. ANC ≥ 1,000/mm^3 (≥1.0 × 10^9/L) within 1 week of lymphodepletion.
14. Adequate pulmonary function, defined as grade ≤ 2 dyspnea and SaO2 ≥ 90% on room air.
15. Adequate cardiac function, defined as left ventricular ejection fraction (LVEF) of ≥50% as assessed by echocardiogram or MUGA scan, or LVEF of 45-49% and clearance by a cardiologist; if patient receives cardiotoxic chemotherapy after enrollment, repeat echocardiogram or MUGA is required to reestablish eligible LVEF.
16. Patients must be fully vaccinated against COVID-19 prior to lymphodepletion. This criterion includes a booster shot if at least 5 months have passed after receiving the second shot of a 2-dose series or 2 months after a single-dose vaccine. Exceptions must be approved by the Alaunos Medical Monitor.
17. Patients may have undergone minor surgical procedures or limited-field radiotherapy provided any major organ toxicities have recovered to ≤ Grade 1.
18. Subjects must not require corticosteroid therapy or dose ≥ 10 mg per day of prednisone or the equivalent. Pulsed corticosteroid dose for disease control is acceptable until the day before the start of lymphodepletion.
19. Must not have received any live vaccines within 30 days prior to enrollment.
20. Patients with known active CNS metastases are excluded; Patients with prior CNS metastatic disease that has been effectively treated and have recovered from the acute effects of radiation therapy or surgery prior to study entry, have discontinued supra physiologic doses of corticosteroid treatment for these metastases (unless required for other than the treatment of brain metastases) for at least 2 weeks and are neurologically stable for 3 months (requires magnetic resonance imaging [MRI] confirmation) may be enrolled, stability for less than 3 months may be allowed after discussion with the

Alaunos Medical Monitor. Any question pertaining to this criterion should be discussed with the Alaunos Medical Monitor.

21. Patients must not have concurrent systemic steroid therapy at a dose of ≥10 mg prednisone daily or equivalent. Physiologic dosing of steroids to treat adrenal insufficiency is permitted. Topical or inhaled steroids are permitted.
22. Patients must not have a history of severe immediate hypersensitivity reaction to cyclophosphamide, fludarabine, [redacted] bendamustine (for subjects if required for lymphodepletion), or dimethyl sulfoxide (DMSO).
23. Patients must not have a severe chronic respiratory condition as determined by the following:

    Symptoms of severe respiratory dysfunction or pleural effusion other than a malignant pleural effusion treatable by drainage with or without pleurodesis.

24. Patients must not have a history of a bleeding disorder or unexplained major bleeding diathesis. Note: Subjects receiving anticoagulation must have been on therapy for at least 4 weeks from the most recent diagnosis of VTE (e.g. DVT, PE) at time of lymphodepletion or TCR-T cell infusion.

    Note: Patients must be deemed clinically stable to have anticoagulant therapy held for the institutional standard (determined by individual patient characteristics) prior to and after apheresis.

    Note: Subjects that are receiving maintenance anti-coagulation therapy that are unable to be transitioned to a low molecular weight heparin (LMWHs) therapy (e.g., dalteparin, enoxaparin, etc.) prior to lymphodepletion and remain on LMWH through 30 days post-TCR-T infusion, will be excluded.
25. [redacted]
26. Patients must not have any major bronchial occlusion or pulmonary bleeding not amenable to palliation. More than two weeks must have elapsed between any prior palliation for major bronchial occlusion or bleeding and enrollment.
27. Patients with psychiatric illness/social situations at the time of treatment that would limit compliance with study requirements are excluded.
28. Patients with a prior history or concurrent malignancy whose natural history or treatment does not have the potential to interfere with either the safety or efficacy assessment of the investigational regimen may be included after discussion with Sponsor, Alaunos Medical Monitor, or designee
29. Patients must not have a history of any one of the following cardiovascular conditions within the past 6 months: Class III or IV heart failure as defined by the NYHA (Refer to

Appendix A), cardiac angioplasty or stenting, myocardial infarction, unstable angina, or other clinically significant cardiac disease.

30. Patients must not have any active unstable or clinically significant medical condition that would, in the opinion of the Principal Investigator (PI) in consultation as warranted with medical monitor, result in risks to safety of a subject and/or their compliance with the protocol. Examples include, but are not limited to, active interstitial lung disease (ILD)/pneumonitis or a history of ILD/pneumonitis requiring chronic treatment with systemic steroids, uncontrolled asthma, or colitis.

6.1.6. Eligibility Criteria for TCR-T Cell Drug Product Infusion:

Prior to administration of TCR-T cell drug product, subjects will have to meet the following criteria (within approximately 24 hours):

1. CRP and ferritin results within normal institutional limits. Abnormal results to be discussed with medical monitor prior to TCR-T cell administration.
2. Clinical Performance Status of ECOG 0 or 1.
3. No active infection requiring systemic therapy or causing fever (temperature > 38.1˚C (100.6 ˚F)) or subjects with unexplained fever (temperature > 38.1˚C (100.6 ˚F)) within 7 days prior to the day of investigational product administration.
4. Neurotoxicity, if present, *e.g.*, following fludarabine administration, must be resolved to grade ≤ 1. If >grade 1, approval from the Alaunos Medical Monitor is required.
5. Serum creatinine within institutional limits; OR Calculated creatinine clearance (ClCr) (e.g., Cockcroft-Gault equation) ≥ 45.0 mL/min and not dialysis dependent.
6. Adequate pulmonary function, defined as grade ≤ 2 dyspnea and SaO2 ≥ 90% on room air.
7. ANC < 1,000/mm^3 (< 1.0 × 10^9/L) within 72 hours prior to dosing of TCR-T cell administration. Subjects with ANC ≥1,000/mm^3 (< 1.0 × 10^9/L) must be discussed with the medical monitor prior to TCR-T cell drug product administration.
8. Must not have received any live vaccines within 30 days prior to enrollment.

6.2. Withdrawal of Subjects from Study Treatment and/or Study

A subject may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, compliance, or administrative reasons. At the time of discontinuing from the study (e.g., due to disease progression, initiation of new-anticancer therapy, withdraw consent, etc.), if possible, an early discontinuation visit should be conducted and enrollment in the LTFU should occur if the subject received TCR-T cell drug product infusion. Refer to the Schedule of Assessments (Table 12 and Table 13) for assessments to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

The early discontinuation visit applies only to participants who are enrolled and then are prematurely withdrawn from the study. Subjects should be questioned regarding their reason for withdrawal. These reasons must be documented in the subject's medical record and on the eCRF.

If a subject withdraws from the study, he/she may request destruction of any remaining samples, but data already generated from the samples will continue to be available and may be used to protect the integrity of existing analyses. The investigator must document any such requests in the site study records.

If the participant withdraws from the study and withdraws consent (Section 6.2.1) for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The sponsor may retain and continue to use any data or materials collected before such withdrawal of consent.

When a participant withdraws from the study because of an SAE, the SAE must be recorded on the eCRF and reported on the Clinical Trial (CT) SAE Report. Every effort should be made to follow discontinued subjects for ongoing treatment-related AE/SAEs until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up.

Lack of completion of all or any of the withdrawal/early termination procedures will not be viewed as protocol deviations so long as the participant's safety was preserved.

6.2.1. Withdrawal of Consent:

Subjects who request to discontinue after receiving TCR-T cell drug product will be followed for progression and survival. In addition, every effort should be made to follow discontinued subjects for ongoing treatment-related AE/SAEs until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up. The only exception to this is when a participant specifically withdraws consent for any further contact with him or her or persons previously authorized by the participant to provide this information. Subjects should notify the investigator in writing of the decision to withdraw consent from future follow-up, whenever possible. The withdrawal of consent should be explained in detail in the medical records by the investigator, as to whether the withdrawal is only from further receipt of investigational product or also from study procedures and/or post-treatment study follow-up and entered on the appropriate CRF page. Subject information garnered from public databases (e.g., SSDI death index) may be included in the database.

6.2.1.1. Reasons for Removal prior to Treatment with TCR-T Cell Drug Product

Reasons for removal from protocol required investigational products or procedures include any of the following:

- Objective disease progression;
- Global deterioration of health status requiring discontinuation;
- Unacceptable toxicity;
- Pregnancy;
- Significant protocol violation;
- Lost to follow-up;
- Participant refused further treatment;
- Study terminated by sponsor;

- Death.

6.2.1.2. Withdrawal of Informed Consent for Data and Biological Samples

Alaunos ensures that biological samples are returned to the source or destroyed at the end of a specified period as described in the informed consent. If a subject withdraws consent to the use of donated biological samples, the samples will be disposed of/destroyed, and the action documented. If samples are already analyzed, Alaunos is not obliged to destroy the results of this research. As collection of the biological samples (except for the optional sample for genetics at screening) is an integral part of the study, then the subject is withdrawn from further study participation.

The Principal Investigator:

- Ensures subjects' withdrawal of informed consent to the use of donated samples is notified immediately to Alaunos.
- Ensures that biological samples from that subject, if stored at the study site, are immediately identified, disposed of/destroyed, and the action documented.
- Ensures the organization(s) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed, the action documented and the signed document returned to the study site.
- Ensures that the subject and Alaunos are informed about the sample disposal.

Alaunos ensures the organization(s) holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed and the action documented and returned to the study site.

6.2.1.3. End of Study

An individual subject will be considered to have completed the study if the subject was followed through their last protocol-specified visit/assessment (including telephone contact). Subjects will be considered not to have completed the study if consent was withdrawn or the subject was lost to follow-up.

The end of the study ("study completion") is defined as the date of the last protocol-specified visit/assessment (including telephone contact) for the last subject in the study. This date will be approximately 25 months after the last subject begins treatment or when the sponsor stops the study, whichever occurs earlier.

6.2.1.4. Lost to Follow-up

A subject will be considered lost to follow-up if s/he repeatedly fails to return for scheduled visits and is unable to be contacted by the study center.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The study center must attempt to contact the participant and reschedule the missed visit within 1 business day following the missed scheduled visit and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain

whether the subject wishes to and/or should continue in the study. Study patient should be asked if they want to continue to complete study assessments per protocol follow-up visits via telephone (only if in-person assessments are not required at a visit), allow site to check subject's medical records or permanently stop study participation, including site not allowed to check study patient's medical records.

- Before a subject is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the subject (the site should attempt to get in contact with the subject by telephone three times and if the subject is still unresponsive send a certified letter to the subject's last known mailing address, search of state death index or local equivalent methods). Site is expected to use a variety of contact attempts to follow-up with the subject. Each contact attempts should be documented in the subject's medical record. Telephone calls to the study patient and/or caregivers should be completed at different times on different days of the week. If site does not speak with a person during one of the telephone calls and only leaves a voice message, the telephone call will not be considered 1 of the 3 contact attempts (maximum of 5 total telephone calls).
- Should the subject continue to be unreachable (after 5 telephone calls and no response to certified letter), s/he will be considered to have withdrawn from the study.

6.3. Replacement of Subjects

Subjects who receive lymphodepletion, but do not receive TCR-T cell drug product (e.g., manufacturing failure, change in clinical status) will be replaced. Subjects who receive TCR-T cell drug product and discontinue by Day 28 may be replaced. All subjects who receive TCR-T cell drug product will be included in the overall safety assessment.

6.4. Premature Termination of Study or Study Site

The sponsor has the right to close the study at any time, although this should occur only after mutual consultation between the sponsor and the investigators. The Institutional Review Board (IRB) or Independent Ethics Committee (IEC) must be informed of such action. Should the study or center be closed prematurely, all study materials (completed, partially completed, and blank electronic case report forms (eCRF), study medication, etc.) must be stored or disposed of according to the sponsor's instructions. Events that may trigger premature termination of the study or closure of a center include but are not limited to the following: new toxicity findings; interim analysis results; noncompliance with the protocol; changes in the development plans for the study drug; slow recruitment; and poor-quality data.

7. INVESTIGATIONAL PRODUCTS

7.1. Description of T-Cell Drug Product

The TCR-T cell therapy will be comprised of autologous patient-specific peripheral blood T lymphocytes genetically modified to express neoantigen TCR(s) targeting specific mutations. [redacted] The Phase I part of the study will examine up to 4 dose levels. Dosing will begin at DL1. Refer to Table 7 for defined dose levels.

The proposed dose levels are based on previous experience at the NCI (National Cancer Institute) demonstrating that doses of TCR-T cell drug product below $1x10^9$ cells are largely ineffective and up to $1.5x10^{11}$ cells can be given safely and result in clinical regressions of metastatic disease (Lu et al., 2017). Doses up to $1.5x10^{11}$ cells are also administered in an ongoing NCI study with autologous PBL transduced with retroviral constructs encoding alpha/beta chains of tumor specific TCRs (ClinicalTrials.gov Identifier: NCT04102436).

[redacted]

7.3. Handling and Storage

Study drugs must be stored in a restricted access area under the storage conditions indicated in the Investigator's Brochure and/or Pharmacy Manual. All necessary precautions while handling potentially toxic compounds must be strictly followed.

All cell manipulation procedures will be performed in a Good Manufacturing Practice (GMP) manufacturing site in accordance with current GMPs following process-specific procedures and batch records as described in the Pharmacy Manual. Cryopreserved TCR-T cell drug product will be shipped to and stored at the clinical site prior to administration. Details related to the storage and thawing of material prior to infusion can be found in the Pharmacy Manual. The final transposed cell product will be delivered to the Patient Care Unit and administered to the subject within the established stability window provided in the Pharmacy Manual.

7.4. Accountability and Dispensation

The investigator must maintain accurate records accounting for the receipt and dispensation of study drugs and investigational product. The investigational materials are to be prescribed only by the investigator or the sub-investigators named on FDA Form 1572 and may only be dispensed by authorized personnel at the institution(s) listed therein. Under no circumstances will the PI allow the investigational drug(s) to be used for purposes or in subjects other than as directed by the protocol.

Any unused apheresis product, unused blood specimens, and unused manufactured cells will be banked at Alaunos and/or a GMP licensed facility (Alaunos designee) and may be used for future research studies. Peripheral blood samples will also be collected from subjects who consent to provide additional samples for undefined future research at time points shown in Treatment Plan.

7.5. Retreatment with TCR-T Cell Drug Product

Eligibility for repeat treatments will be considered on a case-by-case basis and must be discussed with the Alaunos Medical Monitor. Patients obtaining any response except progressive disease are potentially eligible for a repeat treatment. Retreatment will be at least 2 months after the original treatment. The dose of TCR-T cells administered during repeat treatments will be at the dose level currently enrolling patients receiving initial TCR-T cell drug product infusions. The repeat treatments will include the same apheresis collection, lymphodepleting chemotherapy and manufacturing process as the initial treatment. If patients were initially matched to multiple TCRs a discussion between the investigator and Sponsor should occur to determine which TCR will be utilized for re-treatment.

Patients must not have experienced a DLT with their first treatment and must meet the same eligibility requirements listed in Section 6.1. The patients must undergo screening evaluation as listed in Table 12 except infectious disease serology, and brain MRI are not required to be repeated unless clinically indicated. Follow-up testing for retreatment will be the same as for the first treatment. In the case where the planned infusion is with the same library TCR as the first infusion, patients must have molecular testing to confirm matching epitope (i.e., genomic testing and HLA typing of tumor tissue collected after initial TCR-T treatment).

The patient may be re-enrolled on this treatment study as a new patient to allow this re-treatment, and these patients will be considered in the total sample size for this study. A maximum of 2 total treatments can be administered to any one patient, and at least 2 months must elapse between the first treatment and the second treatment.

DLTs that occur in the cohort of patients receiving a repeat treatment will not affect the dose escalation of patients receiving an initial treatment. However, excessive DLTs among retreatment subjects will result in a discontinuation of this practice. Specifically, if 2 of the first 3 patients re-treated, 3 or more of the first 6 re-treated patients, 4 or more of the first 9 retreated patients, or greater than 1/3 of the total patients receiving a repeat treatment experience a DLT during repeat treatment, then repeat treatments will be discontinued altogether.

8. TREATMENT PLAN

An overview of the treatment schedule is shown in Table 8.

Table 8 Treatment Schedule

Lymphodepletion Day	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**					
TCR-T Cell Infusion Day								**0**	**1**	**2**	**3**	**4**
Cyclophosphamide (60 mg/kg)[1, 2]	X	X										
Mesna[3]	X	X										
Fludarabine (25 mg/m[1, 2])	X	X	X	X	X							
Bendamustine (90mg/m^2/day)[2]	X	X										
Tocilizumab (600mg)[4]			X									
TCR-T cell drug product								X				
[redacted]								[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

[1] At the discretion of the investigator in heavily pre-treated or irradiated subjects who are expected to have lower bone marrow reserve, a lower dose regimen of Cyclophosphamide 30 mg/kg /Fludarabine 25 mg/m^2 may be administered

[2] In the event the patient had previous intolerance to cyclophosphamide or due to supply issues, bendamustine 90 mg/m^2/day for 2 days or bendamustine 70 mg/m^2/day + fludarabine 30 mg/m^2/day for 3 days may be substituted with approval from the Alaunos Medical Monitor.

[3] Mesna should be administered according to institutional procedure.

[4] Prophylactic tocilizumab administration will be given as a single, intravenous dose (600mg) on Day -5. NOTE: Substitutions may be considered due to supply issues after discussion between the investigator and Medical Monitor.

[redacted]

8.1. Bridging Therapy (while awaiting manufacture and delivery of TCR-T cell product to site)

Treatment for the patient's tumor while they are waiting for administration of TCR-T cell product will be permitted if medically indicated in the judgement of the investigator. The choice of therapy will be investigator driven. Following treatment and prior to the administration of TCR-T cell product, the patient will be re-evaluated for eligibility to continue to the lymphodepletion phase of the study (enroll) as well as whether or not a lymphodepletion regimen is needed (e.g., TLC less than 0.1x10^3/uL).

8.1.1. Bridging Therapy

Bridging therapy will be permitted after discussion with the Alaunos Medical Monitor. The choice of bridging therapy will be at the discretion of the investigator. Prior to leukapheresis, the following should be considered:

- Avoid lymphotoxic treatments
- Avoid immunosuppressive treatment in the 2-3 weeks preceding leukapheresis

After leukapheresis, treatment may include one or more of the following:

- Radiation therapy for local or bulky disease
- Appropriately tapered corticosteroids (Note: a 7-day washout period of supraphysiologic doses prior to switch dose administration)
- Non-chemotherapy (targeted) agents
- Chemotherapy

8.2. Lymphodepleting Chemotherapy

After eligibility criteria for lymphodepletion has been confirmed, lymphodepleting chemotherapy treatment with cyclophosphamide, mesna and fludarabine will be administered according to the schedule and dose described in Table 8.

Additional information can be found in the respective approved labels. Chemotherapy infusions may be slowed or delayed as medically indicated per standard of care requirements. Administration of diuretics, electrolyte replacement, and hydration and monitoring of electrolytes should all be performed as clinically indicated.

At the discretion of the investigator, heavily pre-treated or radiated patients who do not have as much bone marrow reserve may be treated with a lower dose lymphodepleting regimen per Table 8 . In the event the patient had previous intolerance to cyclophosphamide or at the discretion of the investigator, bendamustine 90 mg/m^2/day for 2 days or bendamustine 70 mg/m^2/day + fludarabine 30 mg/m^2/day for 3 days may be substituted with approval from the Alaunos Medical Monitor.

During the course of the study alternative lymphodepletion regimens may be explored after discussion with the investigators and Sponsor, for example cyclophosphamide 1000mg/m^2 (Days -5 to -3) fludarabine 30mg/m^2 over 4 days (Day -6 to Day -3).

Alternatively, patients may be given a lymphodepleting regimen of cyclophosphamide 500 mg/m2 and fludarabine 30 mg/m^2 on 3 consecutive days, respectively, on an out-patient basis. Also, after discussion between the investigator and the Sponsor, fludarabine and cyclophosphamide may be given on the same day concurrently.

Expected toxicities of the suggested lymphodepletion regimen include:

- Cytopenias
- Anemia
- Infections
- Cardiac arrhythmias
- Infusion related reactions (fludarabine)
- Neurotoxicity (fludarabine)
- Hemorrhagic cystitis (cyclophosphamide)

As noted previously, subjects who have sufficient lymphodepletion from bridging therapy may not need additional lymphodepletion prior to infusion of TCR-T therapy. Other methods of lymphodepletion must be discussed with Sponsor, Alaunos Medical Monitor or designee before other methods of lymphodepletion are used by site.

8.2.1. Cyclophosphamide

Cyclophosphamide is a nitrogen mustard-derivative, polyfunctional alkylating agent used to induce lymphopenia prior to TCR-T cell infusion. Institutional guidelines for handling, reconstitution and administration should be followed. Additional information regarding formulation, storage, stability, and administration of cyclophosphamide can be found in the respective approved label. Cyclophosphamide can cause temporary hair loss, nausea, vomiting, diarrhea, and lowering of the blood cell counts. Antiemetics should be used to minimize nausea and vomiting. At the dose of cyclophosphamide used in this study, these symptoms are expected to be minor.

8.2.2. Fludarabine

Fludarabine is an antimetabolite used to induce lymphopenia prior to TCR-T cell infusion. Institutional guidelines for handling, reconstitution and administration should be followed. Additional information regarding formulation, storage, stability, and administration of fludarabine can be found in the approved label. Fludarabine can cause lowering of blood counts, suppression of the immune system, nausea and vomiting, fever, hypersensitivity reaction, tumor lysis, transient elevation in serum transaminases, hemolysis, and neurotoxicity at doses higher than administered in this study.

8.2.3. Bendamustine

Bendamustine is an alkylating agent used to induce lymphopenia prior to TCR-T cell infusion. The chemical name of bendamustine hydrochloride is 1H-benzimidazole-2-butanoic acid, 5-[bis(2-chloroethyl)amino]-1 methyl-, monohydrochloride. Its empirical molecular formula is C16H21Cl2N3O2 · HCl. Additional information regarding formulation, storage, stability, and administration of bendamustine can be found in the respective approved label.

8.2.4. Mesna

Mesna is a detoxifying agent used to inhibit the hemorrhagic cystitis induced by chemotherapy. The active ingredient mesna is a synthetic sulfhydryl compound designated as sodium-2-mercaptoethane sulfonate with a molecular formula of $C_2H_5NaO_3S_2$. Mesna should be administered per institutional guidelines. Additional information regarding formulation, storage, stability, and administration of mesna can be found in the approved label.

8.2.5. Tocilizumab

Tocilizumab is an interleukin-6 (IL-6) receptor antagonist indicated for the treatment of cytokine release syndrome. Institutional guidelines for handling, reconstitution, and administration should be followed. Additional information regarding formulation, storage, stability, and administration of tocilizumab can be found in the respective approved label. Tocilizumab can cause serious infections, gastrointestinal perforations, lab parameters (neutropenia, thrombocytopenia, elevated

liver enzymes and lipid abnormalities), immunosuppression, hypersensitivity reactions, including anaphylaxis, demyelinating disorders, active hepatic disease, and hepatic impairment. Additional information regarding formulation, storage, stability, and administration of tocilizumab can be found in the approved label. NOTE: Substitutions may be considered due to supply issues after discussion between the investigator and Medical Monitor.

8.4. Supportive Medications

Supportive medications may be administered per institutional standard of care following lymphodepleting chemotherapy and TCR-T cell infusion. These concomitant medications are commercially available and are described in Concomitant Medications Section 10.1.

8.5. TCR-T Cell Drug Product

8.5.1. TCR-T Cell Drug Product Pre-medication

Pre-medication is recommended approximately 30 minutes prior to TCR-T cell drug product infusion and will include acetaminophen 650-1000 mg orally and diphenhydramine 12.5 mg IV or 25 to 50 mg orally (or equivalent). Alternatively, subjects can be pre-medicated with paracetamol and diphenhydramine or another H1 antihistamine within 30 to 60 minutes prior to infusion. Corticosteroids should not be used as a pre-medication at any time except in the case of an emergency.

8.5.2. TCR-T Cell Drug Product Dosing

The phase I part of this study will utilize a dose escalation plan to determine the feasibility, safety, and maximum tolerated dose (MTD) or recommended phase II dose (RP2D) of TCR-T cell drug product. The study will examine up to four dose levels as shown in Table 7. The dose escalation plan is defined in Section 5.1.1.

8.5.3. TCR-T Cell Infusion (Day 0)

As shown in the Schedule of Assessments (Table 12), the TCR-T cell drug product will be infused on Day 0. Refer to Pharmacy Manual for additional details regarding thawing of drug product, drug product handling, and administration guidelines.

Prior to thawing the drug product and before infusion, the TCR-T cell drug product identity label must be double-checked by two authorized staff (MD or RN), and an identification of the product and documentation of administration are entered in the subject's chart. The TCR-T cell drug product will be infused in the Patient Care Unit at the assigned dose level defined in Table 7. Infusion durations may be up to 180 minutes or as clinically determined by an investigator for subject safety; bags will be gently agitated during infusion to prevent clumping. Dose splitting may be considered in certain situations (e.g. subject experiences CRS or IRR) following consultation with the Alaunos Medical Monitor.

Mild infusion reactions should be managed expectantly with antipyretics, antihistamines, and antiemetics per institutional standard of care.

The following procedures must be followed for infusion of TCR-T cell drug product:

1. Vital signs, oxygen saturation by pulse oximeter, laboratory examinations and urinalysis will be obtained pre-infusion on the day of infusion.
2. TCR-T cell drug product will be infused via central venous catheter or via a peripherally inserted IV.
3. Vital signs (temperature, heart rate, blood pressure, and respiratory rate) and oxygen saturation will be obtained on all subjects as follows:
    - Start of infusion;
    - Every 15 mins (± 5 mins) during the first hour of infusion;
    - Then, every 30 mins (± 5 mins) until 1 hour after completion of infusion;
    - Then, every hour (± 10 mins) as indicated by subject's condition.

Should a subject fail to meet TCR-T cell drug product eligibility criteria as specified in Section 6.1.6, the TCR-T cell drug product infusion must be delayed until the subject meets the eligibility criteria. If the TCR-T cell drug product infusion is delayed > 2 weeks, lymphodepletion must be repeated, unless otherwise agreed between the Investigator and Alaunos's Medical Monitor or designee. In all cases of TCR-T cell drug product infusion delays, Alaunos's Medical Monitor or designee should be contacted for guidance.

CRP and ferritin levels in combination with EASIX score measured on the day of TCR-T cell drug product infusion or shortly thereafter may be associated with increased risk of severe CRS and ICANS (Greenbaum et al., 2021).

In addition to the above criteria, if the subject's temperature is ≥ 38.0°C within 48 hours prior to TCR-T cell drug product infusion, or clinically meaningful increased CRP or ferritin levels the Alaunos's Medical Monitor or designee must provide approval before proceeding with the TCR-T cell drug product infusion.

8.5.4. Days 0-4 (Day 0 = Day of TCR-T Cell Drug Product Infusion)

[redacted]

8.6. Study Stopping and Pausing Rules

The study will be paused and further investigation initiated if:

- Occurrence of Grade 4 DLTs in 2 subjects at any time during the conduct of the study trial;
- One treatment related death occurs after the administration of TCR-T cell drug product;
- Death not related to disease progression;
- Development of a secondary malignancy, Epstein-Barr virus (EBV) lymphoma or polyclonal lymphoproliferative disease (PLPD) in an EBV negative subject. Note: accrual of EBV positive subjects will be halted if this is observed.

If any of the above events occur, enrollment of new subjects will be paused, pending review by the SRC. The SRC will recommend if changes to the enrollment of additional subjects are required, including but not limited to amending the protocol prior to enrollment of additional subjects, or discontinuing enrollment in the study.

In the event that any stopping rule is met, the FDA and Institutional Review Board will be informed in a timely manner. Alaunos will discuss the investigation and the data with the FDA to identify next steps, which may include stopping the study.

9. SAFETY MONITORING AND TOXICITY MANAGEMENT

9.1. Known Adverse Events Related to the Lymphodepleting Chemotherapy

Known adverse events related to Cyclophosphamide, Fludarabine and Bendamustine can be found in the study approved labels, Pharmacy Manual and Investigator's Brochure.

9.2. Known Adverse Events Related to Tocilizumab

Known adverse events related to tocilizumab can be found in the approved label and Investigator's Brochure.

9.3. Known Adverse Events Related to Aldesleukin

Known adverse events related to aldesleukin (IL-2) and guidance on their management are listed in the approved labels and Appendix F.

9.4. Known Adverse Events Related to the T-Cell Therapy

The investigational product, TCR-T cell drug product engineered using the *Sleeping Beauty* system, has not been previously used in humans, thus there are no known risks associated with this therapy. The risks with T-cell therapies in general include but are not limited to the following as described below. For subjects with suspected autoimmune toxicities, institutional guidelines for evaluation and management of suspected immune-mediated toxicities should be utilized. Institutional guidelines are not intended to replace the medical judgement of the treating physicians and should supplement other guidance provided in this protocol and Investigator Brochure.

9.4.1. Insertional Mutagenesis

There is a potential long-term toxicity that is a general concern of genetically manipulating T cells and potential for genotoxicity using integrating vectors. This relates to the risk of insertional mutagenesis. To date, there has been no genotoxicity detected in T cells genetically modified by *Sleeping Beauty* non-viral gene transfer (Micklethwaite et al., 2021). This will be monitored in this protocol as described in Section 14.4 (Assessment of Insertional Mutagenesis in the Event of Emergent Malignancies) and continue to be monitored on the LTFU protocol (TCR001-202).

9.4.2. Cytokine Release Syndrome (CRS)

For the purpose of this study, the ASTCT Consensus guidelines should be used for grading of cytokine release syndrome (Lee et al., 2019). Refer to Appendix B for CRS grading. T-cell infusion may produce cytokine release (pro-inflammatory as well as anti-inflammatories) into the blood stream causing a systemic inflammatory reaction most commonly manifested by fever, nausea, vomiting, chills, hypotension, tachycardia, asthenia, headache, pruritus, rash, cough, scratchy throat, dyspnea, fluid overload, acute elevation of liver enzymes among other systemic symptoms.

9.4.3. Neurotoxicity

Immune effector cell (IEC) therapy may result in neurotoxicity, termed IEC-associated neurotoxicity syndrome (ICANS). ICANS may include symptoms such as confusion, aphasia, seizure, and/or cerebral edema. The ASTCT Consensus guidelines should be used for the grading and management of immune effector cell- associated neurotoxicity syndrome (Lee et al., 2019). Refer to Appendix C for Immune Effector Cell-associated Encephalopathy (ICE) scoring and Appendix E for grading of ICANS. A neurology consult is recommended for subjects who develop Grade 1 or higher ICANS.

9.4.4. Tumor Lysis Syndrome (TLS)

Rapid destruction of malignant cells may result in TLS, manifesting as laboratory abnormalities, and in untreated cases renal failure. Subjects will be monitored and treated as per standard guidelines including close monitoring of blood chemistries, IV hydration, allopurinol and/or rasburicase in clinically relevant cases.

9.4.5. Hemophagocytic Lymphohistiocytosis (HLH)/ Macrophage Activation Syndrome (MAS)

Clinical and laboratory features of MAS include fever, increased ferritin levels, pancytopenia, hemophagocytosis in bone marrow or lymph nodes, fibrinolytic coagulopathy, and liver dysfunction. Specific treatment includes administration of tocilizumab and corticosteroids given intravenously. Canakinumab may also be used for additional information. Refer to Appendix D.

9.4.6. Graft-versus-Host Disease (GVHD)

The risk of GVHD is expected to be low for an autologous product but is at present undetermined.

9.4.7. Localized Site Reactions

Injection of agents into tissues carries a potential risk of local reactions that may be characterized as intense immunologic reaction at or near the injection site. By definition, injection site reactions (ISRs) include signs and symptoms of erythema, pruritus, pain, inflammation, rash, induration, and edema at the site where TCR-T cell drug product or aldesleukin are administered. They may occur immediately or within 24-48 hours post injection. Post-infusion reactions will be treated per institutional standard of care. ISRs will not be considered a DLT, but they may be reason for discontinuation from treatment.

9.5. Safety Monitoring and Adverse Effect Management

Parameters used in the safety analysis of all populations will include all laboratory tests, physical examinations, imaging scans, and spontaneous reports of AEs reported by subjects. Each subject will be assessed according to the scheduled study procedures (Table 12 and Table 13) and any unscheduled visits as a result of AEs. Other adverse events will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 criteria.

9.5.1. Severity Grading

As with all signs and symptoms, events should be recorded and graded as AEs according to the NCI CTCAE Version 5.0 criteria. Study stopping rules will not apply to a specific event if it is clearly unrelated to the study treatment.

10. CONCOMITANT THERAPY

Information on concomitant medications including blood products, vitamins, supplements, and cancer treatments (chemo- and immunotherapies, radiation therapy, surgeries, and any associated toxicities) received will be collected through informed consent through the subject's last study visit.

10.1. Permitted Medications

10.1.1. Infection Prophylaxis

As subjects are receiving lymphodepleting chemotherapy, subjects should receive preventive and anti-infection medications for fungal infections, pneumocystis pneumonia, herpes virus, and varicella-zoster virus according to institutional guidelines. In the absence of institutional guidelines, refer to the Infection Prophylaxis Considerations for IEC Therapies (Center, 2020) for recommendations on the following medications:

- **Filgrastim** (Granulocyte Colony-Stimulating Factor, G-CSF, Filgrastim, Neupogen)**:** Filgrastim is recommended to be administered subcutaneously at a dose of 300ug/day per institutional practice to reduce the duration of neutropenia and incidence of infection. Beginning 1 day after TCR-T cell drug product infusion filgrastim should be administered subcutaneously at a dose of 300 μg/day). Filgrastim should be continued daily until ANC >1,000/mm^3 x 3 days or ANC >5,000/mm^3. Dose and route of administration may be altered as clinically indicated.
- **Pentamidine and sulfamethoxazole/trimethoprim (SMZ/TMP):** Pentamidine inhaled or IV within one week prior to TCR-T cell drug product infusion and then transition to sulfamethoxazole/trimethoprim (SMZ/TMP) (preferred post-infusion) by 3-4 weeks if counts have recovered is recommended per institutional standard of care to prevent Pneumocystis Jirovecii Pneumonia. SMZ/TMP should continue for at least one year post TCR-T cell infusion and until CD4 > 200 cells/μL for two consecutive measurements Dapsone (in G6PD deficient patients), or atovaquone may be substituted for SMZ/TMP.
- **Valacyclovir:** Subjects with positive HSV or VZV serology should be given valacyclovir orally per institutional standard of care starting on the day of TCR-T cell drug product infusion. Prophylaxis for pneumocystis, HSV, and VZV will continue for 1-year post-TCR-T cell drug product infusion and may stop after 1 year if the CD4 count is > 200 cells/ μL for two consecutive measures. Acyclovir may be substituted for Valacyclovir to prevent the occurrence of herpes virus infections in patients who cannot take oral medications.
- **Entecavir**: Subjects who are positive for HbsAg or HbcAb should receive entecavir per institutional standard. Entecavir should begin 2 weeks before TCR-T cell drug product infusion and stop 12-24 months post-infusion. Tenofovir alafenamide or Tenofovir disopoxil fumarate may be substituted. Infectious Disease and/or Hepatology should be consulted if not already following. Subjects should be monitored for HBV DNA PCR once a month while on prophylaxis and for a year after stopping.

- **Anti-fungal**
  - **Fluconazole:** It is recommended that low-risk patients receive fluconazole for fungal prophylaxis in accordance with institutional guidelines beginning on the day of TCR-T cell drug product infusion and until infection is controlled and ANC > 0.5 K/μL for 3 consecutive days without growth factor support. The drug may be given via IV in patients unable to take it orally.
  - **Posaconazole:** It is recommended that high-risk patients with leukemia, recent allogenic transplant, prior history of mold infection, neutropenia lasting ≥ 14 days, Grade 3 or 4 CRS/ICANS, those who receive ≥ 3 days of corticosteroids, or those who develop hemophagocytic lymphohistiocytosis (HLH). If corticosteroids are given, continue Posaconazole for at least 1 month after completion of corticosteroids. Posaconazole should begin on the day of TCR-T cell drug product infusion and continue until clinically indicated. Do not stop posaconazole prophylaxis if ANC < 1K/μL. Voriconazole or isavuconazole may be used if the patient had previously been taking them or if Posaconazole is not covered by insurance. In the event Posaconazole, voriconazaole, isavuconazole, or echinocandin are contraindicated or pose affordability/access issues, then use fluconazole for prophylaxis and consider aspergillus antigen testing at least once a week during corticosteroids and for at least a month after completion of corticosteroids. Patients not meeting high risk definitions will be considered to be at low risk for fungal infections and should receive prophylaxis as detailed above.

NOTE: Other anti-infective agents may be substituted at the discretion of the treating physician.

- **COVID Prophylaxis**
  - See Table 9 for recommended management of COVID-19.

Table 9: Management of COVID-19

Patient Status	Recommendations

Does not require hospitalization or supplemental oxygen	For All Patients:
	• Offer symptomatic management.
	• Recommend against the use of dexamethasone or other systemic corticosteroids in the absence of another indication.
	For All Patients:
	• Preferred therapies, which are listed in order of preference:
	  • Ritonavir-boosted nirmatrelvir (Paxlovid)
	  • Remdesivir
	• Alternative therapies for use ONLY when neither of the preferred therapies are available, feasible to use, or clinically appropriate. Alternative therapies are listed in alphabetical order:
	  • Bebtelovimab
	  • Molnupiravir

Discharged from hospital inpatient setting, in stable condition, and does not require supplemental oxygen	Discontinue the use of remdesivir, dexamethasone, or baricitinib after hospital discharge.
Discharged from hospital inpatient setting and requires supplemental oxygen (for those who are stable enough for discharge but still require oxygen)	There is insufficient evidence to recommend either for or against the continued use of remdesivir or dexamethasone.
Discharged from emergency department despite new or increasing need for supplemental oxygen (when hospital resources are limited, inpatient admission is not possible, and close follow-up is ensured)	Initiate dexamethasone 6 mg PO orally administered once daily for the duration of supplemental oxygen (dexamethasone use should not exceed 10 days) with careful monitoring for adverse events. ***Because remdesivir is recommended for patients with similar oxygen needs who are hospitalized, clinicians may consider using it in this setting. As remdesivir requires intravenous infusions for up to 5 consecutive days, there may be logistical constraints to administering remdesivir in the outpatient setting.***

10.1.2. Empiric Antibiotics

Subjects should be started on broad-spectrum antibiotics on the day of TCR-T cell drug product infusion in accordance with current institutional guidelines or at a minimum, a fever of 38.3°C (100.9°F) once or two temperatures ≥ 38.0°C (100.4°F) at least one hour apart, and an ANC <500/mm^3. Treatment with antibiotics should continue until ANC>500/mm^3 for 3 consecutive days without growth factor support. Infectious disease consultation should be obtained for all subjects with unexplained fever or any infectious complications and if the patient is allergic to quinolones and cephalosporins. Antibiotic coverage for central venous catheters may be provided at the discretion of the investigator.

10.1.3. Blood Product Support

Using daily CBCs as a guide, the subject will receive platelets and packed red blood cells as needed. All blood products will be irradiated and transfused per institutional guidelines and/or standard of care.

10.1.4. Medication for Infusion-related Reactions (IRRs)

IRRs may occur with either TCR-T cell drug product [redacted]. It may be difficult to distinguish this from mild CRS. IRR is typically characterized by fever, chills (rigor), and less commonly, hypotension. The Investigator should follow institutional practices for primary prophylaxis.

It is recommended that patients be pre-treated with acetaminophen (650 to 1000 mg) and diphenhydramine 12.5 mg (IV) or 25 to 50 mg (oral) or equivalent approximately 30 minutes prior to TCR-T cell drug product administration. Two additional doses of acetaminophen may be given every 4 to 6 hours as needed. However, the Investigator should follow institutional practices if the local standard of care is different. All pretreatment and other medications given must be recorded in the subject's eCRF.

IRR will not be considered a DLT, but it may be reason for discontinuation from treatment.

Suggested management of severe IRR symptoms is as follows:

- Allergic reaction/anaphylaxis: Stop product infusion and administer corticosteroids (**as per institutional standard**), antihistamines, famotidine, and aggressive life support, as needed. If corticosteroids are to be utilized, Sponsor must be notified within 24-48hrs.
- Bradycardia: Monitor closely, slow or stop product infusion, and continue hydration.
- Bronchospasm: Stop product infusion and administer oxygen and a bronchodilator. If severe, add corticosteroids, antihistamines, and famotidine.
- Chest tightness: Administer oxygen and slow or stop product infusion.
- Chills: Stop product infusion. If severe, administer meperidine ± hydrocortisone (**after discussion with medical monitor**). Consider blood cultures and product cultures.

- Dyspnea: Administer oxygen, slow or stop product infusion, review fluid balance, and consider diuretic.
- Erythema, rash: If hives, treat as an allergic reaction. Monitor and slow product infusion if needed.
- Fever: Consider blood cultures and product cultures. Administer acetaminophen and slow product infusion.
- Flushing: Slow product infusion and monitor; if worsens, treat as an allergic reaction.
- Headache: Slow product infusion and administer analgesia and oxygen.
- Hematuria (can occur after the infusion): Continue post-infusion hydration and monitor urine output.
- Hypertension: Slow or stop product infusion. Administer antihypertensive (hydralazine) ± diuretics.
- Hypotension: Consider medications to support blood pressure. Slow or stop product infusion and administer fluid bolus.
- Nausea/vomiting: Administer antiemetics (eg, lorazepam).
- Seizures: Stop product infusion and administer anticonvulsants and normal saline.

10.1.5. Medication for Injection Site Reactions (ISRs)

By definition, ISR includes signs and symptoms of erythema, pruritus, pain, inflammation, rash, induration, and [redacted] (Refer to Appendix F). It may occur immediately or within 24 to 48 hours post injection.

Institutional practices should be followed for management of ISRs. ISRs will not be considered a DLT, but it may be a reason for discontinuation from treatment.

10.1.6. Medication for Hypersensitivity Type 1 and Type 3

Type 1 hypersensitivity or allergic reactions are theoretically possible in response to an injected protein and may be manifested by shortness of breath, rash, anaphylaxis, or angioedema.

Immune complex-mediated Type 3 hypersensitivity reactions are similar to Type 1 hypersensitivity reactions but are likely to be delayed and may include, in addition to the aforementioned symptoms, polyarthritis, myalgia, fever, or glomerulonephritis.

The patient should be treated symptomatically with supportive care, antihistamines, and continued monitoring. Use of corticosteroids should be discussed with the Sponsor given their potential interference with TCR-T cell drug product. Symptomatic treatment should be documented in the eCRF.

10.1.7. Medication for Cytokine Release Syndrome (CRS)

CRS is commonly observed following immune-based biotherapeutics such as CAR-T cells and CD3-engaging bispecific antibodies. Subjects can present with fever, rigor, malaise, headache, nausea/vomiting, or more severe, life-threatening symptoms of hypoxia, pulmonary edema,

tachycardia, hypotension, aphasia, confusion, or seizures. Early recognition of CRS is important (e.g., monitoring of symptoms, cytokines, CRP, and ferritin); however, diagnosis is difficult due to the non-specific symptoms that are observed. Recommendations for treatment and the grading system (Appendix B) is based on (Lee et al., 2019) and is shown in Appendix J.

Premedication (tocilizumab) for CRS prophylaxis in all patients will be administered on Day-5 as part of the subject's pre-conditioning regimen (Section 8.2.5).

10.1.8. Fever (≥ 38.3°C; ≥ 101°F) Antipyretic Therapy

If grade 1 fever dose not respond to antipyretics/analgesics as needed after 24 hours initiate Actemra® (tocilizumab) therapy 8 mg/kg to be repeated every 4 to 6 hours as needed if no improvement in fever after 3 days. Dexamethasone 10 mg therapy may be administered concurrently.

Total acetaminophen (APAP) utilization is not to exceed 2 g/day.

10.1.9. Medication for Immune Effector Cell-associated Neurologic Syndrome (ICANS)

ICANS occurs frequently with CAR T cell therapy and like CRS, is associated with rapid CAR T expansion and high tumor burden as well as other factors. Severe ICANS is associated with increased CSF protein levels, encephalopathy, and other signs of neurotoxicity. Aggressive treatment is suggested and includes administering corticosteroids. Actemra® (tocilizumab) may be administered if neurotoxicity is accompanied by CRS (Appendix J).

10.1.10. Medication for Hemophagocytic Lymphohistiocytosis (HLH)/Macrophage Activation Syndrome (MAS)

Clinical and laboratory features of MAS include fever, increased ferritin levels, pancytopenia, hemophagocytosis in bone marrow or lymph nodes, fibrinolytic coagulopathy and liver dysfunction. Specific treatment includes administration of Actemra® (tocilizumab) and corticosteroids given intravenously. Canakinumab may also be used. Refer to (Center, 2020) for medication for Tumor Lysis Syndrome (TLS).

TLS occurs when tumor cells release their content in the blood stream, either spontaneously or due to treatment, leading to metabolic disturbances including hyperuricemia, hyperkalemia, hypophosphatemia, and hypocalcemia. The incidence and severity of TLS depends on tumor volume, potential for the tumor to lyse and patient characteristics. Management of cardiac and neuromuscular abnormalities and preservation of kidney function are most important.

Allopurinol can be used to reduce hyperuricemia and rasburicase can be used to preserve or improve renal function.

Because hyperkalemia can cause sudden death due to cardiac arrhythmias, patients should limit potassium and phosphorus intake during the TLS risk period (0 to 3 months).

Frequent measurements of serum electrolytes, continuous cardiac monitoring and the administration of oral sodium polystyrene are recommended. Symptomatic hypocalcemia should be treated with calcium supplementation; non-symptomatic hypocalcemia does not require treatment.

10.1.11. Medication for B cell depletion and Hypogammaglobulinemia

It is possible that B cell depletion and/or hypogammaglobulinemia will occur due to the effects of TCR-T cell drug product on normal B cells. Gammaglobulins may be administered for hypogammaglobulinemia according to institutional guidelines. At a minimum, trough IgG levels should be kept above 400 mg/dL, especially in the setting of infection (Center for International Bone Marrow Transplant Research, American Society for Bone and Morrow Transplantation).

10.1.12. Anti-Nausea and Anti-Diarrheal Medication

The choice of the prophylactic drug is up to the Investigator (with Sponsor approval) as well as the duration of treatment.

10.1.13. Pain Medication

Anti-inflammatory and opioid drugs are permitted as needed during the study as long as there is no known or expected drug-drug interaction.

10.1.14. Hematopoietic Growth Factors

The use of growth factors such as granulocyte colony-stimulating factor (G-CSF) is permitted in this study. Primary prophylactic use of growth factors may be permitted during the first 28 days or may be used during this time to treat treatment emergent neutropenia. The use of G-CSF is as indicated by the current ASCO guidelines and National Comprehensive Cancer Network (NCCN) guidelines.

Use of granulocyte/macrophage stimulating growth factor (GM-CSF) is not recommended. High dose pulsed therapy with corticosteroid is also not permitted. In general, use of systemic corticosteroids should occur after discussion with the Sponsor.

Erythropoietin may be used at the Investigator's discretion for the supportive treatment of anemia.

10.1.15. Transfusion Support

Primary transfusion support for anemia may be permitted during screening and for treatment emergent anemia as indicated by the current ASCO and AABB guidelines (Kaufman et al., 2015).

Transfusion support is permitted if completed 24 hours before TCR-T cell drug product dosing.

Primary prophylactic use of transfusion support for thrombocytopenia may be permitted in the screening period if it is completed 24 hours before TCR-T cell drug product dosing. Treatment emergent thrombocytopenia can be treated as indicated in the ASCO guidelines (Bohlius et al., 2019).

Attempts should be made to keep hemoglobin >8.0 g/dL and platelets > 20,000/mm^3. Leukocyte filters should be utilized for all blood and platelet transfusions to decrease sensitization to transfused white blood cell (WBC)'s and decrease the risk of cytomegalovirus (CMV) infection.

10.1.16. Surgery

Caution is advised on theoretical grounds for any surgical procedures during the study. The appropriate interval of time between surgery and the dose of TCR-T cell drug product required to minimize the risk of impaired wound healing and bleeding has not been determined.

10.1.17. Anti-coagulation Therapy

Subjects that are receiving maintenance anti-coagulation therapy should be transitioned to a low molecular weight heparin (LMWHs) therapy (e.g., dalteparin, enoxaparin, etc.) prior to lymphodepletion, and they should remain on the LMWH through 30 days post-TCR-T infusion, at which time they can be transitioned back to their prior anti-coagulation therapy if clinically indicated.

10.1.18. Other Concomitant Medications to Control Side Effects

Concomitant medications to control side effects of therapy may be given per institutional standard of care.

10.2. Prohibited Medications/Therapies

Except for adrenal replacement doses, chronic systemic corticosteroid use is not permitted.

Symptomatic tapering treatment and acute emergency administration, topical, inhaled, eye drops, or local injection of corticosteroids are permitted.

Corticosteroid therapy at a pharmacologic dose (> 5 mg/day of prednisone or equivalent doses of other corticosteroids) and other immunosuppressive drugs must be avoided for 7 days prior to lymphodepletion.

Supratherapeutic systemic corticosteroids are discouraged 7 days prior to TCR-T cell drug product dose.

Immunosuppressive drugs should also be avoided for 3 months after TCR-T cell drug product administration, unless used to manage TCR-T cell product related toxicities.

The use of GM-CSF is prohibited at least 7 days prior to TCR-T cell drug product administration.

Treatment such as chemotherapy, immunotherapy, targeted agents, radiation, and high dose corticosteroid, other than defined/allowed in this protocol, and other investigational agents are prohibited, except as needed for treatment of disease progression after the TCR-T cell drug product infusion.

In addition, live vaccine administration is prohibited during the study, including the treatment period, until recovery of B lymphocytes to normal ranges following the TCR-T cell drug product administration.

If permissibility of a specific medication/treatment is in question, the Medical Monitor or designee should be contacted before the medication/treatment is administered.

11. STUDY PROCEDURES

11.1. Written Informed Consent

The provided written Informed Consent Form (ICF) must be discussed with and signed by the subject before any protocol specific procedures and assessments can be performed. Adequate time for review, discussion of and answering subject's questions should be made available to the subject to allow them to make an informed decision whether they want to voluntarily participate in this clinical trial. The ICF should be discussed in a quiet setting free from distractions. A copy of the signed ICF will be given to the subject and a copy should be filed in the medical record. The original ICF should be kept on file with the study reports. Refer to Section 16.10 for further information. Patients will first sign the pre-screening consent form to determine if they have at least on TCR matching a combination of their somatic mutation and HLA type available in the Alaunos Clinical TCR Library. If the patient has at least one TCR match, they will sign the main consent form before completing screening assessments/procedures.

11.2. Subject Registration

Centralized registration of subjects will be completed according to a process defined by the sponsor. Eligible subjects will be assigned a unique study identification number. Once assigned, a subject's identification number will not be reused.

11.3. Schedule of Assessments and Observations

Screening assessments must be performed within the time frames outlined in Table 12. All study visits must be completed as described in the protocol. Follow-up assessment visit windows are shown in Table 13. Translational research samples should be collected at the visits outlined in Table 14.

11.3.1. Study Tests, Exams, and Procedures

11.3.1.1. Demographics, Medical and Cancer History

Each subject's complete medical history will be documented during screening, including demographic information, relevant medical history, current primary cancer diagnosis, and immunotherapy related biomarker diagnostics (e.g., MSI, MMR, PD-1/PD-L1), if available, prior cancer treatments (chemo- and immunotherapies, radiation therapy, surgeries, and any associated toxicities) including regimen, doses, start and stop dates, any associated residual toxicity, and best response for each regimen.

11.3.1.2. Eastern Cooperative Oncology Group (ECOG) Performance Status

The ECOG Performance Status measures the ability of cancer subjects to perform ordinary tasks. Scores range from 0 to 5 with a lower score meaning that the subject is better able to carry out daily activities. ECOG Performance Status is used to determine a subject's prognosis, to measure changes in a subject's ability to function, or to decide if a subject could be included in a clinical trial. For this trial, subjects must have an ECOG Performance Status score of 0 or 1 at the Screening Visit, apheresis eligibility check, lymphodepletion eligibility check, and TCR-T product infusion eligibility check to be included in the study.

11.3.1.3. Vital Signs

Temperature (oral, tympanic, temporal, or axillary), pulse rate, and blood pressure (BP) will be assessed and recorded either in a supine or seated position. Vital signs should be taken in the same position, if possible, throughout the study. Site is requested to make a notation of the subject's position at time of vital signs collected in site's electronic medical record.

For BP assessment, the same arm (preferably the dominant arm) should be used throughout the study. A BP cuff, which has been properly sized and calibrated, should be used to measure BP. The use of automated devices for measuring BP is acceptable.

BP and pulse measurements should be preceded by at least 5 minutes of rest for the subject in a quiet setting without distractions (e.g., television, cell phones).

Vital signs will consist of 1 pulse and 3 blood pressure measurements (3 consecutive blood pressure readings will be recorded at intervals of at least 1 minute). The average of the 3 blood pressure readings will be recorded on the eCRF. Single blood pressure readings can be performed after 14 days.

On Day 0, vitals should be obtained as per Section 8.5.3. For the remainder of the subject's inpatient stay, starting on Day 1 monitoring of vital signs must be every 4 hours (±30 mins) while awake (starting at the first vital signs taken each day; inpatient encounter assessment). For outpatients, monitoring of vital signs after TCR-T infusion (for at least 14 days post-TCR-T infusion) will be collected at least once daily for the first 7 days and every other day up to 14 days.

Vital sign assessments simultaneously scheduled with electrocardiogram (ECG) assessments should be taken after the ECG assessment. The date/time of each vital sign and pulse oximetry assessment, as well as the position of the assessment, are to be recorded in the eCRF.

11.3.1.4. Oxygen Saturation

Oxygen saturation by pulse oximeter will be obtained according to the Schedule of Assessments. Assessment of oxygen saturation is required prior to, during, and up to an hour after infusion.

11.3.1.5. Weight and Height

Weight and Height (screening only) will be measured and recorded according to Schedule of Assessments.

11.3.1.6. Physical Examination

A full physical examination includes an examination of all major body systems (including general appearance, head, ears, eyes, nose, mouth, throat, neck, thyroid, lungs, heart, breasts, abdomen, neurological examination and musculoskeletal as clinically indicated), weight, which may be performed by a physician, nurse practitioner, or other qualified health care provider, will be required as per the schedule of assessments in Table 12 and Table 13.

The use of individualized nutritional support during the course of this trial may improve important clinical outcomes. Therefore, systematically screening subjects prior to lymphodepletion, independent of their medical condition, followed by a nutritional assessment and introduction of individualized nutritional support in patients at risk or in deficit is required.

Findings should be recorded in the source documents, and any change from baseline considered by the investigation to be clinically significant should be recorded as an AE in the eCRF.

Physical examinations will also include the following neurological examinations at each timepoint:

1. Mentation
2. Cranial nerves
3. Motor
4. Reflexes
5. Sensory
6. Coordination
7. Additional tests may be performed as clinically indicated (e.g., Dix–Hallpike test, HINTS exam [Head-Impulse-Nystagmus-Test of Skew]) Neurologic findings should also be recorded in source documents, and any change from baseline considered by the Investigator to be clinically significant should be recorded as an AE in the eCRF.

11.3.1.7. Cardiac Evaluation

11.3.1.7.1. Electrocardiogram (ECG)

A single 12-lead ECG will be obtained as outlined in Table 12 and Table 13 using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and ST. Additional triplicate recordings should be taken and evaluated by a qualified medical person to confirm a prolonged QTc.

11.3.1.7.2. Echocardiogram or MUGA scan

An echocardiogram or MUGA scan will be obtained at Screening.

11.3.1.7.3. Pulmonary Function Testing (Unscheduled Only)

For subjects with a prolonged history of cigarette smoking (> 20 pack-year smoking history, with cessation within the past two years), NSCLC, clinically significant respiratory dysfunction, or other clinical indications which may include thoracic surgeries, conventional pulmonary function testing may be performed as a screen for underlying respiratory abnormalities and to provide baseline lung function measurements. Parameters should include conventional forced expiratory volume in one second (FEV1), forced vital capacity (FVC), total lung capacity (TLC) by spirometry, and also diffusing capacity of carbon monoxide (simpler single breath method is acceptable) (DLCO SB).

11.3.2. Clinical Laboratory Assessments

Refer to the Schedule of Assessments in Table 12 and Table 13 for the timing and frequency of clinical laboratory assessments. Refer to

Table 10 for additional details on the analytes for each laboratory test.

Hematology and blood chemistry will be drawn at the timepoints described in the SOA and analyzed at local laboratories.

All laboratory tests at any time during the study, which have abnormal result values that are considered clinically significant (AESIs or SAEs) should be repeated, on a schedule as determined by the Investigator, until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or medical monitor. If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, an attempt at determining the etiology should be identified and the Sponsor notified.

All protocol-required laboratory assessments, as defined below, must be conducted in accordance with the Laboratory Manual and as outlined in Table 12 and Table 13.

Additional local laboratory assessments outside of the timepoints outlined in Table 12 and Table 13 may be obtained for patient safety evaluation at the discretion of the Investigator.

The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the subject's disease unless judged by the Investigator to be more severe than expected for the subject's condition.

The below analyses will be performed at the clinical research site (

Table 10).

Table 10. Laboratory Assessments Performed Locally

Laboratory Assessments	Parameters	
Hematology	Platelet Count Red Blood Cell Count (RBC) Hemoglobin (Hb or Hgb) Hematocrit (HCT) Red Blood Cell Indices: Mean corpuscular volume (MCV) Mean corpuscular hemoglobin (MCH) Mean corpuscular hemoglobin concentration (MCHC) % Reticulocytes	White Blood Cell Count (absolute) with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils Immature cells absolute count and percent (metamyelocytes, myelocytes, promyelocytes, blasts, etc.)

Laboratory Assessments	Parameters	
Clinical Chemistry	BUN Creatinine Glucose non-fasting Magnesium (Total Mg) Chloride Potassium Sodium Calcium (Total) Lipase Albumin Uric acid CRP Amylase	AST / SGOT ALT / SGPT ALP Lactate dehydrogenase Total and direct bilirubin GGT Triglycerides Creatine Kinase as clinically indicated Total Protein Inorganic Phosphorus Potassium Total CO_2
Coagulation	INR PT APTT or PTT Ferritin ESR	Thrombin time (as clinically indicated) Fibrinogen
Urinalysis	Color Appearance Occult Blood Protein Bilirubin Urobilinogen Ketones Leukocyte esterase Microscopy* of urine sediment (if clinically indicated, i.e.., if urine dipstick is positive for occult blood, protein, nitrites or leukocyte esterase)	pH Nitrite urine Specific gravity Glucose
Viral serology as clinically indicated**	HIV I and II Hepatitis C Virus (quantitative) HbsAg: HbsAg (hepatitis B surface antigen) HbcAb (hepatitis B core antibody) HbsAb (hepatitis B surface antibody) HbeAg (hepatitis B type e antigen)	**Optional Infectious Disease Screening as clinically indicated:** Herpes virus 6 and 7 EBV T spot to assess for exposure or history of tuberculosis anti-HTLV antibody HTLV I/II Ab RPR syphilis CMV IgG and IgM West Nile Virus nucleic acid test T Cruzi antibody Varicella Zoster Virus

Laboratory Assessments	Parameters
Thyroid	free triiodothyronine (FT3) free thyroxine (FT4) thyroid stimulating hormone (TSH)
Other Laboratory Tests	**Pregnancy Tests:** FSH Serum pregnancy test or urine pregnancy test **Cytokines:** The following may be included in the local testing panel: pro-inflammatory and immune modulating cytokines: IL- 6, TNF-α, and IFN-γ; immune effector molecules: Granzyme A, Granzyme B, Perforin, and sFasL; and chemokines: MIP-1α MCP-1 and haptoglobin Optional cytokines that may be tested include, but are not limited to: IL-8, IL-1β, IL-2, IL-7, IL-10, IP-10, GM-CSF, and IL-15 **TBNK:** TBNK (T cell, B cell, and NK cell counts)
Urine Microscopy*	Crystalluria calcium oxalate and/or hippurate crystals Leukocyturia White and red cell casts Hyaline casts Granular cast Waxy casts Fatty casts Broad casts (Broad waxy casts)
CSF (Refer to Section 11.3.7)	Cerebrospinal pressure Proteins WBC cells/cell subsets Cytokines Viruses (including HHV 6 and 7), bacteria, other organisms if suspected

ALP = alkaline phosphatase; ALT = alanine aminotransferase; APTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CMV = cytomegalovirus; CO_2 = carbon dioxide; CRP = C-reactive protein; EBV = Epstein-Barr virus; ESR= erythrocyte sedimentation rate; FSH = follicle-stimulating hormone; GGT = gamma-glutamyl transpeptidase ; HBV = hepatitis B; HCV = hepatitis C; HHV = Human herpes virus; HIV = human immunodeficiency virus; HTLV = human T cell lymphotropic virus; INR = international normalized ratio; LDH = lactate dehydrogenase; MCH = mean cell hemoglobin; MCV = mean cell volume; PT = prothrombin time; PTT= partial prothrombin time; RBC = red blood cell; RPR = rapid plasma reagin; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; WBC = white blood cell

*NOTE: Subjects with urine dipstick results that are positive for occult blood, protein, nitrites or leukocyte esterase will have urine microscopy sample collected

***NOTE:* Subjects with hepatitis B (HbsAg+) who have controlled infection (serum hepatitis B virus DNA PCR that is below the limit of detection AND receiving anti-viral therapy for hepatitis B) are permitted. Subjects with

controlled infections must undergo periodic monitoring of HBV DNA. Subjects must remain on anti-viral therapy for at least 6 months beyond the last dose of investigational study drug.

NOTE: Subjects who are hepatitis C virus antibody positive (HCV Ab+) who have controlled infection (undetectable HCV RNA by PCR either spontaneously or in response to a successful prior course of anti-HCV therapy) are permitted.

11.3.2.1. Hemophagocytic Lymphohystiocytosis (HLH) and Macrophage Activation Syndrome (MAS) Assessment

Subjects should be assessed for HLH and MAS at the timepoints specified in Table 12 and Table 13. HLH and MAS assessments will be performed at screening, prior to lymphodepletion (Day -8) and Day -1. If HLH/MAS is suspected during the study, serum ferritin, triglycerides, haptoglobin will be measured as clinically indicated. Refer to Center, 2020 and Appendix D for diagnostic criteria of HLH and MAS.

- Clinical and laboratory features of MAS include fever, increased ferritin levels, pancytopenia, hemophagocytosis in bone marrow or lymph nodes, fibrinolytic coagulopathy, and liver dysfunction. Specific treatment includes administration of Actemra® (tocilizumab) and corticosteroids given intravenously. Canakinumab may also be used.

11.3.3. HLA Class I and Class II Allele Determination

A blood sample will be collected and submitted to a CLIA-certified laboratory for testing using next generation sequencing for HLA class I and class II genes as part of the initial pre-screening if results are not already available to meet inclusion criteria.

11.3.4. Tumor Neoantigen Determination

To determine somatic mutations, tumor tissue sample (collected during biopsy or resection conducted as part of standard-of-care treatment) that in some instances may be paired with a blood sample, archived tumor tissue, or a liquid biopsy will be sent to a CLIA-certified (or accredited) laboratory for molecular genetic test (e.g., OncomineTM) as part of the initial pre-screening if such diagnostic results are not already available.

11.3.5. Imaging

Tumor response will be obtained using imaging scans including CT or MRI and evaluated as defined by RECIST guidelines (v1.1) (Eisenhauer et al., 2009) for assessment of tumor burden and disease progression. Follow-up imaging studies will be performed using the same imaging technique utilized at screening. Refer to Section 12 for further details. Scans will be provided to the central imaging vendor in the event the sponsor wants to perform central reads.

11.3.6. Assessment of Cytokine Release Syndrome (CRS)

Assessment of CRS includes grading of the following CRS parameters: fever, hypotension, hypoxia according to the ASTCT Grading guidance (refer to Appendix B). CRS grade should be determined at least twice daily where indicated in Table 12 and Table 13 and any time there is a change in subject's status.

11.3.7. Cerebrospinal Fluid (CSF)

Subjects with symptoms of central nervous system malignancy such as new onset of severe headaches, neck stiffness, or any focal neurologic findings on physical exam will have lumbar puncture performed at the screening visit for examination of cerebral spinal fluid. Refer to

Table 10 for CSF testing parameters. Lumbar puncture may be performed as applicable for patients with new onset of ≥ grade 2 neurotoxicities post-TCR-T cell drug product administration.

11.3.8. Assessment of Immune Effector Cell Associated Neurotoxicity Syndrome (ICANS) and Immune-effector Cell Encephalopathy (ICE) Score

A thorough neurologic exam, including imaging of the brain (preferably MRI with and without contrast but CT without contrast is acceptable if MRI cannot be performed) to rule out any central nervous system disease and also to serve as a baseline for comparison in case the subject develops ICANS; diagnostic lumbar puncture, and ICE Score (refer to Appendix C and Appendix E). Assessment and grading for ICANS should be completed at least every 12 hours where indicated in Table 12 and Table 13 including the ICE scoring. Care giver can be trained to administer this test if subject is outpatient. This information may be requested from the care giver.

11.3.9. Immunosuppression Utilization

Use of IL-6 antagonists and alternative immunosuppressive agents (including but not limited to tocilizumab, siltuximab, anakinra) for the management of CRS and ICANS will be recorded and analyzed throughout the study.

11.3.10. Handwriting Sample and Temperature Log

Subjects should be instructed to complete daily temperature readings and handwriting samples in the Handwriting and Temperature log from time of discharge through Day 28. Subjects should be instructed to return the log when they return for their Week 4 visit. Additional samples may be collected as clinically indicated. Caregiver should be trained in this process and may be asked to return these logs to the study coordinator. Both the Handwriting Sample and the Temperature Log will be submitted centrally to allow for Medical Monitor review.

11.3.11. Tumor Markers

Tumor markers will include the following per tumor subgroup listed below in Table 11.

Note: If a conventional tumor marker is found to be elevated at baseline, a repeat scheduled/unscheduled sample should be obtained to assess for decrease to baseline/ normalization if indicated to confirm a complete clinical response in conjunction with radiologic studies in accordance with the RECIST v1.1 guideline.

Table 11. Subgroup Specific Tumor Markers

	Subgroup					
Tumor Marker	**1: Ovarian**	**2: Endometrial**	**3: Colorectal**	**4: Pancreatic**	**5: NSCLC**	**6: Cholangiocarcinoma**
alpha-fetoprotein (AFP)					X	X
Calretinin	X					
Cancer Antigen 125 (CA-125)	X	X				
Carcinoembryonic Antigen (CEA)			X	X		X
C-Reactive Protein (CRP)			X	X		
Cancer Antigen 19-9 (CA19-9)			X	X		X
CK19, K19					X	
Microsatellite instability			X			

11.3.12. Collection of Biospecimens for Translational Research Assessments

Blood and tissue specimens for research will be collected throughout the study to evaluate the metrics as outlined in Section 14.1. Samples will be collected according to the schedule specified in Table 14. Procedures regarding the collection, processing, storage, and shipment of biospecimens are detailed in the Study Laboratory Manual.

11.3.13. Adverse Events Assessment and Grading

Adverse events will be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 criteria. Reporting from the time of informed consent until lymphodepletion will only include AEs and SAEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw). AEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw) will be recorded as adverse events in the CRF as outlined in Section 13.6. SAEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw) will be recorded in the CRF as outlined in Section 13.6 and reported to Sponsor as defined in Section 13.8. AEs and SAEs considered not protocol-related should be considered medical history.

The reporting period of all adverse events and serious adverse events will begin from the start of lymphodepletion through end of study regardless of relationship to study treatment. Refer to Section 13 for more details.

11.3.14. Concomitant Medications

Information on concomitant medications, including all medications, blood products, vitamins, and other supplements, will be collected from informed consent through the subject's last study visit.

11.3.15. Schedule of Assessments

The schedule of assessments is provided in Table 12 and Table 13.

Table 12. Schedule of Assessments – Pre- TCR-T Cell Drug Product Infusion

Study Period	**Pre-Screening[A]**	**Screening[1]**	**Pre-Treatment**			**Treatment**							
			Pre-Apheresis	**Apheresis[32]**		**Lymphodepletion**							**Pre-TCR T-cell drug product infusion**
Assessment					**Day -8**	**Day -7**	**Day -6**	**Day -5**	**Day -4**	**Day -3**	**Day -2**	**Day -1**	**Day 0**
Visit Window		≤ 2 weeks from signing the screening consent	≤ 2 days prior to apheresis										
Informed Consent[2]	X[A]	X											
Medical History[3]	X	X											
Physical Exam[4]		X			X							X	X
Nutrition Consultation					X[4]								
ECOG PS	X	X			X							X	X
Weight (Height at screening only)		X				X							X
Vital Signs[5]		X	X		X	X	X	X	X	X		X	X
Oxygen Saturation[6]		X	X*		X	X	X	X	X	X		X	X
Pregnancy Test[7]		X	X		X								
Urinalysis[8]		X			X							X	
Telemetry (optional)													X
ECHO or MUGA[9]		X	X*		X[9]								
ECG[10]		X			X								X
Adverse Events[11]	CONTINOUS												
Concomitant Medications[12]	CONTINOUS												

Study Period	**Pre-Screening[A]**	**Screening[1]**	**Pre-Treatment**			**Treatment**							
			Pre-Apheresis	**Apheresis[32]**		**Lymphodepletion**							**Pre-TCR T-cell drug product infusion**
Assessment					**Day -8**	**Day -7**	**Day -6**	**Day -5**	**Day -4**	**Day -3**	**Day -2**	**Day -1**	**Day 0**
Visit Window		≤ 2 weeks from signing the screening consent	≤ 2 days prior to apheresis										
Immunosuppression Utilization	CONTINOUS												
Overall Survival	CONTINOUS												
Infectious Disease Testing[13]		X											
Hematology[14]		X	X**		X[14]	X	X	X	X	X		X	X
Blood Chemistry[14]		X	X*		X[14]	X	X	X	X	X		X	X
Coagulation Panel[14]					X[14]	X	X	X	X	X			
TBNK					X[14]								
Thyroid Panel[14]					X[14]								
CT or MRI[15]		X			X[16]								
Neoantigen Determination[17]	X												
HLA Allele Determination[17]	X												
Eligibility[18]	X	X	X		X							X	X
Enrollment[18]					X								
Tumor Markers[19]					X								
Lumber Puncture[20]		X											
Local assessment of cytokines[14]					X							X	

Study Period	**Pre-Screening[A]**	**Screening[1]**	**Pre-Treatment**			**Treatment**							
			Pre-Apheresis	**Apheresis[32]**		**Lymphodepletion**							**Pre-TCR T-cell drug product infusion**
Assessment					**Day -8**	**Day -7**	**Day -6**	**Day -5**	**Day -4**	**Day -3**	**Day -2**	**Day -1**	**Day 0**
Visit Window		≤ 2 weeks from signing the screening consent	≤ 2 days prior to apheresis										
Hospitalization[21]												X	X
HLH/MAS Assessment[22]		X				X						X	
Assessment of ICANS including ICE score[23]												X	X
Handwriting Sample and Temperature Log[164]													
Bridging Therapy[25]				X	X								
Infection Prophylaxis[17]					X	X	X	X	X	X	X	X	X
Apheresis[18]				X									
Lymphodepletion [19]						X	X	X	X	X			
CRS Prophylaxis[20]								X					
Injection Site Reaction Assessment[30]													
TCR-T Pre-medication[21]													X

[A] Patients will initially sign the pre-screening consent form to determine if the subject has at least one TCR matching a combination of their somatic mutation(s) and HLA type is available in Alaunos's Clinical TCR library. If subjects don't have at least one TCR match identified, they will be discontinued and will not move forward with further screening for the study.

[*] Assessments completed for the screening eligibility review will satisfy this criterion if apheresis is scheduled within 28 days of the initial screening

[**] Assessments completed for the screening eligibility review will satisfy this criterion if apheresis is scheduled within 7 days of the initial screening

[1] Screening assessments must be performed within 2 weeks after the subject signs the main consent form.

[2] Informed consent must be obtained prior to undergoing any study specific procedures. The main consents for the protocol TCR001-201 and the consent for protocol TCR001-202 (LTFU Protocol) must be obtained at the same time.

[3] Medical history includes demographic information, relevant medical history, current primary cancer diagnosis, and immunotherapy related biomarker diagnostics (e.g., MSI, MMR, PD-1/PD-L1), if available, prior cancer treatments (chemo- and immunotherapies, radiation therapy, surgeries, and any associated toxicities) including regimen, doses, start and stop dates, any associated residual toxicity, and best response for each regimen.

[4] A complete physical examination including, but not limited to cardiovascular, dermatological, gastrointestinal, musculoskeletal, neurological, pulmonary systems. Refer to Section 11.3.1.6 for full guidance on physical examinations. Patients with symptoms of central nervous system malignancy such as new onset severe headaches, neck stiffness, or any focal neurologic findings on PE will have lumbar puncture performed at the screening visit for examination of cerebral spinal fluid. Symptom directed PEs should be performed as appropriate at each time point and on an as needed basis for assessment of AEs. Symptom directed PE can be performed only if clinically indicated. Toxicity assessment will be ongoing and any toxicities or infections will be evaluated. The use of individualized nutritional support during the course of this trial may improve important clinical outcomes. Therefore, systematically screening subjects prior to lymphodepletion, independent of their medical condition, followed by a nutritional assessment and introduction of individualized nutritional support in patients at risk or in deficit is required.

[5] Vital signs should be collected before blood collection on days where laboratory tests are collected. Vital signs include body temperature (oral, tympanic, temporal or axillary), blood pressure, and pulse rate to be recorded in the sitting position after 5 minutes of rest. Vital signs will consist of 1 pulse and 3 blood pressure measurements (3 consecutive blood pressure readings will be recorded at intervals of at least 1 minute). The average of the 3 blood pressure readings will be recorded on the eCRF. Single blood pressure readings can be performed after 7 days. On Day 0, vitals should be obtained as per Section 8.5.3 . For the remainder of the subject's inpatient stay, starting on Day 1 monitoring of vital signs must be every 4 hours (±30 mins) while awake (starting at the first vital signs taken each day; inpatient encounter assessment). For outpatients, monitoring of vital signs after TCR-T infusion (for at least 14 days post-TCR-T infusion) will be collected at least once daily for the first 7 days and every other day up to 14 days. Vital sign assessments simultaneously scheduled with electrocardiogram (ECG) assessments should be taken after the ECG assessment. The date/time of each vital sign and pulse oximetry assessment, as well as the position of the assessment, are to be recorded in the eCRF.

[6] Oxygen saturation by pulse oximeter will be obtained. Assessment of oxygen saturation is required prior to, during and up to an hour after infusion.

[7] Females of childbearing potential will have a serum or urine pregnancy test at the Screening Visit and a serum pregnancy test within 7 days prior to lymphodepletion, with a negative pregnancy outcome prior to treatment. Females of non-childbearing potential is defined as: Achieved postmenopausal status, defined as follows: cessation of regular menses for at least 12 consecutive months with no alternative pathological or physiological cause; status may be confirmed with a serum follicle stimulating hormone (FSH) level confirming the postmenopausal state, have undergone a documented hysterectomy and/or bilateral oophorectomy, or have medically confirmed ovarian failure.

[8] The urinalysis panel (dipstick) is required and includes color, appearance, pH, specific gravity, glucose, protein/albumin, and presence of blood, ketones, bilirubin, urobilirubin, nitrates, and leukocyte esterase. In addition, a microscopic exam for casts, crystals, and cells will be done if clinically indicated (i.e., if urine dipstick is positive for occult blood, protein, nitrites or leukocyte esterase).

[9] A baseline ECHO or MUGA must be performed to measure left ventricular ejection fraction (LVEF). The same modality will be used throughout the study. The ECHO or MUGA collected to confirm eligibility prior to lymphodepletion can be collected within 28 days prior to Day -8.

[10] At each time point, 12 lead ECG will be performed to determine heart rate, PR, QRS, QT, ST and mean QTcF intervals. On Day 0, triplicate ECG will be performed prior to TCR-T cell drug product administration and at the end of infusion. At other time points, a single ECG can be performed. If the mean QTcF is prolonged (>500 msec), or > than 60 msec change from baseline of QTc, the ECG should be re-evaluated by a qualified person at the institution for confirmation, and triplicate ECGs may be performed as clinically indicated.

11 Adverse events will be assessed according to the NCI CTCAE v5.0 criteria. Reporting from the time of informed consent until lymphodepletion will only include AEs and SAEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw). AEs that have an attribution of at least possibly

related to a study procedure (e.g., blood draw) will be recorded as adverse events in the CRF as outlined in Section 13.6. SAEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw) will be recorded in the CRF as outlined in Section 13.6 and reported to Sponsor as defined in Section 13.8. AEs and SAEs considered not protocol-related should be considered medical history. The reporting period of all adverse events and serious adverse events will begin from the start of lymphodepletion through end of study regardless of relationship to study treatment. Refer to Section 13 for more details. SAEs must be reported within 24-hours of awareness. After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up. Refer to Section 13 Safety Assessments for more information on AE/SAE reporting including what is not considered an AE/SAE.

[12]Concomitant medications will be monitored and recorded throughout the study. Medications received in the period preceding consent (~28 days), in addition to those ongoing at screening, will be captured in the eCRF. Concomitant medications include all medications, blood products, vitamins and other supplements and will be collected from informed consent through the subject's last visit.

[13]Serology testing for HIV, Hepatitis B, Hepatitis C, Cytomegalovirus, Herpes Simplex Virus, Epstein-Barr Virus, Varicella Zoster Virus, and other viruses according to institutional practice.

[14]Refer to Table 10 for analytes. Blood samples collected to confirm eligibility prior to lymphodepletion may be collected within the 3 days prior to Day -8

[15]Appropriate cancer staging procedures should be performed during screening per RECIST v1.1. For the purpose of this clinical trial, the following imaging is expected at screening: a. CT of the chest, and CT (or MRI) of the abdomen b. MRI (or CT) of the brain, if brain metastases are known or suspected c. CT or MRI of other anatomical regions as clinically indicated. All imaging should be of diagnostic quality and include IV contrast. Disease sites are to be imaged throughout the study using the same method(s) used at screening. Chest and abdomen imaging are required for all follow-up imaging time points; images of the brain and other anatomical regions should be acquired on follow-up if positive at screening and as clinically indicated.

[16]Subjects that are treated with bridging therapy will have an additional CT or MRI to serve as a baseline assessment. The CT or MRI collected to confirm eligibility prior to lymphodepletion can be collected within 7 days prior to Day -8 (preferred) or at Day -2.

[17]For somatic mutation determination, prior genetic testing results, archival tissue, or a new tumor biopsy and a blood sample (if being performed as standard-of-care) is required. If no prior mutation results are available, and a re-biopsy is not feasible, a liquid biopsy must be performed for somatic mutation determination. Results of a high resolution, HLA class I and II typing from a CLIA laboratory will be collected if previously performed. In the absence of such a result, a blood sample will be collected and submitted to a CLIA-certified or -accredited laboratory for testing.

[18]All patients will need to meet the screening eligibility criteria requirements. Once deemed eligible, the subject's dose level will be assigned by the Sponsor. Subjects will be considered enrolled into the study once eligibility for lymphodepletion has been approved by the Alaunos Medical Monitor or designee. Subject eligibility criteria should be re-assessed prior to treatment with TCR-T cell drug product to ensure the safety of the subject. Refer to Section 6.1.

[19]Tumor markers will be assessed as listed in Table 10 . If markers are not elevated at baseline, additional sample collection is not required.

[20]Subjects with symptoms of central nervous system malignancy such as new onset severe headaches, neck stiffness, or any focal neurologic findings on physical exam will have lumbar puncture performed at the screening visit for examination of cerebral spinal fluid. Refer to Section 11.3.7 for additional requirements. Lumbar puncture may be performed as applicable for patients with new onset of ≥ grade 2 neurotoxicities post TCR-T cell drug product administration.

[21]Lymphodepletion will occur on an out-patient basis unless hospitalization is clinically determined to be in the best interest of the patient. Subjects may be hospitalized from Day -1 through Day 7 or longer if medically indicated. Duration of hospitalization may be decreased once safety profile has been determined. Subjects must stay within close proximity of the treating hospital for 28 days after TCR-T cell drug product administration.

[22]HLH and MAS assessments will be performed at screening, Day -7 prior to lymphodepletion and Day -1. If HLH/MAS is suspected, serum ferritin, triglycerides, haptoglobin will be measured.

[23]Neurological assessment: ASTCT Grading of ICANS: Q12 hours while in hospital. ICE score does not have to be collected in the hospital and only upon discharge. When not collected in the log, ICE will be captured in the hospital source documents. If this is not within institutional practice, continue the recording on the log.

[24]Subjects should be instructed to complete daily temperature readings and handwriting samples in Handwriting and Temperature log from time of discharge through Day 28. Subjects should be instructed to return the log when they return for their Week 4 visit.
[25]Subjects will be allowed to receive bridging therapy following apheresis after consultation with the Alaunos Medical Monitor. The consultation with the Alaunos Medical Monitor will determine the type and duration of bridging therapy based on the subject's tumor type. Subjects who have sufficient lymphodepletion from bridging therapy do not need additional lymphodepletion as described in Section 8.2 prior to infusion of TCR-T cell drug product.
[26]Infection prophylaxis should be given according to institutional guidelines. In the absence of institutional guidelines, refer to Section 10.1.1 for recommendations on prophylaxis medications.
[27]Apheresis must be approved by Alaunos to accommodate receipt and processing of apheresis at the manufacturing facility.
[28]Lymphodepleting chemotherapy will follow the schedule outlined in Table 7 in Section 8.2. Lymphodepletion may commence following notification from Alaunos that the subject's TCR-T cell drug product has been manufactured and Certification of Analysis is available.
[29]Subjects will receive a single, intravenous dose (600mg) of tocilizumab as part of their preconditioning regimen. NOTE: Substitutions may be considered due to supply issues after discussion between the investigator and Alaunos Medical Monitor.
[30]Subjects should be monitored for injection site reactions at 1 and 3hrs post- infusion of TCR-T cell drug product [redacted] at 1 and 3hrs post. Refer to Section 9.4.7 and Section 10.1.5 for further details.
[31]All subjects should receive TCR-T cell drug product pre-medication as outlined in Section 8.5.1.
[32] The apheresis collection will occur at any time during the subject's clinical course after the subject meets the eligibility criteria for apheresis collection. Manufacturing will begin once receipt, inspection, and release of the leukapheresis product is completed and a manufacturing slot is available.

Table 13. Schedule of Assessment – Post-TCR-T Cell Drug Product Infusion

Study Period	Treatment									Follow-Up				Un-scheduled Visit
	TCR-T Cell Drug Product Infusion	Post TCR-T Cell Drug Product Infusion												
Assessment	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Week 2 & 4 (±3 days)**	**Week 6 (±1 week)**	**Week 12 (±2 weeks)**	**Every 3 months[1] (±2 weeks)**	**Early Discontinuation Visit[24]**	
Physical Exam[2]		X	X	X	X	X	X	X	X	X	X	X	X	X
ECOG PS									X	X	X	X		X
Weight (Height at screening only)														X
Vital Signs[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Oxygen Saturation [4]	X	X	X	X	X	X	X	X	X	X				X
Pregnancy Test[5]														X
Urinalysis[6]									X	X	X	X	X	X
Telemetry (optional)		X	X	X										X
ECHO or MUGA[7]														X
ECG [8]		X	X	X									X	X
Adverse Events[9]	CONTINUOUS													
Concomitant Medications[10]	CONTINUOUS													
Immunosuppression Utilization	CONTINUOUS													

Study Period	**Treatment**									**Follow-Up**				**Un-scheduled Visit**
	TCR-T Cell Drug Product Infusion	**Post TCR-T Cell Drug Product Infusion**												
Assessment	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Week 2 & 4 (±3 days)**	**Week 6 (±1 week)**	**Week 12 (±2 weeks)**	**Every 3 months[1] (±2 weeks)**	**Early Discontinuation Visit[24]**	
Overall Survival	CONTINUOUS													
Infectious Disease Testing [11]														X
Hematology[12]		X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Chemistry[12]		X	X	X	X	X	X	X	X	X	X	X	X	X
Coagulation panel[12]		X			X			X	X	X	X	X	X	X
TBNK		X	X	X	X	X	X	X	X	X	X	X		X
Thyroid Panel[12]									X	X	X	X		X
CT or MRI[13]										X	X	X		X
Tumor Markers[14]											X	X		X
Assessment of CRS[15]		X	X	X	X	X	X	X						X
Lumbar Puncture[16]														X
Local assessment of cytokines[12]		X	X	X	X	X	X	X						X
Hospitalization[17]	X	X	X	X	X	X	X	X						
HLH/MAS Assessment[18]														X

Study Period	Treatment									Follow-Up				Un-scheduled Visit
	TCR-T Cell Drug Product Infusion	Post TCR-T Cell Drug Product Infusion												
Assessment	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Week 2 & 4 (±3 days)**	**Week 6 (±1 week)**	**Week 12 (±2 weeks)**	**Every 3 months[1] (±2 weeks)**	**Early Discontinuation Visit[24]**	
Assessment of ICANS including ICE score[19]		X	X	X	X	X	X	X	X	X				X
Handwriting Sample and Temperature Log[20]		X	X	X	X	X	X	X	X	X				X
Infection Prophylaxis[21]	→													
[redacted]														
Injection Site Reaction Assessment[23]	X	X	X	X	X									X
TCR-T cell drug product infusion	X													

[1] Visits occur every 3 months from Week 12 until subjects complete the 2-year follow-up or discontinue due to disease progression.

[2] A complete physical examination including, but not limited to cardiovascular, dermatological, gastrointestinal, musculoskeletal, neurological, pulmonary systems. Refer to Section 11.3.1.6 for full guidance on physical examinations. Patients with symptoms of central nervous system malignancy such as new onset severe headaches, neck stiffness, or any focal neurologic findings on PE will have lumbar puncture performed at the screening visit for examination of cerebral spinal fluid. Symptom directed Pes should be performed as appropriate at each time point and on an as needed basis for assessment of AEs. Symptom directed PE can be performed only if clinically indicated. Toxicity assessment will be ongoing and any toxicities or infections will be evaluated.

[3] Vital signs should be collected before blood collection on days where laboratory tests are collected. Vital signs include body temperature (oral, tympanic, temporal or axillary), blood pressure, and pulse rate to be recorded in the sitting position after 5 minutes of rest. Vital signs will consist of 1 pulse and 3 blood pressure measurements (3 consecutive blood pressure readings will be recorded at intervals of at least 1 minute). The average of the 3 blood pressure readings will be recorded on the eCRF. Single blood pressure readings can be performed after 7 days. On Day 0, vitals should be obtained as per Section 8.5.3 . For the remainder of the subject's inpatient stay, starting on Day 1 monitoring of vital signs must be every 4 hours (±30 mins) while awake (starting at the first vital signs taken each day; inpatient encounter assessment). For outpatients, monitoring of vital signs after TCR-T infusion (for at least 14 days post-TCR-T infusion) will be collected at least once daily for the first 7 days and every other day up to 14 days. Vital sign assessments simultaneously scheduled with electrocardiogram (ECG) assessments

should be taken after the ECG assessment. The date/time of each vital sign and pulse oximetry assessment, as well as the position of the assessment, are to be recorded in the eCRF.

[4] Oxygen saturation by pulse oximeter will be obtained. Assessment of oxygen saturation is required prior to, during and up to an hour after infusion.

[5] Females of childbearing potential will have a serum or urine pregnancy test at the Screening Visit and a serum pregnancy test within 7 days prior to lymphodepletion, with a negative pregnancy outcome prior to TCR-T cell drug product infusion.

[6] The urinalysis panel (dipstick) is required and includes color, appearance, pH, specific gravity, glucose, protein/albumin, and presence of blood, ketones, bilirubin, urobilirubin, nitrates, and leukocyte esterase. In addition, a microscopic exam for casts, crystals, and cells will be done if clinically indicated (i.e.., if urine dipstick is positive for occult blood, protein, nitrites or leukocyte esterase).

[7] A baseline ECHO or MUGA must be performed to measure left ventricular ejection fraction (LVEF). The same modality will be used throughout the study.

[8] At each time point, 12 lead ECG will be performed to determine mean QTcF interval. On Day 0, triplicate ECG will be performed prior to TCR-T administration and at the end of infusion. At other time points, a single ECG can be performed. If the mean QTcF is prolonged (>500 msec), or > than 60 msec change from baseline of QTc, the ECG should be re-evaluated by a qualified person at the institution for confirmation, and triplicate ECGs may be performed as clinically indicated.

[9] Adverse events will be assessed according to the NCI CTCAE v5.0 criteria. Reporting from the time of informed consent until lymphodepletion will only include AEs and SAEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw). AEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw) will be recorded as adverse events in the CRF as outlined in Section 13.6. SAEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw) will be recorded in the CRF as outlined in Section 13.6 and reported to Sponsor as defined in Section 13.8. AEs and SAEs considered not protocol-related should be considered medical history. The reporting period of all adverse events and serious adverse events will begin from the start of lymphodepletion through end of study regardless of relationship to study treatment. Refer to Section 13 for more details. SAEs must be reported within 24-hours of awareness. After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up. Refer to Section 13 Safety Assessments for more information on AE/SAE reporting including what is not considered an AE/SAE.

[10] Concomitant medications will be monitored and recorded throughout the study. Medications received in the period preceding consent (~28 days), in addition to those ongoing at screening, will be captured in the eCRF. Concomitant medications include all medications, blood products, vitamins and other supplements and will be collected from informed consent through the subject's last visit

[11] Serology testing for HIV, Hepatitis B, Hepatitis C, Cytomegalovirus, Herpes Simplex Virus, Epstein-Barr Virus, Varicella Zoster Virus, and other viruses according to institutional practice

[12] Refer to Table 10 for analytes.

[13] Appropriate cancer staging procedures should be performed during screening per RECIST v1.1. For the purpose of this clinical trial, the following imaging is expected at screening: a. CT of the chest, and CT (or MRI) of the abdomen b. MRI (or CT) of the brain, if brain metastases are known or suspected c. CT or MRI of other anatomical regions as clinically indicated. All imaging should be of diagnostic quality and include IV contrast. Disease sites are to be imaged throughout the study using the same method(s) used at screening. Chest and abdomen imaging are required for all follow-up imaging time points; images of the brain and other anatomical regions should be acquired on follow-up if positive at screening and as clinically indicated.

[14] Tumor markers will be assessed as listed in Table 11. If markers are not elevated at baseline, additional sample collection is not required.

[15] Cytokine release syndrome grading should be assessed per ASTCT Consensus Guidelines. Refer to Appendix B. Assessment and grading of CRS should occur Q12 hours while in hospital. Toxicity assessment will also include HLH and MAS which may occur in the setting of CRS.

[16] Subjects with symptoms of central nervous system malignancy such as new onset severe headaches, neck stiffness, or any focal neurologic findings on physical exam will have lumbar puncture performed at the screening visit for examination of cerebral spinal fluid. Refer to Section 11.3.7 for additional requirements. Lumbar puncture may be performed as applicable for patients with new onset of ≥ grade 2 neurotoxicities post TCR-T cell drug product administration.

[17]Lymphodepletion will occur on an out-patient basis unless hospitalization is clinically determined to be in the best interest of the patient. Subjects may be hospitalized from Day -1 through Day 7 or longer if medically indicated. Duration of hospitalization may be decreased once safety profile has been determined. Subjects must stay within close proximity of the treating hospital for 28 days after TCR-T cell administration.

[18]HLH and MAS assessments will be performed at screening, Day -7 prior to lymphodepletion and Day -1. If HLH/MAS is suspected, serum ferritin, triglycerides, haptoglobin will be measured.

[19]Neurological assessment: ASTCT Grading of ICANS: Q12 hours while in hospital. ICE score does not have to be collected in the hospital and only upon discharge. When not collected in the log, ICE will be captured in the hospital source documents. If this is not within institutional practice, continue the recoding on the log.

[20]Subjects should be instructed to complete daily temperature readings and handwriting samples in Handwriting and Temperature log from time of discharge through Day 28. Subjects should be instructed to return the log when they return for their Week 4 visit.

[21]Infection prophylaxis should be given according to institutional guidelines. In the absence of guidelines, refer to Section 10.1.1 for recommendations on prophylaxis medications.

[redacted]

[23]Subjects should be monitored for injection site reactions at 1 and 3hrs post- infusion of TCR-T cell drug product [redacted]. Refer to Section 9.4.7, Section 10.1.4, and Section 10.1.5 for further details.

[24]Early discontinuation is at the time the subject discontinues from the study (e.g., due to disease progression, initiation of new-anticancer therapy, withdraw consent, etc.). Subjects should be questioned regarding their reason for withdrawal. These reasons must be documented in the subject's medical record and on the eCRF.

Table 14. Schedule of Sample Collection for Translational Research Assessments

	Baseline		Pre-TCR -T Cell Drug Product Infusion	Post TCR-T Cell Drug Product Infusion									Follow-Up					
				Days (± days)									Weeks (± weeks)		Months		Unscheduled	
Sample Collection	**After MM sign off on screening eligibility**	**≤ 7 Days Prior to Lympho-depletion**	**Day 0[1]**	**0 (+30 minutes)[2]**	**1**	**2**	**4**	**7**	**10 (± 2)**	**14 (± 2)**	**21 (± 2)**	**28 (± 2)**	**6 (± 1)**	**12 (± 2)**	**6, 9,12 (± 2 weeks)**	**15, 18, 21, 24 (± 2 weeks)**	**Progression or Suspected Progression[10]**	**SAE[3]**
Peripheral Blood																		
TCR-T Cellular Kinetics[4]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
TCR-T Insertion-Site Clonality		X													X[5]	X[5]		
Immunophenotyping		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Gene Expression		X	X	X						X		X	X	X	X		X	X
Genomic Analysis		X	X	X						X		X	X	X			X	X
Immunogenicity (anti-drug T cells)		X													X[6]			X
Biorepository[7]		X						X		X		X		X			X	
Serum																		
Cytokines		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
Immunogenicity (anti-drug antibodies)		X													X[5]			X
Plasma (Streck)																		
ctDNA		X									X		X	X	X	X	X	X
Tissue																		
Tumor Tissue	X[8]													X[9]			X	

[1] Within 4 hours prior to TCR-T cell drug product infusion

[2] Within 30 minutes after completion of TCR-T cell drug product infusion. Infusion length will vary based on dose level and could be up to 180minutes, but collection time should be adjusted accordingly to the previous sentence of this footnote.

[3] Samples should be collected at the time of SAEs (within 1 hour [±1 hour] of awareness) that are known to be related to TCR-T cell drug product as specified in Section 9.3.

[4] Subjects will be evaluated TCR-T cell drug product persistence [redacted] and TCR-T cell drug product transposon insertion-site clonality until the end of the study or until 2 consecutive tests demonstrate that there is no TCR-T cell drug product persistenc [redacted]).

[5] Months 6, 12, 18 and 24 (±2 weeks) only

[6] Month 6 (±2 weeks) only

[7] Samples for exploratory biomarker analysis

[8]Tumor biopsies performed for the purposes of providing baseline tumor tissue may be performed at any time after subjects meet screening eligibility criteria and at least a week prior to lymphodepletion. If the lesion intended for biopsy is locate in the lung, the risk of pneumothorax should be discussed with the Alaunos Medical Monitor. Baseline tumor tissue may be archival tumor tissue (≤6 months old relative to date of enrollment). Archival tissue >6 months relative to the date of enrollment may be accepted after discussion with Sponsor.

[9] Tumor biopsy of non-target lesion must be collected prior to Week 12 tumor imaging scan.

[10] Samples will be collected at time of progression or suspected progression.

11.3.15.1. Contraception

All participants of child-bearing potential must practice effective birth control while on study. Acceptable forms of birth control for female subjects include hormonal birth control, intrauterine device, diaphragm with spermicide, condom with spermicide, or abstinence, for 30 days from the time of the TCR-T cell infusion. If the participant is a female and becomes pregnant or suspects pregnancy, she must immediately notify her doctor. If the participant becomes pregnant during this study, she will be taken off this study. Men who are of child-bearing potential must use effective birth control while on the study. The acceptable form of birth control for male subjects is condom with spermicide, for 30 days from the time of the TCR-T cell infusion. If the male participant fathers a child or suspects that he has fathered a child while on the study, he must immediately notify his doctor. If a subject should become pregnant while on-study, guidance for submitting a Pregnancy Form can be found in Section 13.11

11.3.16. Retention of Unused Subject Material

The unused apheresis product, biorepository blood specimens, and unused manufactured cells will be stored at Alaunos and/or its affiliates and may be used for future retreatment or research studies which may include one or more of the following: cryopreservation, phenotyping, genotyping, genetic modification, propagation of cells and derivatives for the purposes of improving understanding of immunotherapy of cancer. If the subject refuses consent, the cells will be destroyed.

11.3.17. Long-Term Follow-Up

Enrolled research participants who receive TCR-T cell drug product will be required to participate in the long-term follow-up (LTFU) protocol (TCR001-202) per the guidelines set forth by the FDA's Biologic Response Modifiers Advisory Committee (BRMAC) that apply to gene transfer studies. Current recommendations from the FDA require a minimum of 15 years of follow-up after infusion of genetically modified cells. Since the long-term effects of gene and cell therapy are unknown, after receiving an infusion of genetically modified T cells, the subject will be required to participate in a study for LTFU of gene and cell therapy subjects and sign the associated consent/assent form. This participation is mandatory. If the research participant dies, then an autopsy will be requested, but is not mandatory. Subjects will begin the LTFU protocol once they complete the follow-up period or discontinue from this study (e.g., due to disease progression, initiation of new-anticancer therapy, withdraw consent, etc.).

12. TUMOR RESPONSE ASSESSMENTS

12.1. Tumor Response

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (v1.1) (Eisenhauer et al., 2009). Target and non-target lesions should be selected and measured as per the RECIST guideline.

12.2. Tumor Response Evaluation

Tumor response will be assessed locally using RECIST (v1.1) criteria (refer to Appendix I). Subjects should be imaged throughout the study using the same method(s) and techniques as were used for the screening images. Post-treatment tumor biopsies may occur as available and at the discretion of the investigator. A repeat scan to confirm response should be completed at least 4 weeks (per RECIST) after first documentation of response.

Final progression determinations will be made by the investigator. Every effort should be made to follow subjects radiographically until disease progression is confirmed per RECIST criteria.

Subjects without confirmed disease progression should continue to have tumor assessments approximately every 12 weeks until disease progression has been identified and confirmed. If subjects have yet to progress when this treatment protocol is complete, i.e., 2 years post TCR-T cell drug product infusion, they will continue to be scanned until progression on the LTFU protocol TCR001-202.

All radiological studies acquired at all scheduled time points and any additional (unscheduled) radiological images acquired to evaluate for potential metastatic disease will be sent to an Independent Review Facility (IRF) for storage and may be analyzed at a later date. All scans sent to the IRF must be in original Digital Imaging and Communications in Medicine (DICOM) format. Electronic transfer of scan files (via file transfer protocol [FTP], hypertext transfer protocol [HTTP], or similar means) is preferred, although transfer on physical media (such as digital versatile disc [DVD] or compact disc [CDs] is acceptable. For digital media, each disk should contain one timepoint for one patient. The site is expected to maintain a copy of digital data for the retention period applicable to the protocol, Good Clinical Practice (GCP), and federal, international and/or state legal and medical requirements. Sponsor and or designee will retain the media for the life of the study.

If desired by the Sponsor, the IRF may evaluate imaging studies and supportive clinical data in a central and independent fashion. The IRF will be comprised of board-certified radiologists and nuclear medicine physicians who will identify baseline lesions and assign post-baseline timepoint responses. Details regarding IRF member qualification, training, methods, procedures, and other issues relevant to IRF will be described in the IRF Charter, if needed.

The interpretation of scans in subjects treated with TCR-T cell drug product has an inherent uncertainty that stems from the pseudo-progression phenomena. Pseudo-progression is a term used to describe the appearance of radiographic disease progression due to increase contrast enhancement without true tumor progression. The increase in contrast enhancement can be influenced by several parameters including differences in radiologic technique, the amount of contrast agent used, the timing of the contrast agent administration relative to the imaging,

infarction, treatment related inflammation, seizure activity, sub-acute radiation effects, radiation necrosis, and corticosteroid use. Consideration of these factors by experts and clinical experience is likely to identify these subjects.

12.2.1. Imaging Acquisition Parameters

The following image acquisition parameters may be recorded as applicable: tomographic slice thickness and reconstruction interval, pulse sequence, contrast agent (including brand name), contrast agent dose and route of administration, contrast agent injection start and stop times, and contrast agent injector type (e.g., manual or power/auto injector).

12.2.2. Evaluation of Target Lesions – RECIST 1.1

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

12.2.3. Evaluation of Non-Target Lesions – RECIST 1.1

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).

NOTE: If tumor markers are initially above the upper normal limit, they must normalize for a subject to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances.

13. SAFETY ASSESSMENTS

Safety will be evaluated based on frequency and severity of AEs, SAEs, and findings from laboratory tests, electrocardiograms, vital signs, and physical/neurologic examinations. The severity of AEs will be graded using NCI CTCAE v5.0.

Reporting from the time of informed consent until lymphodepletion will only include AEs and SAEs that have an attribution of at least *possibly related* to a study procedure (e.g., blood draw). AEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw) will be recorded as adverse events in the CRF as outlined in Section 13.6 . SAEs that have an attribution of at least possibly related to a study procedure (e.g., blood draw) will be recorded in the CRF as outlined in Section 13.6 and reported to Sponsor as defined in Section 13.8. AEs and SAEs considered not protocol-related should be considered medical history.

The reporting period of all adverse events and serious adverse events will begin from the start of lymphodepletion through end of study regardless of relationship to study treatment.

- *Protocol-related:* The event occurred due to a procedure or intervention that was described in the protocol for which there is no alternative etiology present in the subject's medical record.
- *Not protocol-related:* The event is related to an etiology other than the study procedure (the alternative etiology must be documented in the study subject's medical record).

Worsening of signs and symptoms of the malignancy under study should be recorded as AEs in the appropriate section of the CRF. Disease progression assessed by measurement of malignant lesions on radiographs or other methods should not be reported as an AE.

Events not considered to be reportable SAEs:

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.
- Abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of preexisting disease(s) or condition(s) present or detected at the start of the study that do not worsen.

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up.

All SAEs must be reported to Alaunos Safety within 24 hours of awareness. Email the SAE Report Form (and any supporting documentation) to TCRSAE@alaunos.com

Documents can also be sent via fax to: +1-888-922-5939

For medical questions call the Alaunos Safety Hotline: + 1-866-990-2291

Questions or assistance with completing forms can be directed to Alaunos Therapeutics, Drug Safety and Pharmacovigilance: TCRSAE@alaunos.com

Additional data concerning the SAE (e.g., diagnostic test reports, hospital summaries, etc.) must be promptly reported (within 24 hours of receipt) to the sponsor or sponsor's designee, until resolution of the SAE. Should the FDA or National Regulatory Authorities require that the sponsor submit additional data on the event, the investigator will be asked to provide those data to the sponsor in a timely fashion.

13.1. Adverse Events and Definitions

13.1.1. Adverse Event

Any untoward medical occurrence associated with the use of a study drug in humans, whether considered drug related. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medical treatment or procedure, and any worsening of a pre-existing condition regardless of causality to study drug. An AE is also known as an adverse experience. Abnormal laboratory assessments that are an unexpected outcome of lymphodepleting chemotherapy administered before TCR-T cell drug product infusion will be recorded. Since a decreased lymphocyte count is not only expected but is also the desired therapeutic outcome of LD, decreased lymphocyte count and dependent decreased white blood cell count will be recorded, but will not be considered adverse events.

For non-serious events that occur prior to the administration of any study drug (i.e., after informed consent and before administration), an assessment of protocol relatedness must be made. Only those considered protocol-related should be entered as AEs. Events considered not protocol-related should be considered medical history.

- *Protocol-related:* The event occurred due to a procedure or intervention that was described in the protocol for which there is no alternative etiology present in the subject's medical record.
- *Not protocol-related:* The event is related to an etiology other than the study procedure (the alternative etiology must be documented in the study subject's medical record).

Worsening of signs and symptoms of the malignancy under study should be recorded as AEs in the appropriate section of the CRF. Disease progression assessed by measurement of malignant lesions on radiographs or other methods should not be reported as an AE.

13.1.2. Suspected Adverse Reaction

A *suspected adverse reaction* is any AE for which there is evidence to suggest a causal relationship (reasonable possibility) between the drug and the AE. A suspected adverse reaction implies less certainty about causality than an adverse reaction.

13.1.3. Adverse Reaction

An *adverse reaction* is any AE caused by a drug. Adverse reactions are a subset of all suspected adverse reactions which provide a reason to conclude that the study drug caused the event.

13.1.4. Unexpected Adverse Reaction

Any AE that is (a) not listed in the Reference Safety Information (RSI) of Investigator's Brochures, (b) not listed with the specificity and severity that is being observed, (c) not consistent with the risk information described in the general investigational plan or elsewhere in the current application (in the absence of an investigator brochure), and (d) listed as occurring with a class of drugs, but not specifically mentioned as occurring with the particular drug under investigation.

13.2. Evaluation of Adverse Events

Adverse events include:

- Suspected adverse drug reactions
- Reactions from study drug overdose, abuse, withdrawal, sensitivity, or toxicity
- Significant changes or abnormalities in signs, symptoms, clinical laboratory results, or physiological testing when compared to baseline. This includes any worsening of a pre-existing condition temporally associated with the use of a study drug.
- Other untoward medical events, regardless of their relationship to the study drug, such as injury, events that require surgery, accidents, extensions of symptoms, or apparently unrelated illnesses
- Worsening of signs and symptoms of the malignancy under study should be recorded as AEs in the appropriate section of the CRF.

The following considerations apply when identifying an AE:

- Anticipated day-to-day fluctuations of pre-existing conditions, including the disease under study, that do not represent a clinically significant exacerbation or worsening need not be considered AEs.
- If a constellation of symptoms results in a confirmed diagnosis, the diagnosis (not the symptoms) should be recorded as the AE.
- If a diagnosis cannot be established, the symptoms should be recorded as the AEs.
- If an ongoing symptom has been included in the medical history, an associated severity grade and frequency should also be documented so that a worsening in severity or frequency of a symptom can be readily identified as an AE.

- Disease progression assessed by measurement of malignant lesions on radiographs or other methods should not be reported as an AE. Disease progression assessed by measurement of malignant lesions on radiographs or other methods should not be reported as an AE.

After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up.

13.3. Determination of Seriousness

13.3.1. Serious Adverse Event

An AE is considered an SAE if at least one of the following conditions applies:

- *Death* occurring during the study. In addition, this includes a reported death at any time post-study that is thought to be related to study drug administration.
- *Life-threatening*: An AE that places the subject, in the view of the investigator, at immediate risk of death from the event as it occurred (i.e., this does not include a reaction that, had it occurred in a more severe form, might have caused death).
- *Permanent, persistent, or significant disability*: A disability is defined as any substantial disruption of a person's ability to conduct normal life functions.
- *Inpatient hospitalization or prolongation of existing hospitalization* (in general, for at least a 24-hour stay). Hospitalizations for routine blood transfusions, for an elective or diagnostic procedure, or for surgery for a pre-existing condition that has not worsened are not considered SAEs. Emergency room visits that do not result in a hospital admission are not considered as SAEs.
- *A congenital anomaly/birth defect*, that is, a fixed, permanent impairment established at or before birth.
- *An important medical event* that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they jeopardize the subject and require medical or surgical intervention to prevent a life-threatening situation, hospitalization or death.

Events not considered to be reportable SAEs:

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.
- Abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition.

- Medical or surgical procedure (e.g., endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of preexisting disease(s) or condition(s) present or detected at the start of the study that do not worsen.

13.3.2. Non-Serious Adverse Event

An AE that does not fulfill the criteria for a SAE is classified as a non-serious AE.

13.4. Determination of Severity

The severity of AEs will be assessed according to the NCI CTCAE Version 5.0. If an AE is not specifically defined in the NCI CTCAE, Version 5.0, the investigator will determine the severity of an AE based on the following recommended general definitions (NCI 2017):

- *Mild* (Grade 1): The AE is noticeable to the subject but does not interfere with routine activity. The AE does not require intervention.
- *Moderate* (Grade 2): The AE interferes with routine activity but responds to symptomatic therapy or rest. The AE may require minimal, local, or noninvasive intervention.
- *Severe* (Grade 3): The AE significantly limits the subject's ability to perform routine activities despite symptomatic therapy. In addition, the AE may necessitate the subject's hospitalization or prolongation of hospitalization.
- *Life-threatening* (Grade 4): The AE requires urgent intervention to resolve. The subject is at immediate risk of death.
- *Death* (Grade 5): The subject dies as a direct result of the AE complication or condition.

13.5. Determination of Causality

The investigator will use medical consideration, based on his/her clinical judgment, to determine the potential relationship of the AE to the study drugs. Assessment of causality will be based upon the following:

- Alternative possible causes of the AE, including other drugs, other host, environmental factors, and the subject's underlying disease or comorbid conditions
- The temporal sequence between the exposure to study drug and the AE
- Whether the clinical or laboratory manifestations of the AE are consistent with known actions or previously reported toxicity of the study drug or similar drugs

These relationship assessments indicate a "Not Related" study drug:

- *None:* The event is related to an etiology other than the investigational product (the alternative etiology must be documented in the study subject's medical record and/or SAE form).
- *Unlikely or Remote*: The event is unlikely to be related to the study drug and likely to be related to factors other than the study drug.

These relationship assessments indicate a "Related" study drug:

- *Possible*: There is an association between the event and the study drug and there is a plausible mechanism for the event to be related to the study drug; but there may also be an alternative etiology, such as characteristics of the subject's clinical status or underlying disease.
- *Probable*: There is an association between the event and the study drug and a plausible mechanism for the event to be related to the study drug. The event could not be reasonably explained by known characteristics of the subject's clinical status or an alternative etiology is not apparent.
- *Definite*: There is an association between the event and the study drug. A plausible mechanism exists for the event to be related to the study drug and causes other than the study drug have been ruled out.

For non-serious events that occur prior to the administration of any study drug (i.e., after informed consent and before administration), an assessment of protocol relatedness must be made. Only those considered protocol-related should be entered as AEs. Events considered not protocol-related should be considered medical history. Only those that are assessed by the investigator as protocol-related should be reported to the sponsor.

- *Protocol-related:* The event occurred due to a procedure or intervention that was described in the protocol for which there is no alternative etiology present in the subject's medical record.
- *Not protocol-related:* The event is related to an etiology other than the study procedure (the alternative etiology must be documented in the study subject's medical record).

13.6. Documenting Adverse Events

All AEs, including SAEs, are to be accurately recorded on the Adverse Event CRF. Each event will be assessed for serious criteria, severity, and causality (Section 13.3, Section 13.4, and Section 13.5). The date of onset, as well as the duration of the event will be recorded. In addition, treatments provided to the subject, actions taken with the study drugs, and the outcome of the AE will also be noted.

13.7. Dose-limiting Toxicity Assessment

Severity of AEs will be graded according to NCI CTCAE Version 5.0. For the purpose of dose escalation, any of the AEs listed below that occur within 28 days after the infusion which are attributable to study treatment and unrelated to tumor, intercurrent illness, or concomitant medications will be classified as DLTs. Due to the requirement for lymphodepletion prior to

investigational product administration, and the expected toxicities associated with lymphodepletion, hematological toxicities will not be considered DLTs unless specified below.

Clinically important or persistent toxicities that are not included in the definitions below may also be considered a DLT following review by the CRC. All DLTs need to represent a clinically significant shift from baseline.

13.7.1. Dose-limiting Toxicity (DLT) Definitions

A DLT is a study intervention-related AE occurring within the DLT window (Day 0 to Day 28), defined based on NCI CTCAE Version 5.0 as the following:

Hematologic

- Grade 4 neutropenia lasting ≥ 14 days
- Grade 4 thrombocytopenia
- Grade 3 thrombocytopenia associated with clinically significant bleeding,
- ≥ Grade 3 febrile neutropenia associated with hemodynamic compromise or objective evidence of infection.

Non-hematologic

- CRS grade 3 or 4 that does not improve to grade ≤ 2 within 72 hours despite appropriate treatment.
- Grade 3 Immune-effector cell-associated neurotoxicity syndrome (ICANS) that does not return to grade ≤2 or lower with 7 days.
- Any grade 5 adverse event (not due to disease progression or to a known cause other than TCR-T (e.g., automobile accident)).
- Grade 3 hepatic toxicity that does not return to grade ≤ 2 or lower within 7 days.
- Grade 3 toxicity involving vital organs that does not improve to grade ≤ 2 or lower within 7 days of onset.
- Grade 4 toxicity not previously specified.

In addition to the related AEs mentioned above, any related grade 3 toxicity and all grade 4 toxicities will be considered DLTs except the following:

- Grade 3 anemia that is not associated with other clinically significant complications
- Expected chemotherapy toxicities due to lymphodepletion including cytopenias, fludarabine related adverse reactions, and cyclophosphamide related hemorrhagic cystitis.

13.8. Reporting Serious Adverse Events

13.8.1. Time Frame for Reporting

All SAEs must be reported to Alaunos Safety within 24 hours of awareness. Email the SAE Report Form (and any supporting documentation) to TCRSAE@alaunos.com**.**

Documents can also be sent via fax to: +1-888-922-5939

For medical questions call the Alaunos Safety Hotline: + 1 866-990-2291

Questions or assistance with completing forms can be directed to Alaunos Therapeutics, Drug Safety and Pharmacovigilance: TCRSAE@alaunos.com**.**

Additional data concerning the SAE (e.g., diagnostic test reports, hospital summaries, etc.) must be promptly reported (within 24 hours of receipt) to the sponsor or sponsor's designee, until resolution of the SAE. Should the FDA or National Regulatory Authorities require that the sponsor submit additional data on the event, the investigator will be asked to provide those data to the sponsor in a timely fashion.

The Investigator must sign and date the SAE Report Form. If the person completing the report form is different from the investigator, he/she must sign and date the report as well.

If the investigator is not immediately available to sign the report, it can be submitted with the reporter's signature. **The Investigator signoff is expected within 48 hours**. Once signed, submit the updated SAE Report form. Information to be Provided by the Investigator

Within 24 hours of becoming aware of the SAE, the investigator must notify the sponsor, Alaunos Medical Monitor or designee and transmit information to the sponsor, Alaunos Medical Monitor or designee as follows:

1. Information (initial and follow-up) should be provided on an electronic and/or paper SAE Report form signed and dated by the investigator.
2. The SAE Report form and copies of source documents with subject identifiers redacted will be transmitted by email or fax.
3. A hospital discharge summary should be provided if the subject was hospitalized.

An SAE report will be considered final once all relevant information has been received and reviewed by the sponsor.

The SAE report form is provided in the investigator study files. Refer to the investigator study files for instructions on how to complete these forms. The investigator will provide all the following information related to the event:

- Investigator identification
- Subject identification (e.g., subject number, initials, sex, age, or year of birth)
- Information regarding study procedure (e.g. start/stop time)
- Day of SAE occurrence
- Description of event

- Action taken with the study procedures/treatments in relation to the SAE
- Outcome of the SAE

In addition to the above information, the investigator must provide, for each event term, an assessment of:

- Severity/intensity
- Relationship to the study drug (causality assessment)

13.9. Sponsor and Investigator Responsibility for Reporting Adverse Events

All AEs and SAEs will be reported to Regulatory Authorities (RAs), IRBs/IECs, and investigators in accordance with all applicable global laws and regulations, including but not limited to 21 CFR 312.32. The investigator must submit all Safety Letters received from the sponsor to his/her IRB/IEC per agreements and local requirements. The investigator must keep copies of all safety reports/letters, including correspondence with Alaunos and the IRB/IEC, in the study file.

13.10. Follow-up Information for Adverse Events

Appropriate diagnostic tests should be performed and therapeutic measures, as medically indicated, should be instituted. After the initial AE/SAE report, the investigator is required to proactively follow each subject at subsequent visits/contacts. All SAEs will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up.

13.11. Pregnancies

The sponsor should be immediately notified if subjects become pregnant during the study.

An initial Pregnancy Report form and updates as additional information becomes available are to be completed by the investigator or designee. The Pregnancy Report form and the completion instructions will be provided in the investigator study files. Refer to the investigator study files for details on how to complete these forms.

13.12. Overdose

Investigational product overdose of study subject, with or without associated AEs/SAEs, should be reported within 24 hours of awareness to sponsor (Alaunos Therapeutics, Drug Safety and Pharmacovigilance, TCRSAE@alaunos.com or +1-866-990-2291). All AEs or SAEs as a result of overdose should be reported as described previously in Section 13.6 and Section 13.8.

14. TRANSLATIONAL RESEARCH ASSESSMENTS

To enable investigation of correlative biomarkers, molecular signatures, mechanism of action, or other such translational hypotheses as specified in the study objectives or otherwise, samples will be collected prior to, during, and following TCR-T-cell infusion as outlined in Table 14. The assessments of these samples are outlined in Section 14 subsections below. If clinical circumstance limits the collection of samples from a subject at a given time point(s), samples should be prioritized in the following order: Samples that inform Secondary Objectives, samples that inform Exploratory Objectives, then samples that inform additional translational analyses. If a subject progresses, TCR-T-cell persistence and insertion-site clonality of TCR-T cell drug product assessments will be assessed per the schedule of assessments in the Long-Term Follow-up protocol, as well as Insertional Mutagenesis assessments (Section 14.4) to monitor for delayed adverse events (Food and Drug Administration, 2020). While collection volumes are designed to limit the amount of sample to only that which is required to complete the specified assessment, any unused subject material (e.g., cells, RNA, or DNA) will be retained by the Sponsor for future translational research if the subject consents.

14.1. Assessments from Peripheral Blood and Serum

Blood and serum will be collected and assessed from all subjects with evaluable samples according to the schedule of sample collection shown in Table 14. Refer to the laboratory manual for collection, processing, and shipment of samples. Samples will be analyzed for of the following (including but not limited to):

14.1.1. Translational Assessments in Blood and Serum Related to Secondary Objectives:

- TCR-T-cell persistence, as defined by the duration that TCR-T cell drug product is measurable by VCN in peripheral blood samples.

14.1.2. Translational Assessments in Blood and Serum Related to Exploratory Objectives:

- TCR-T cellular persistence in peripheral blood (e.g., Cmax, Tmax, $AUC_{D0\text{-}D28}$, etc.) over time determined by VCN.
- Insertion-site clonality of TCR-T cell drug product will be measured by tracking the transposon insertion-site(s) of gene-modified cells in peripheral blood samples.
- Serum cytokine concentrations including, but not limited to IFN-γ, IL-6, TNF-α, GM-CSF, IL-2, IL-7, and IL-15 will be determined by multiplex immunoassay.
- Immunogenicity elicited against the transgenic components of the TCR therapy (i.e., anti-drug antibody and T-cell responses) will be assessed by immunoassay of peripheral blood and serum samples.

Data collected from these samples will be analyzed, in part, as they relate to fulfillment of the study objectives as outlined above.

14.1.3. Additional Translational Assessments Planned in Blood

In addition to the assessments above that are related to study objectives, planned study analyses of blood samples include the following:

- Immunophenotyping of immune cell population markers in the peripheral blood will be assessed by flow cytometry. Cell subsets/characteristics assessed may include T-cell subsets (e.g., naïve, central memory, etc.), activated T cells, T-cell exhaustion, classic immune cell subsets (e.g., B cells, NK cells, etc.). Markers assessed may include but are not limited to cluster of differentiation (CD) antigens CD3, CD4, CD8, CD45RA/RO, CD25, CD137 (4-1BB) and CD279 (PD-1).
- Genomic analysis of peripheral blood samples of TCRvβ diversity using NGS or other appropriate methodologies.
- Gene expression analysis (e.g., RNA-Seq) of peripheral blood samples.
- Exploratory biorepository samples will be collected and retained by the Sponsor for future translation research.

14.2. Assessments of Tumor Tissues

Tumor tissue samples will be collected from all subjects at baseline, and on-treatment prior to the 12-week imaging scan. Additionally, an optional biopsy will be requested at the time of confirmed or suspected progression. These timepoints are described in greater detail below (refer to laboratory manual(s) for details related to collection, processing and shipment). Fine needle aspirates (FNA) are not acceptable.

- Baseline tumor tissue is required for all subjects. Tissue may be provided from an archival tumor resection or biopsy sample (most recent tumor sample collected prior to lymphodepletion available and not to have been collected more than 6 months prior to subject enrollment on this study). If archival tissue (refer to Lab Manual for minimum tissue requirement) is not available, then a new biopsy from a non-target lesion must be obtained. See Table 14 for more details.
- On-treatment tumor biopsy collected at Week 12 (±2 weeks) prior to the 12-week imaging scan is required for all subjects. The tissue should be collected from the same lesion as the Baseline sample if possible.
- Progression tumor biopsy collected at time of progression or suspected progression (optional for all subjects; within 1 month of confirmed progressive or suspected disease) as defined in Section 12.2.2 or from unscheduled visits. The tissue should be collected from the same lesion as the On-treatment and/or Baseline biopsy sample if possible.

14.2.1. Translational Assessment of Tumor Tissues Related to Exploratory Objectives

The collection of tumor tissue samples is to enable the investigation of all T cells in the tumor. The following parameters will be evaluated by DNA sequencing, RNA-Seq, IHC, or other suitable methodology in biopsy samples collected at baseline, on-treatment (i.e., Week 12) and progression or suspected progression:

- T-cell infiltration (e.g., total CD3, CD4, and CD8)
- TCR-T cell infiltration
- Changes in the tumor associated with progression such as:
    - Presence of HLA alleles in tumor samples
    - Presence of tumor-specific neoantigens in tumor samples

Additional confirmatory analyses of these samples (e.g., IHC or ISH to verify target expression) may be performed based on emergent data and sufficient sample availability. Data collected from these samples will be analyzed, in part, as they relate to fulfillment of the study objectives as outlined above. Tumor specimens may be evaluated for other cell types or molecular markers driven by emergent data.

14.3. Assessment of Circulating Tumor DNA (ctDNA)

14.3.1. Translational Assessment of ctDNA Related to Exploratory Objectives:

To evaluate the levels of blood-based tumor neoantigen biomarkers, peripheral blood samples collected from all subjects according to the schedule shown in may be analyzed. Refer to the laboratory manual for collection, processing, and shipment of samples for ctDNA analysis. Analysis of ctDNA samples by NGS may include the determination of:

- Presence of neoantigen alleles
- Quantification of variant allele frequency of tumor neoantigen specific gene(s)

These data may be used to assess the appropriateness of ctDNA as a surrogate pharmacodynamic marker.

14.4. Assessment of Insertional Mutagenesis in the Event of Emergent Malignancies

In the event of a new malignancy, unrelated to any current or prior diagnosis, an appropriate sample will be obtained of the tumor (i.e., needle biopsy for solid tumors, bone marrow biopsy or blood sample for hematological malignancies) to assess for potential relatedness to TCR-T cell therapy. Methods for collection, processing, and shipment of such sample(s) will be provided by the Sponsor after consultation with the investigator, should such an event occur. Assessment of this sample will include evaluation of:

- Presence of TCR-T cell drug product by VCN
- Clonality (transposon insertion-sites) of any TCR-T cell drug product present in the sample

Other laboratory assessments may be conducted on the sample as appropriate, based on the nature of the malignancy and the sample obtained. The objective of these analyses is to evaluate the likelihood that the new malignancy is the result of insertional mutagenesis caused by the transposon.

15. STATISTICAL CONSIDERATIONS

This section is a summary of the key, planned reporting and statistical analytic methods of the study data. The Statistical Analysis Plan (SAP) will develop these concepts in detail and will be finalized before database lock.

15.1. Phase I [redacted] Enrollment Rules

15.1.1. Phase I: MTD Determination

The Bayesian optimal interval (BOIN) design (Liu & Yuan, 2015; Yuan, Hess, Hilsenbeck, & Gilbert, 2016) will be utilized in this trial to characterize safety and tolerability, as defined by DLTs (refer to Section 5.3), with the goal of identifying an MTD/RP2D of TCR-T cell drug product.

BOIN escalation/de-escalation rules will be implemented based on DLT data throughout the study for each dose cohort after the accelerated titration of the first dosing cohort. Note, as described in Section 5.1.1, dose index 1 or 2 may apply depending on the ability to manufacture for the enrolled subjects. A BOIN dose finding will follow after the DLT period of last enrolled subject in a dose cohort for the next dosing cohort and will be selected from dose levels that do not exceed safety/toxicity thresholds.

The target toxicity rate for BOIN dose finding is 30% and the maximum sample size is 30. We will enroll and treat patients in dose cohorts of size 3. The BOIN design uses the following rule, optimized to minimize the probability of incorrect dose assignment:

- If the observed DLT rate at the current dose is $\leq$ 25.3%, escalate the dose to the next higher dose level;
- If the observed DLT rate at the current dose is $>$ 35.9%, de-escalate the dose to the next lower dose level;
- Otherwise, stay at the current dose.

The detailed rules and operating characteristics for the BOIN implementation are provided in Appendix H.

[redacted]

15.2. Analysis Sets

For the purposes of analysis, the following analysis sets are defined:

Enrolled	The enrolled analysis set (ENR) will consist of all participants who have received lymphodepletion.
Full Analysis Set	The full analysis set (FAS) will consist of ENR participants who received a TCR-T cell drug product infusion and have at least one valid post-baseline assessment (PK, PD, Efficacy).
Safety Analysis Set	The safety analysis set (SAF) will consist of ENR participants who received a TCR-T cell drug product infusion.
Per Protocol Analysis Set	The per protocol analysis set (PPS) consists of SAF participants who do not have major treatment protocol violation during the DLT observation period. Participants with major treatment deviations during DLT observation period unrelated to safety or tolerability events are not evaluable for the dose-escalation assessment and will be replaced as needed.
Dose Determining Analysis Set	The dose-determining analysis set (DDS) will consist of PPS participants who have completed the planned TCR-T cell drug product infusion and experienced a DLT or completed the DLT observation period.

15.3. Statistical Analyses

The following sections detail key analysis definitions, methods and approaches.

15.3.1. General Considerations

This section summarizes the key, planned reporting and statistical analytic methods of the study data. A forthcoming Statistical Analysis Plan (SAP) will develop these concepts in detail.

In general, continuous variables, including baseline characteristics, will be summarized by reporting the number of observations, mean, standard deviation (SD), median, minimum and maximum and categorical/discrete variables will be summarized using frequency tables showing the number and percentage of patients within a category.

Time-to-event data will be summarized using Kaplan-Meier or Nelson-Aalen statistical techniques, as appropriate, with parameter estimates displayed at pre-defined time points along with the number of censored observations. A 2-sided 95% confidence interval for median time to event estimates will be produced in addition to the event 25th and 75th percentile summary statistic.

Baseline is defined as the last available observation prior to the first administration of study treatment.

In addition, analyses may be performed separately for each individual TCR.

Any deviations from the SAP will be reported in the clinical study report.

15.3.2. Baseline Characteristics

Subject disposition, demographics and other baseline characteristics, medical history, prior therapy, concomitant medications will be summarized by treatment, disease subgroup and overall, as applicable.

15.3.3. Analysis of Phase I Primary Endpoint

Dose Limiting Toxicities (DLTs):

The primary analyses will characterize the incidence of DLTs as defined in Section 5.3. The DLT incidence will be used as a component of the MTD/RP2D selection criteria.

Adverse Events:

Treatment-emergent AEs through 28 days after last protocol therapy will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) Version 13.1 (or higher) System Organ Class and preferred term. The incidences and percentages of participants experiencing each AE preferred term will be summarized with descriptive statistics. AEs will also be summarized by NCI CTCAE, Version 5.0, by grade and by causality (attribution to study treatment).

Laboratory abnormalities:

Laboratory results will be classified according to NCI CTCAE, Version 5.0. Laboratory results not corresponding to an NCI CTCAE term will not be graded. Incidences of laboratory abnormalities will be summarized with descriptive statistics.

15.3.4. Analysis of Phase I Secondary Endpoints

The Phase I secondary endpoint for evaluating the duration of persistence of TCR-T cell drug product by VCN is defined as the length of time from the date of infusion of TCR-T cell product to the date of the last measurable (i.e., ≥ LOQ) TCR-T cell level by VCN.

Final T cell count, including percent viability, for appropriate clinical dose will be evaluated and details will be provided in the SAP.

15.3.5. Analysis of Phase I Exploratory Endpoint

Exploratory endpoints for analysis will be performed in the FAS.

Analysis of exploratory endpoints may be performed separately by dose level and tumor type, or where appropriate combined. Details of the analyses will be described in the SAP.

Objective Response Rate (ORR):

Objective Response Rate (ORR) is defined as the proportion of FAS subjects achieving a confirmed PR or CR according to RECIST 1.1 and iRECIST during study. All objective responses (CR or PR) require confirmation by a repeat tumor assessment at least 4 weeks (28

days) after the response is first observed in order for the response to be considered confirmed. An estimate of the ORR is determined by the proportion of confirmed objective responses and will be summarized separately for each of the dose levels.

[redacted]

15.3.9. Safety Analyses

Adverse Events

Treatment-emergent AEs through 30 days after last protocol therapy will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) Version 13.1 (or higher) System Organ Class and preferred term. The incidences and percentages of patients experiencing each AE preferred term will be summarized with descriptive statistics. AEs will also be summarized by NCI CTCAE, v5.0, by grade and by causality (attribution to study treatment).

AEs resulting in dose modification or treatment discontinuation will be summarized by preferred terms.

Laboratory Evaluations

Laboratory results will be classified according to NCI CTCAE, Version 5.0. Laboratory results not corresponding to an NCI CTCAE term will not be graded. Incidences of laboratory abnormalities will be summarized with descriptive statistics. Vital signs and physical examination results will be summarized with descriptive statistics.

Patient listing of laboratory values and grading will be provided by laboratory parameter and dose level. The incidence of lab abnormalities will be shown by parameter, dose level and overall.

16. STUDY MANAGEMENT

16.1. Electronic Case Report Forms and Source Documentation

For each subject, eCRFs and corresponding source records will be maintained at each clinical site. The sponsor or designee will provide the study sites with secure access to and sufficient training on the electronic data capture (EDC) application, to permit site personnel to enter or correct information in the eCRFs for the subjects for whom they are responsible.

The eCRFs should be completed in a timely manner, and every effort should be made to have forms completed and up to date in anticipation of a visit by the sponsor's monitor. Specific instructions will be provided to the site. All requested information must be entered on the eCRF in the spaces provided. If an item is not available or is not applicable, it should be documented as such; do not leave a space blank.

It is the investigator's responsibility to ensure the accuracy, completeness, clarity, and timeliness of the data reported in the subject's eCRF. Through the EDC application, the investigator must provide formal approval of all subject information in the eCRFs and changes to the eCRFs to endorse the final submitted data for the subjects for whom he/she is responsible. The audit trail entry will show the user's identification information and the date and time of any corrections.

eCRF completion may be delegated to other study personnel included on study site's Delegation of Authority/Responsibility Log; however, such delegation must be documented in writing and approved by site's Principal Investigator on site's Delegation of Authority/Responsibility Log before the delegated study personnel completes any actions for this study. If, for any reason, certain data are lacking to complete an individual report form, the investigator will provide a written statement explaining the reasons for the lack of data.

Sponsor or designee will retain the eCRF data and corresponding audit trails. A copy of the final archival eCRF in the form of a compact disk or other electronic media will be placed in the investigator's study file.

16.2. Good Clinical Practice

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines, applicable regulatory requirements, and Alaunos policies.

16.3. Sponsor Monitoring

After satisfactory receipt of all necessary regulatory paperwork, the sponsor's monitor will arrange that all study material be delivered to the study site at a mutually convenient time. A site initiation visit (SIV) by Alaunos and its monitoring personnel will be made. At this meeting, all personnel expected to be involved in the conduct of the study will undergo an orientation to include review of study protocol, instruction for eCRF completion and overall responsibilities, including those for drug accountability and study file maintenance.

Throughout the course of the study, the sponsor's monitor will make frequent contact with the investigator, and this will include telephone and/or onsite visits. During these visits, eCRFs will

be reviewed for completeness and adherence to protocol. As part of the data audit, it is expected that source documents (e.g., hospital records, office records) will be made available for review by the Medical Monitor. The monitor also will perform drug accountability checks and may periodically request review of the investigator's study file to assure completeness of documentation in all respects of study conduct.

Upon study completion, the monitor will arrange for a final review of the study files, after which the file should be secured by storage for the appropriate period as specified in Section 16.6. The investigator or appointed delegate will receive the sponsor's representative during these onsite visits and will cooperate in providing the documents for inspection and responding to inquiries that may arise as part of this review. The investigator will also permit inspection of the study files by authorized representatives of the FDA.

16.4. Duration of the Study

The duration of accrual from the time of initiating subject enrollment until the completion of survival follow-up is anticipated to be approximately 7 years.

The overall duration is expected to be up to 2 years and 1 month for an individual subject, including the following:

- Screening period of up to 2 weeks
- Study treatment period of approximately 5 weeks (Days -7 through Day 28)
- The Follow-up Period includes assessment of tumor response at 6 weeks (± 1 week), and then every 3 months (± 2 weeks) for up to two-years post-TCR-T cell drug product infusion.

In addition, subjects who receive investigational product without objective evidence of disease progression during the 2-year follow-up period will continue to be followed until progression has been documented via the Long-Term Follow-Up protocol (TCR001-202).

16.5. Audits and Inspections

Authorized representatives of Alaunos, a regulatory authority, and/or an Independent Ethics Committee or an Institutional Review Board may visit the site to perform audits or inspections, including source data verification. The purpose of a Alaunos audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice guidelines of the International Conference on Harmonization, and any applicable regulatory requirements. The investigator should contact Alaunos immediately if contacted by a regulatory agency about an inspection.

16.6. Retention of Records

Records of drug disposition, eCRFs, and reports of the clinical trial must be maintained by the investigator for a period of at least 2 years following the date on which the investigational product is approved by FDA for marketing for the purposes that were investigated in the study. If

no application is to be filed or if the application is not approved for such indication, the records must be stored for two additional years and then returned to Alaunos.

16.7. Communication with Supporting Companies and Laboratories

Anonymized data and/or specimens may be generated, shared and/or stored with the sponsor, Alaunos, and its contracted vendors. Data may also be shared with the investigators/programs at the clinical site/institution who are involved with patient care and manufacturing of TCR-T cell drug product and/or testing of samples collected during the study.

16.8. Institutional Review Board/ Independent Ethics Committee

This protocol and the study ICF must be reviewed and approved by the IRB/IEC prior to the start of the study, and a copy of the approval letter supplied to Alaunos. During the study, the investigator shall make timely and accurate reports to the IRB/IEC on study progress at intervals not exceeding 1 year, as well as satisfying any other local IRB/IEC reporting regulations. Copies of all reports to, and correspondence with, the IRB/IEC must be provided to Alaunos. Furthermore, within 3 months of the completion or early termination of the study, a final report should be made to the IRB/IEC and Alaunos by the investigator.

All protocol revisions must originate with and be documented by Alaunos. If the requested revision is an amendment, the investigator must sign it. The investigator must submit the amendment to his/her IRB/IEC for review and approval prior to implementation. Documentation of approval signed by the chairperson or designee of the IRB/IEC must be sent to Alaunos.

It is the investigator's obligation to maintain an IRB/IEC correspondence file and to make this available for review to Alaunos representatives as part of the routine study-monitoring process.

16.9. Confidentiality and Health Insurance Portability and Accountability Act Information

The written ICF will explain that study data will be stored in a database, maintaining confidentiality in accordance with national data legislation. All data processed by Alaunos, or its representatives, will be identified by subject number and study code.

The written ICF will also explain that, for data verification purposes, authorized representatives of Alaunos, a regulatory authority (FDA), and/or the IRB/IEC may require direct access to parts of the hospital or clinic records relevant to the study that include the subject's medical history.

The written ICF will be accompanied by or include a separate document incorporating United States Health Insurance Portability and Accountability Act (HIPAA)-compliant wording by which the subjects authorize the use and disclosure of their Protected Health Information.

16.10. Informed Consent

16.10.1. Informed Consent Requirements

The investigator or his/her staff will explain the nature of the study, its purpose and associated procedures, the expected duration, and the potential risks involved to the prospective subject prior to enrollment. The ICF should also indicate that, by signature, the prospective subject (or

where appropriate, a legal guardian) permits access to relevant medical records by the sponsor and by representatives of the FDA. If a prospective subject does not understand English, an appropriate translation of the ICF into the subject's primary language must be made available. The investigator or designee will obtain written, informed, and witnessed consent. The prospective subject will have ample time and opportunity to ask questions. The prospective subject will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide a reason for this decision. Following the discussion regarding the study, the prospective subject will be asked if he/she is willing to personally sign and date a statement of informed consent. Only if the prospective subject voluntarily agrees to sign the informed consent statement and has done so, is he/she enrolled into the study. A copy of the signed and dated ICF will be provided to each prospective subject. The signed ICF is to remain in the investigator's file.

The ICF and any other written information provided to the subjects will be revised whenever important new information becomes available that may be relevant to the subject's consent, or if there is an amendment to the protocol that necessitates a change to the content of the study's ICF. The investigator will inform the subject of changes in a timely manner and will ask the subject to confirm continuation of his/her participation in the study by his/her signature on the revised ICF, if applicable. Any written ICF and written information must receive IRB/IEC approval/favorable opinion in advance of use.

Alaunos will provide a sample subject ICF for modification by the investigator as appropriate.

17. LIST OF REFERENCES

Bohlius, J., Bohlke, K., Castelli, R., Djulbegovic, B., Lustberg, M. B., Martino, M., . . . Lazo-Langner, A. (2019). Management of Cancer-Associated Anemia With Erythropoiesis-Stimulating Agents: ASCO/ASH Clinical Practice Guideline Update. *Journal of Clinical Oncology, 37*(15), 1336-1351. doi:10.1200/jco.18.02142

Cafri, G., Yossef, R., Pasetto, A., Deniger, D. C., Lu, Y. C., Parkhurst, M., . . . Rosenberg, S. A. (2019). Memory T cells targeting oncogenic mutations detected in peripheral blood of epithelial cancer patients. *Nat Commun, 10*(1), 449. doi:10.1038/s41467-019-08304-z

Center, U. o. T. M. A. C. (2020). IEC Therapy Toxicity Assessment and Management (also known as CARTOX)-Adult. Retrieved from https://www.mdanderson.org/documents/for-physicians/algorithms/clinical-management/clin-management-cytokine-release-web-algorithm.pdf

Davis, J. L., Theoret, M. R., Zheng, Z., Lamers, C., Rosenberg, S. A., & Morgan, R. A. (2010). Development of Human Anti-Murine T-cell Receptor Antibodies in Both Responding and Non-responding Patients Enrolled in TCR Gene Therapy Trials. *Clin Cancer Res, 16*(23), 5852-5861. doi:10.1158/1078-0432.CCR-10-1280

Deniger, D. C., Pasetto, A., Robbins, P. F., Gartner, J. J., Prickett, T. D., Paria, B. C., . . . Rosenberg, S. A. (2018). T-cell Responses to TP53 "Hotspot" Mutations and Unique Neoantigens Expressed by Human Ovarian Cancers. *Clin Cancer Res, 24*(22), 5562-5573. doi:10.1158/1078-0432.CCR-18-0573

Deniger, D. C., Pasetto, A., Tran, E., Parkhurst, M. R., Cohen, C. J., Robbins, P. F., . . . Rosenberg, S. A. (2016). Stable, Nonviral Expression of Mutated Tumor Neoantigen-specific T-cell Receptors Using the Sleeping Beauty Transposon/Transposase System. *Mole Ther*, 1-12.

Dutcher, J. P., Schwartzentruber, D. J., Kaufman, H. L., Agarwala, S. S., Tarhini, A. A., Lowder, J. N., & Atkins, M. B. (2014). High dose interleukin-2 (Aldesleukin) - expert consensus on best management practices-2014. *J Immunother Cancer, 2*(1), 26. doi:10.1186/s40425-014-0026-0

Eisenhauer, E. A., Therasse, P., J, B., Schwartz, L. H., Sargent, D., Ford, R., . . . Verweij, J. (2009). New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *European Journal of Cancer, 45*, 228-247.

Field, A. C., Vink, C., Gabriel, R., Al-Subki, R., Schmidt, M., Goulden, N., . . . Qasim, W. (2013). Comparison of lentiviral and sleeping beauty mediated alphabeta T cell receptor gene transfer. *PLoS One, 8*(6), e68201. doi:10.1371/journal.pone.0068201

Food and Drug Administration, C. f. B. E. a. R. (2020). Guidance for Industry: Long Term Follow-Up After Administration of Human Gene Therapy Products. Retrieved from https://www.fda.gov/media/113768/download

Gattinoni, L., Powell, D. J., Jr., Rosenberg, S. A., & Restifo, N. P. (2006). Adoptive immunotherapy for cancer: building on success. *Nature reviews. Immunology, 6*(5), 383-393. doi:10.1038/nri1842

Greenbaum, U., Strati, P., Saliba, R. M., Torres, J., Rondon, G., Nieto, Y., . . . Ahmed, S. (2021). CRP and ferritin in addition to the EASIX score predict CAR-T-related toxicity. *Blood Adv, 5*(14), 2799-2806. doi:10.1182/bloodadvances.2021004575

Hackett, P. B., Largaespada, D. A., & Cooper, L. J. N. (2010). A Transposon and Transposase System for Human Application. *Mol Ther, 18*(4), 674-683. doi:doi:10.1038/mt.2010.2

Ivanova, A. (2021). Simon's Two-Stage design Application. Retrieved from http://cancer.unc.edu/biostatistics/program/ivanova/SimonsTwoStageDesign.aspx

Ivics, Z., Hackett, P. B., Plasterk, R. H., & Izsvak, Z. (1997). Molecular Reconstruction of Sleeping Beauty, a Tc1-like Transposon from Fish, and Its Transposition in Human Cells. *Cell Press, 91*, 501-510.

Jena, B., Dotti, G., & Cooper, L. J. (2010). Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. *Blood, 116*(7), 1035-1044. doi:10.1182/blood-2010-01-043737

Kaufman, R. M., Djulbegovic, B., Gernsheimer, T., Kleinman, S., Tinmouth, A. T., Capocelli, K. E., . . . Aabb. (2015). Platelet transfusion: a clinical practice guideline from the AABB. *Ann Intern Med, 162*(3), 205-213. doi:10.7326/M14-1589

Kebriaei, P., Huls, H., Jena, B., Munsell, M., Jackson, R., Lee, D. A., . . . Cooper, L. J. (2012). Infusing CD19-directed T cells to augment disease control in patients undergoing autologous hematopoietic stem-cell transplantation for advanced B-lymphoid malignancies. *Hum Gene Ther, 23*(5), 444-450. doi:10.1089/hum.2011.167

Lee, D. W., Santomasso, B. D., Locke, F. L., Ghobadi, A., Turtle, C. J., Brudno, J. N., . . . Neelapu, S. S. (2019). ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biol Blood Marrow Transplant, 25*(4), 625-638. doi:10.1016/j.bbmt.2018.12.758

Liu, S., & Yuan, Y. (2015). Bayesian optimal interval designs for phase I clinical trials. *Journal of the Royal Statistical Society: Series C (Applied Statistics), 64*(3), 507-523. doi:https://doi.org/10.1111/rssc.12089

Lu, Y. C., Parker, L. L., Lu, T., Zheng, Z., Toomey, M. A., White, D. E., . . . Rosenberg, S. A. (2017). Treatment of Patients With Metastatic Cancer Using a Major Histocompatibility Complex Class II-Restricted T-Cell Receptor Targeting the Cancer Germline Antigen MAGE-A3. *J Clin Oncol, 35*(29), 3322-3329. doi:10.1200/JCO.2017.74.5463

Malekzadeh, P., Pasetto, A., Robbins, P. F., Parkhurst, M. R., Paria, B. C., Jia, L., . . . Deniger, D. C. (2019). Neoantigen screening identifies broad TP53 mutant immunogenicity in patients with epithelial cancers. *J Clin Invest, 129*(3), 1109-1114. doi:10.1172/JCI123791

Micklethwaite, K. P., Gowrishankar, K., Gloss, B. S., Li, Z., Street, J. A., Moezzi, L., . . . Blyth, E. (2021). Investigation of product derived lymphoma following infusion of piggyBac modified CD19 chimeric antigen receptor T-cells. *Blood*. doi:10.1182/blood.2021010858

Peng, P. D., Cohen, C. J., Yang, S., Hsu, C., Jones, S., Zhao, Y., . . . Morgan, R. A. (2009). Efficient nonviral Sleeping Beauty transposon-based TCR gene transfer to peripheral blood lymphocytes confers antigen-specific antitumor reactivity. *Gene Ther, 16*(8), 1042-1049. doi:10.1038/gt.2009.54

[redacted]

Rohaan, M. W., Wilgenhof, S., & Haanen, J. B. A. G. (2019). Adoptive cellular therapies: the current landscape. *Virchows Archiv : an international journal of pathology, 474*(4), 449-461. doi:10.1007/s00428-018-2484-0

Rosenberg, S. A., & Restifo, N. P. (2015). Adoptive cell transfer as personalized immunotherapy for human cancer. *Science (New York, N.Y.), 348*(6230), 62-68. doi:10.1126/science.aaa4967

Schwartz, R. N., Stover, L., & Dutcher, J. P. (2002). Managing toxicities of high-dose interleukin-2. *Oncology (Williston Park), 16*(11 Suppl 13), 11-20. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12469935

Simon, R. (1989). Optimal two-stage designs for phase II clinical trials. *Control Clin Trials, 10*(1), 1-10. doi:10.1016/0197-2456(89)90015-9

Singh, H., Huls, H., Kebriaei, P., & Cooper, L. J. N. (2014). A new approach to gene therapy using Sleeping Beauty to genetically modify clinical-grade T cells to target CDE19. *Immunological Reviews, 257*, 181-190.

Singh, H., Serrano, L. M., Pfeiffer, T., Olivares, S., McNamara, G., Smith, D. D., . . . Cooper, L. J. N. (2007). Combining Adoptive Cellular and Immunocytokine Therapies to Improve Treatment of B-Lineage Malignancy. *Cancer Research, 67*(6), 2872. doi:10.1158/0008-5472.CAN-06-2283

Tran, E., Robbins, P. F., & Rosenberg, S. A. (2017). 'Final common pathway' of human cancer immunotherapy: targeting random somatic mutations. *Nat Immunol, 18*(3), 255-262. doi:10.1038/ni.3682

Wang, S., O'Rourke, D. M., Chawla, S., Verma, G., Nasrallah, M. P., Morrissette, J. J. D., . . . Mohan, S. (2019). Multiparametric magnetic resonance imaging in the assessment of anti-EGFRvIII chimeric antigen receptor T cell therapy in patients with recurretn glioblastoma. *British J Cancer, 120*, 54-56.

Yuan, Y., Hess, K. R., Hilsenbeck, S. G., & Gilbert, M. R. (2016). Bayesian Optimal Interval Design: A Simple and Well-Performing Design for Phase I Oncology Trials. *Clin Cancer Res, 22*(17), 4291-4301. doi:10.1158/1078-0432.CCR-16-0592

18. APPENDICES

APPENDIX A. NEW YORK HEART ASSOCIATION (NYHA) CLASSIFICATION OF CARDIAC DISEASE

The following table presents the NYHA classification of cardiac disease.

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

APPENDIX B. ASTCT GRADING FOR CYTOKINE RELEASE SYNDROME[1]

(Note: CRS grade should be determined at least twice daily and any time there is a change in patient's status)

CRS parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever[2]	Yes	Yes	Yes	Yes
		With		
Hypotension[3]	None	Requiring IV fluids but not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
		And/Or		
Hypoxia[3]	None	Requiring low-flow O_2 via nasal cannula[4] or blow-by	Requiring O_2 via high-flow nasal cannula[4], facemask, nonrebreather mask, or Venturi mask	Requiring O_2 via positive pressure (e.g., CPAP, BiPAP, and mechanical ventilation)

Adapted from (Lee et al., 2019)

CPAP=continuous positive airway pressure
BiPAP=bilevel positive airway pressure

[1]Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading.
[2] Fever is defined as temperature ≥ 38 °C not attributable to any other cause. In patients who have CRS then receive antipyretics or anti-cytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.
[3] CRS grade is determined by 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.
[4] Low-flow nasal cannula is defined as oxygen delivered at ≤ 6 L/min. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at > 6 L/min.

APPENDIX C. IMMUNE EFFECTOR CELL-ASSOCIATED ENCEPHALOPATHY (ICE) SCORE

ICE Assessment
Orientation: orientation to year, month, city, hospital: 4 points (1 point each)
Naming: ability to name 3 objects (e.g., clock, pen, button): 3 points (1 point each)
Following commands: ability to follow simple commands (e.g., "Show me 2 fingers" or "Close your eyes and stick out your tongue"): 1 point
Writing: ability to write a standard sentence (e.g., "Our national bird is the bald eagle"): 1 point
Attention: ability to count backwards from 100 by 10: 1 point

Score 10: No Impairment
Score 7-9: Grade 1 ICANS
Score 3-6: Grade 2 ICANS
Score 0-2: Grade 3[1] ICANS
Score 0 due to patient unarousable or unable to perform ICE assessment: Grade 4 ICANS

[1]A patient with an ICE score of 0 may be classified as having Grade 3 ICANS if the patient is awake with global aphasia or may be classified as having Grade 4 ICANS if the patient is unarousable

APPENDIX D. DIAGNOSTIC CRITERIA FOR IEC-ASSOCIATED FULMINANT HEMOPHAGOCYTIC LYMPHOHISTIOCYTES (HLH)/MACROPHAGE ACTIVATION SYNDROME (MAS)

- Consider HLH/MAS if a subject has a peak ferritin > 10,000ng/mL during the CRS phase and develops any two of the following organ toxicities after IEC therapy
  - ≥ Grade 3 increase in bilirubin, aspartate transaminase, or alanine transaminase[1]
  - ≥ Grade 3 oliguria or increase in creatinine[1]
  - ≥ Grade 3 pulmonary edema[1]
  - Presence of hemophagocytosis by morphology and/or CD68 immunohistochemistry in bone marrow or organs
- If HLH/MAS is suspected, obtain baseline fasting triglyceride level and serum soluble IL-2 receptor

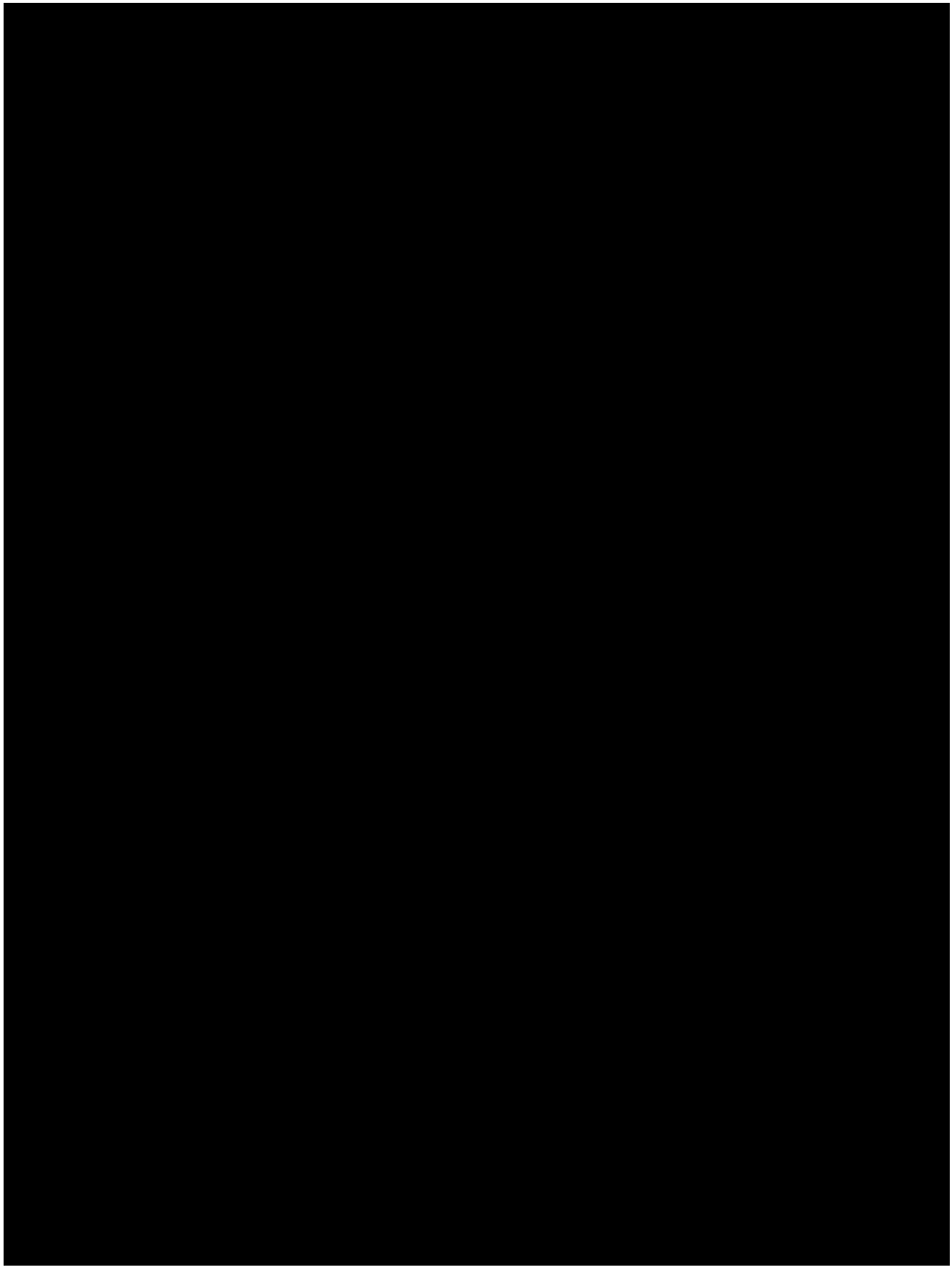
[1] Grading per Common Terminology Criteria for Adverse Events, NCI CTCAE Version 5.0

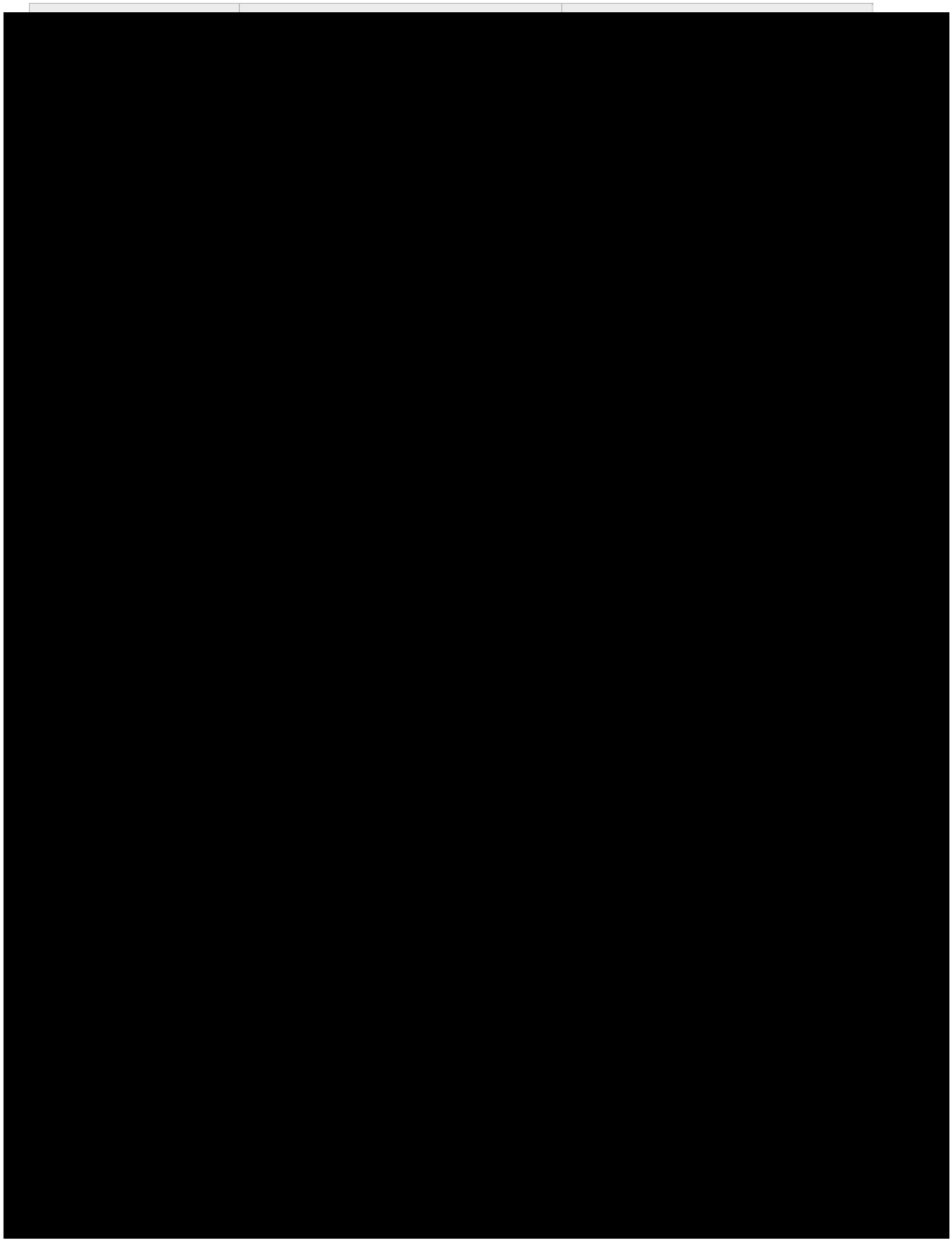
APPENDIX E. ASTCT GRADING OF ICANS[1]

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE score[2]	7-9	3-6	0[3]-2	0[3] (patient is unarousable and unable to perform ICE)
Depressed levels of consciousness[4]	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 findings[5]	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis
Elevated intracranial pressure (ICP)[6]/cerebral edema	N/A	N/A	Focal/local edema on neuroimaging[7]	Diffuse cerebral edema on neuroimaging; Decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad

Adapted from (Lee et al., 2019)

[1.] ICANS grading is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause; for example, a patient with an ICE score of 3 who has a generalized seizure is classified as grade 3 ICANS.
N/A indicates not applicable.
[2.] Refer to Appendix D for ICE Score.
[3.] A patient with an ICE score of 0 may be classified as having Grade 3 ICANS if the patient is awake with global aphasia or Grade 4 ICANS if the patient is unarousable
[4.] Depressed level of consciousness should not be attributable to any other cause (e.g., sedating medication)
[5] Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading.
[6.] Opthamology may be consulted to assess for papilledema if concern for elevated ICP, but otherwise not needed for all patients
[7] 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.





Issue	Considerations	Management[1]
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APPENDIX H. SUPPLEMENTARY STATISTICAL METHODS FOR PHASE I DOSE ESCALATION [redacted] STAGED FUTILITY

Phase I:

The Bayesian optimal interval (BOIN) design (Liu & Yuan, 2015; Yuan et al., 2016) will be used to find the MTD.

The target toxicity rate for the MTD is $\phi = 0.3$ and the maximum sample size is 12. We will enroll and treat patients in dose cohorts of size 3. DLTs are defined in Section 5.3, and only those DLTs that occur within **the first cycle** will be used for dose finding. As shown in Figure 5, the BOIN design uses the following rule, optimized to minimize the probability of incorrect dose assignment, to guide dose escalation/de-escalation:

- if the observed DLT rate at the current dose is ≤ 0.253, escalate the dose to the next higher dose level;
- if the observed DLT rate at the current dose is > 0.359, de-escalate the dose to the next lower dose level;
- otherwise, stay at the current dose.

For the purpose of overdose control, doses j and higher levels will be eliminated from further examination if $\Pr(p_j > 0.3 \mid \text{data}) > 0.95$ and at least 3 evaluable patients have been treated at dose level j, where p_j is the true DLT rate of dose level $j, j = 1,\cdots, 4$. This posterior probability is evaluated based on the beta-binomial model $y_j \mid p_j \sim \text{binomial}(p_j)$ with $p_j \sim \text{uniform}(0,1)$, where y_j is the number of patients experienced DLT at dose level j. When the lowest dose is eliminated, stop the trial for safety. The probability cutoff 0.95 is chosen to be consistent with the common practice that when the target DLT rate <=1/6, a dose with 2/3 patients experienced DLT is eliminated. The above dose escalation/de-escalation and elimination rule can be equivalently presented in Table 15, which will be used to conduct the trial.

The steps to implement the BOIN design are described as follows:

1. Perform accelerated titration as follows. Treat the first patient at dose level 2 and escalate the dose in the one-patient-per-dose-level fashion until any of the following events is observed: (i) the first instance of DLT, (ii) the second instance of moderate (grade 2) toxicity, or (iii) the highest dose level is reached. Then, treat 2 additional subjects at the current dose level. Hereafter, subjects are treated in dose cohorts of size 3 as described in steps 2 and 3.

2. To assign a dose to the next dose cohort of patients, conduct dose escalation/de-escalation according to the rule displayed in Table 15. When using Table 15, please note the following:

    a. “Eliminate” means eliminate the current and higher doses from the trial to prevent treating any future patients at these doses because they are overly toxic.

b. When we eliminate a dose, automatically de-escalate the dose to the next lower level. When the lowest dose is eliminated, stop the trial for safety. In this case, no dose should be selected as the MTD.

c. If none of the actions (i.e., escalation, de-escalation or elimination) is triggered, treat the new patients at the current dose.

d. If the current dose is the lowest dose and the rule indicates dose de-escalation, treat the new patients at the lowest dose unless the number of DLTs reaches the elimination boundary, at which point terminate the trial for safety.

e. If the current dose is the highest dose and the rule indicates dose escalation, treat the new patients at the highest dose.

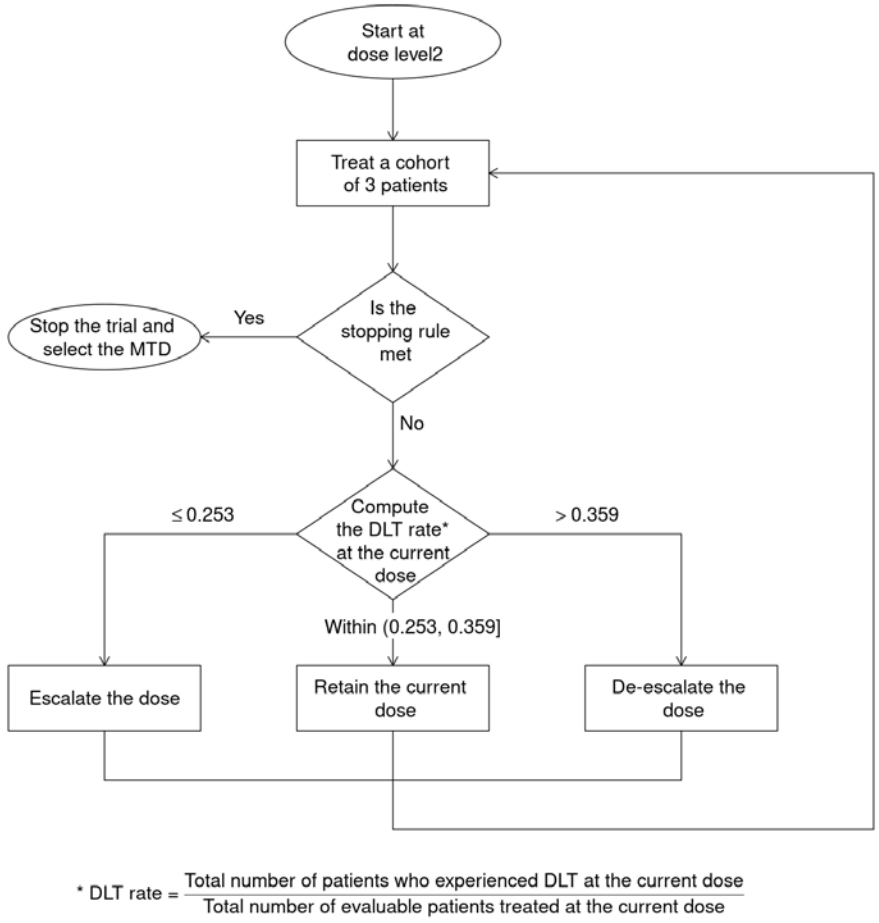
3. Repeat step 2 until the maximum sample size of 12 is reached, or stop the trial if the number of evaluable patients treated at the current dose reaches 9 and the decision according to Table 15 is to stay at the current dose.

Table 15. Dose escalation/de-escalation rule for the BOIN design

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Number of evaluable patients treated at current dose	1	2	3	4	5	6	7	8	9
Escalate if # of DLT <=	0	0	0	1	1	1	1	2	2
Deescalate if # of DLT >=	1	1	2	2	2	3	3	3	4
Eliminate if # of DLT >=	NA	NA	3	3	4	4	5	5	5

Note. “# of DLT” is the number of patients with at least 1 DLT. When none of the actions (i.e., escalate, de-escalate or eliminate) is triggered, stay at the current dose for treating the next dose cohort of patients. “NA” means that a dose cannot be eliminated before treating 3 evaluable patients.

Figure 5. Flowchart for trial conduct using the BOIN design



After the trial is completed, select the MTD based on isotonic regression as specified in (Liu & Yuan, 2015). This computation is implemented by the shiny app available at http://www.trialdesign.org. Specifically, select as the MTD the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there are ties, select the higher dose level when the isotonic estimate is lower than the target toxicity rate and select the lower dose level when the isotonic estimate is greater than or equal to the target toxicity rate.

Operation Characteristics

Table 16 shows the operating characteristics of the trial design based on 1000 simulations of the trial using shiny app "BOIN" (BOIN V2.6.5.0) available at http://www.trialdesign.org. The operating characteristics show that the design selects the true MTD, if any, with high probability and allocates more patients to the dose levels with the DLT rate closest to the target of 0.3.

Table 16. Operating characteristics of the BOIN design

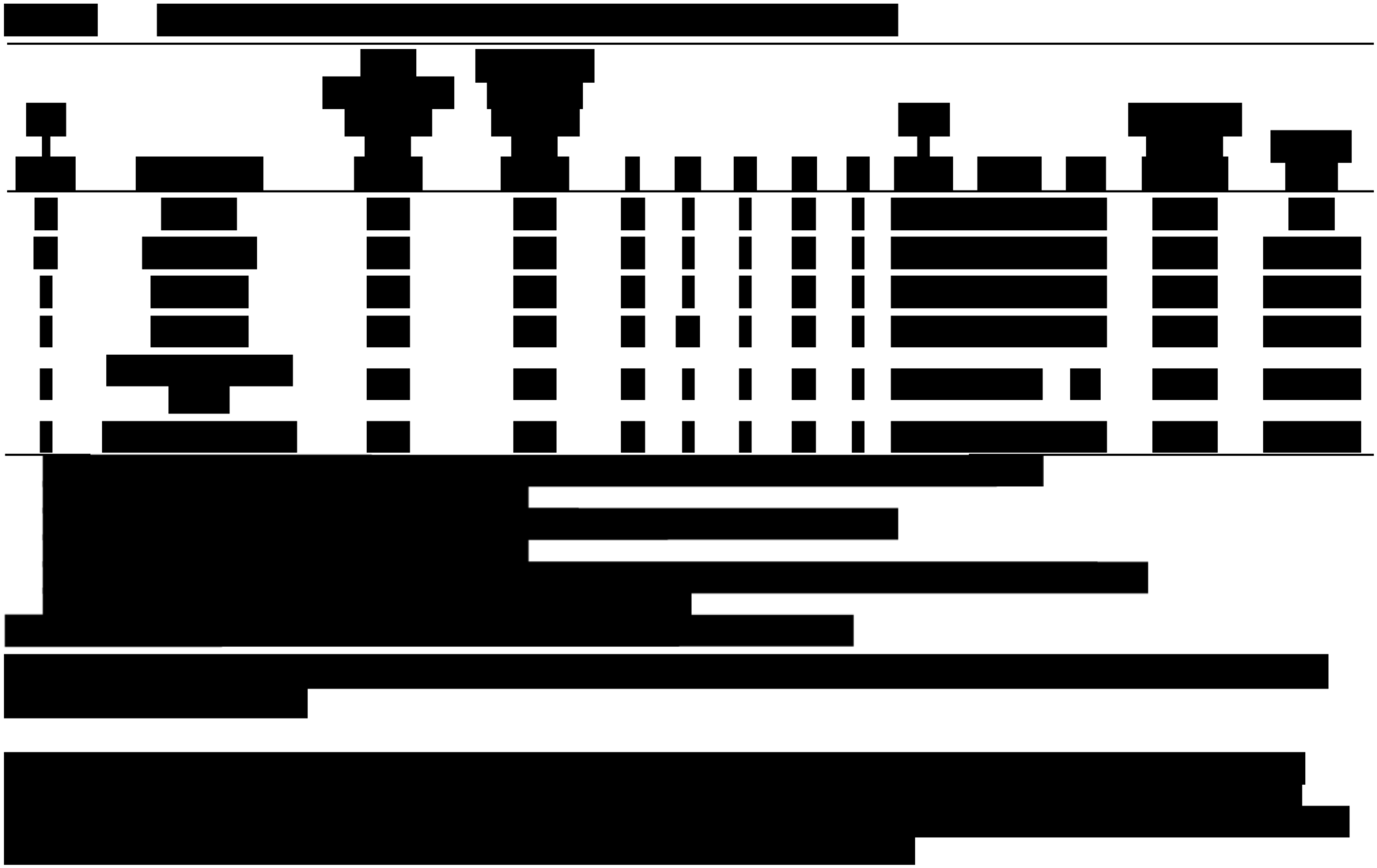
	1	2	3	4	Number of Patients	% Early Stopping
Scenario 1						
True DLT Rate	0.3	0.47	0.55	0.64		
Selection %	44.3	34.5	13.5	2.6		5.1
% Pts Treated	29.2	38.4	21.8	10.6	11.8	
Scenario 2						
True DLT Rate	0.11	0.3	0.45	0.67		
Selection %	13.4	47.4	35.8	3.2		0.2
% Pts Treated	11.1	36.7	34.6	17.6	11.9	
Scenario 3						
True DLT Rate	0.02	0.13	0.3	0.47		
Selection %	0.1	20.9	47.8	31.2		0
% Pts Treated	1.1	23.5	37.1	38.3	11.8	
Scenario 4						
True DLT Rate	0.05	0.1	0.15	0.3		
Selection %	0.3	4.6	23.9	71.2		0
% Pts Treated	0.8	15.4	26	57.8	11.5	

Note: "% Early Stopping"" refers to early stopping due to excessive DLT.

Table 17. Trial and Design Specifications

Parameter	Value
Number of doses	4
Starting dose	2
Max sample size	12
Dose cohort size	3
Stop trial if # patients assigned to single dose reaches	9
Use accelerated titration	TRUE
Target toxicity probability	0.3
Use the default alternatives to minimize decision errors	FALSE
Alternative (unacceptable high toxicity) for optimization	0.42
Alternative (unacceptable low toxicity) for optimization	0.21
Eliminate dose threshold	0.95
Impose a more stringent safety stopping rule	FALSE
Require the isotonic estimate of the DLT probability for the dose selected as the MTD less than the de-escalation boundary	FALSE
Number of repetitions per scenario	1000
Random number generator seed	6

[redacted]



APPENDIX I. PROTOCOL CRITERIA FOR RECIST (RESPONSE EVALUATION CRITERIA IN SOLID TUMORS) VERSION 1.1 AND IRECIST (IMMUNE RECIST) GUIDELINES

Response and progression will be evaluated in this study using the revised international criteria (1.1) proposed by the RECIST (Response Evaluation Criteria in Solid Tumors) committee as well as the modified iRECIST guidelines. Investigators should note the different requirements for confirmatory scans as well as follow up for the two criteria.

1 RECIST 1.1 Response and Evaluation Endpoints

1.1 *Measurable Disease*. Measurable tumor lesions (nodal, subcutaneous, lung parenchyma, solid organ metastases) are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥10 mm with CT scan or clinical examination.Bone lesions are considered measurable only if assessed by CT scan and have an identifiable soft tissue component that meets these requirements (soft tissue component ≥10 mm by CT scan). *Malignant lymph nodes* must be ≥15 mm in the short axis to be considered measurable; only the short axis will be measured and followed. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Previously irradiated lesions are not considered measurable unless progression has been documented in the lesion.

1.2 *Non-measurable Disease*. All other lesions (or sites of disease), including small lesions are considered non-measurable disease. Bone lesions without a measurable soft tissue component, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, lymphangitic involvement of lung or skin and abdominal masses followed by clinical examination are all non-measurable. Lesions in previously irradiated areas are non-measurable, unless progression has been demonstrated.

1.3 *Target Lesions*. When more than one measurable tumor lesion is present at baseline all lesions up to a maximum of 5 lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. Note that pathological nodes must meet the criterion of a short axis of ≥15 mm by CT scan and only the short axis of these nodes will contribute to the baseline sum. All other pathological nodes (those with short axis≥10 mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10 mm are considered non-pathological and should not be recorded or followed. At baseline, the sum of the target lesions (longest diameter of tumor lesions plus short axis of lymph nodes:

overall maximum of 5) isto be recorded. After baseline, a value should be provided on the CRF for all identified target lesions for each assessment, even if very small. If extremely small and faint lesions cannot be accurately measured but are deemed to be present, a default value of 5 mm may be used. If lesions are too small to measure and indeed are believedto be absent, a default value of 0 mm may be used.

1.4 *Non-target Lesions*. All non-measurable lesions (or sites of disease) plus any measurable lesions over and above those listed as target lesions are considered *non-target lesions*. Measurements are not required but these lesions should be noted at baseline and should be followed as "present" or "absent".

1.5 *Response*.

All patients will have their BEST RESPONSE from the start of study treatment until the end of study classified as outlined below:

Complete Response (CR): disappearance of target and non-target lesions and normalization of tumor markers. Pathological lymph nodes must have short axis measures <10 mm (Note: continue to record the measurement even if <10 mm and considered CR). Residual lesions (other than nodes <10 mm) thought to be non-malignant should be further investigated (by cytology specialized imaging or other techniques as appropriate for individual cases before CR can be accepted. Confirmation of response is only required in non-randomized studies.

Partial Response (PR): at least a 30% decrease in the sum of measures (longest diameter for tumor lesionsand short axis measure for nodes) of target lesions, taking as reference the baseline sum of diameters. Non target lesions must be non-PD. Confirmation of response is only required in non-randomized studies.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PDtaking as reference the smallest sum of diameters on study.

Progressive Disease (PD): at least a 20% increase in the sum of diameters of measured lesions taking as references the smallest sum of diameters recorded on study (including baseline) AND an absolute increase of ≥5 mm. Appearance of new lesions will also constitute progressive disease (including lesions in previously unassessed areas). In exceptional circumstances, unequivocal progression of non-target disease may be accepted as evidence of disease progression, where the overall tumor burden has increased sufficiently to merit discontinuation of assessment or where the tumor burden appears to have increased by at least 73% in volume. Modest increases in the size of one or more non-target lesions are NOT considered unequivocal progression. If the evidence of PD is equivocal (target or non-target), assessment may continue until the next assessment, but if confirmed, the earlier date must be used.

Table 19: **Integration of target, non-target and new lesions into response assessment**

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Response for thisCategory also Requires**
Target lesions ± non target lesions				
CR	CR	No	CR	Normalization of tumor markers,tumor nodes <10 mm
CR	Non-CR/Non-PD	No	PR	
CR	Not all evaluated	No	PR	
PR	Non-PD/ not all evaluated	No	PR	
SD	Non-PD/ not all evaluated	No	SD	Documented at least once ≥4 wks. from baseline
Not all evaluated	Non-PD	No	NE	
PD	Any	Any	PD	
Any	PD	Any	PD	
Any	Any	Yes	PD	
Non target lesions ONLY				
No Target	**CR**	**No**	**CR**	**Normalization of tumor markers, tumor nodes <10 mm**
No Target	Non-CR/non-PD	No	Non-CR/non-PD	
No Target	Not all evaluated	No	NE	
No Target	Unequivocal PD	Any	PD	
No Target	Any	Yes*	PD	

Note: Patients with a global deterioration of health status requiring discontinuation of assessment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration". This is a reason for stopping therapy, but is NOT objective PD. Every effort should be made to document the objective progression even after discontinuation from the core study.
*Investigators should record all new lesions; if the new lesion is felt to be equivocal, assessment may be continued pending further assessments – see table 2.

2 iRECIST Response Assessment

Overall response will also be assessed using iRECIST. Immunotherapeutics may result in infiltration of immune cells leading to transient increase in the size in malignant lesions, or undetectable lesions becoming detectable. The criteria are identical to those of RECIST 1.1 in many respects but have been adapted to account for instances where an increase in tumor burden, or the appearance of new lesions, does not reflect true tumor progression.

Key differences are described below. All responses defined using iRECIST criteria are designated with a prefix. iRECIST time-point and best overall responses will be recorded separately.

2.1 Confirming Progression

Unlike RECIST 1.1, iRECIST requires the confirmation of progression and uses the terms iUPD (unconfirmed progression) and iCPD (confirmed progression). Confirmatory scans should be performed at least 4 weeks, but no longer than 8 weeks after iUPD.

iCPD is confirmed if further increase in tumor burden, compared to the last assessment, is seen as evidenced by one or more of the following:

- Continued increase in tumor burden (from iUPD) where RECIST 1.1 definitions of progression had been met (from nadir) in target, non-target disease or new lesions
    - Progression in target disease worsens with an increase of at least 5 mm in the absolute value of the sum
    - Continued unequivocal progression in non-target disease with an increase in tumor burden
    - Increase in size of previously identified new lesion (s) (an increase of at least 5 mm in the absolute value of the sum of those considered to be target new lesions) or additional new lesions.
- RECIST 1.1 criteria are met in lesion types (target or non-target or new lesions) where progression was not previously identified, including the appearance of additional new lesions.

If iUPD is not confirmed at the next assessment, then the appropriate response will be assigned (iUPD if the criteria are still met, but no worsening, or iSD, iPR or iCR if those criteria are met compared to baseline). As can be seen in table 2, the prior documentation of iUPD does not preclude assigning iCR,iPR, or iSD in subsequent time-point assessments or as best overall response (BOR) providing that iCPD isnot documented at the next assessment after iUPD.

2.2 New lesions

New lesions should be assessed and measured as they appear using RECIST 1.1 criteria (maximum of 5 lesions, no more than 2 per site, at least 10 mm in long axis (or 15 mm in short axis for nodal lesions) and recorded as New Lesions-Target (NLT) and New Lesion-Non-Target (NLNT) to allow clear differentiation from baseline target and non-target lesions.

New lesions may either meet the criteria of NLT or NLNT to drive iUPD (or iCPD). However, the measurements of target lesions should NOT be included in the sum of measures of original target lesions identified at baseline. Rather, these measurements will be collected on a separate table in the case record form.

PD is confirmed in the New Lesion category if the next imaging assessment, conducted at least 4 weeks (but not more than 8 weeks) after iUPD confirms further progression from iUPD with either an increase of at least 5 mm in the absolute value of the sum of NLT OR an increase (but not necessarily

unequivocal increase) in the size of NLNT lesions OR the appearance of additional new lesions

Table 20: Time-point (TP) iResponse

Target Lesions*	Non-TargetLesions*	New Lesions*	Time Point Response	
			No prior iUPD**	**Prior iUPD**; *****
iCR	iCR	No	iCR	iCR
iCR	Non-iCR/Non-iUPD	No	iPR	iPR
iPR	Non-iCR/Non-iUPD	No	iPR	iPR
iSD	Non-iCR/Non-iUPD	No	iSD	iSD
iUPD with no change OR decrease from last TP	iUPD with nochange ORdecrease from last TP	Yes	NA	NLs confirms iCPD if NLs were previously identified and increase in size (≥5 mm in SOM for NLT or any increase for NLNT) or number. If no change in NLs (size or number) from last TP, remains iUPD
iSD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based in further increase in size of NT disease (need not meet RECIST 1.1 criteria for unequivocal PD)
iUPD	Non-iCR/Non-iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on: o further increase in SOM of at least 5 mm, otherwise remainsiUPD
iUPD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increasein: o previously identified T lesion iUPD SOM ≥5 mm and / or o NT lesion iUPD (prior assessment - need not be unequivocalPD)
iUPD	iUPD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on further increasein: o previously identified T lesion iUPD ≥5 mm and / or o previously identified NT lesion iUPD (need not be unequivocal) and /or o size or number of new lesions previously identified
Non-iUPD/PD	Non-iUPD/PD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on o increase in size or number of new lesions previously identified

* Using RECIST 1.1 principles. If no PSPD occurs, RECIST 1.1 and iRECIST categories for CR, PR and SD wouldbe the same. ** in any lesion category. *** previously identified in assessment immediately prior to this TP.

All patients will have their iBOR from the start of study treatment until the end of study classified as outlined below.

Table 21: iRECIST Best Overall Response (iBOR)

TPR1	TPR2	TPR3	TPR4	TPR5	iBOR
iCR	iCR, iPR,iUPD, NE	iCR, iPR, iUPD, NE	iUPD	iCPD	iCR
iUPD	iPR, iSD, NE	iCR	iCR, iPR, iSD, iUPD, NE	iCR, iPR, iSD, iUPD, iCPD, NE	iCR
iUPD	iPR	iPR, iSD, iUPD, NE	iPR, iSD, iUPD, NE, iCPD	iPR, iSD, iUPD, NE, iCPD	iPR
iUPD	iSD, NE	PR	iPR, iSD, iUPD, NE	iPR, iSD, iUPD, iCPD, NE	iPR
iUPD	iSD	iSD, iUPD, NE	iSD, iUPD, iCPD, NE	iSD, iUPD, ICPD, NE	iSD
iUPD	iCPD	Anything	Anything	Anything	iCPD
iUPD	iUPD	iCPD	Anything	Anything	iCPD
iUPD	NE	NE	NE	NE	iUPD

- Table assumes a randomised study where confirmation of CR or PR is not required.
- NE = not evaluable that cycle.
- Designation "I" for BOR can be used to indicate prior iUPD to aid in data interpretation.
- For patients with non-target disease only at baseline, only CR or non-CR/non-PD can be assigned at each TPR but is notshown in the table for ease of presentation.

5 Response and Stable Disease Duration (RECIST 1.1 and iRECIST)

Response duration will be measured from the time measurement criteria for CR/PR or iCR/iPR (whichever is first recorded) are first met until the first date that recurrent or progressive disease is objectively documented, taking as reference the smallest measurements recorded on study (including baseline).

Stable disease duration will be measured from the time of start of treatment until the criteria for progression are met, taking as reference the smallest sum on study (including baseline).

6 Methods of Measurement

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Assessments should be identified on a calendar schedule and should not be affected by delays in therapy. While on study, all lesions recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g., 2 mm).If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned. For lesions which fragment/split add together the longest diameters of the fragmented portions; for lesions which coalesce, measure the maximal longest diameterfor the "merged lesion".

6.1 *Clinical Lesions*. Clinical lesions are not useful in assessment of lesion size and should not be used as a method of measurement and will not be an allowed modality for lesion determinations in this study. For qualitative use of skin lesions, documentation by colorphotography including a ruler to estimate

the size of the lesion is recommended. If feasible, imaging is preferred.*Chest X-ray*. Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. Chest X-ray is not useful in assessment of lesion size and should not be used as a method of measurement and will not be an allowed modality for lesion determinations in this study.

6.2 *CT, MRI*. CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm,the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans, liver lesions). While PET scans are not considered adequate to measure lesions, PET-CT scans may be used providing that the measures are obtained from the CT scan and the CT scan is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast).

6.3 *Ultrasound*. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. If new lesions are identified by ultrasound during the study, confirmation by CT is advised.

6.4 *Endoscopy, Laparoscopy*. The utilization of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies areobtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

6.5 *Tumor Markers*. Tumor markers alone cannot be used to assess objective tumor response. Note: If markers are initially above the upper normal limit, they may need to normalize for a patient to be considered in complete response. Note: this should be discussed with the Alaunos Medical Monitor.

6.6 *Cytology, Histology*. When effusions are known to be a potential adverse effect of treatment (e.g., with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is advised to differentiate between response or stable disease and progressive disease

APPENDIX J. IMMUNE-EFFECTOR CELL TOXICITY ASSESSMENT AND MANAGEMENT

Immune-effector cell toxicity assessment and management should be performed per the CARTOX criteria (https://www.mdanderson.org/documents/for-physicians/algorithms/clinical-management/clin-management-cytokine-release-web-algorithm.pdf).

Table 22: Management of CRS

CRS Grade	CRS Parameter	Management		
		Diagnostic Work-Up	**Supportive Care**	**Anti-IEC Therapies**
Grade 1	**Fever**	• Assess for infection with blood and urine cultures, and chest radiography • Cardiac telemetry and pulse oximetry	• Acetaminophen and hypothermia blanket as needed for the treatment of fever • Ibuprofen if fever is not controlled with above; use with caution or avoid with thrombocytopenia or renal dysfunction • Empiric broad-spectrum antibiotics and consider filgrastim products if neutropenic • Maintenance IV fluids for hydration • Symptomatic management of constitutional symptoms and organ toxicities as per standard guidelines • If not on seizure prophylaxis, initiate levetiracetam 500 mg PO twice daily	• Administer tocilizumab[1] for 1 dose for persistent fever lasting greater than 3 days

[1] **See Table 24 for Dosing of IL-6 Antagonists and Alternative Agents**

Management of CRS - *continued*

CRS Grade	CRS Parameter	Management		
		Diagnostic Work-up	**Supportive Care**	**Anti-IEC Therapies**
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 – 1,000 mL 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[1] for 1 dose **and** consider dexamethasone 4 - 10 mg 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 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, face mask, or positive pressure ventilation, refer to further management as in Grade 3 or 4 CRS • Symptomatic management of fever as in Grade 1 CRS • Symptomatic management of constitutional symptoms and organ toxicities as per standard guidelines	• Administer tocilizumab[1] for 1 dose **and** consider dexamethasone 4 - 10 mg 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 doses in a 24-hour period

[1] See Table 24 for Dosing of IL-6 Antagonists and Alternative Agents

Management of CRS - *continued*

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

[1] See Table 24 for Dosing of IL-6 Antagonists and Alternative Agents
[2] VASST Trial vasopressor equivalent equation: norepinephrine equivalent dose = [norepinephrine (mcg/minute)] + [dopamine (mcg/kg/minute) / 2] + [epinephrine (mcg/minute)] + [phenylephrine (mcg/minute) / 10]

Management of CRS - *continued*

CRS Grade	CRS Parameter	Management		
		Diagnostic Work-up	**Supportive Care**	**Anti-IEC Therapies**
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[1] as in Grade 2 CRS if not administered previously; tocilizumab may be repeated every 8 hours for up to 3 doses in a 24-hour period • Methylprednisolone 1,000 mg/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 (see Table 24 including activation of safety switches if applicable
	Hypoxia	• Monitor oxygen saturation while on mechanical ventilation • Fever work-up if not previously performed ◦ Assess for infection with blood and urine cultures, and chest radiography	• Transfer patient to ICU • Positive pressure ventilation including CPAP, BiPAP, mechanical ventilation • Symptomatic management of fever as in Grade 1 CRS • Symptomatic management of constitutional symptoms and organ toxicities as per standard guidelines	• Tocilizumab[1] as in Grade 2 CRS if not administered previously; tocilizumab may be repeated every 8 hours for up to 3 doses in a 24-hour period • Methylprednisolone 1,000 mg/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 (see Table 24) including activation of safety switches if applicable

[1] See Table 24 for Dosing of IL-6 Antagonists and Alternative Agents

Table 23: Management of ICANS

ICANS Grade	Sign or symptom	Management		
		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 • ICE Score assessment every 6 hours or more frequently if clinically indicated • EEG • Consider diagnostic lumbar puncture if other causes of encephalopathy are suspected (*e.g.,* infections, autoimmune, leptomeningeal disease) ◦ Add a meningitis-encephalitis panel from CSF in patients with neurologic symptoms that persist or worsen after ICANS therapy and/or if symptoms start after corticosteroids	• 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 (0.25-0.5 mg IV every 8 hours) or haloperidol (0.5 mg IV every 6 hours) may be used with careful monitoring for agitated patients • If no seizures on EEG, continue prophylactic levetiracetam • If EEG shows focal or generalized convulsive or non-convulsive seizure or convulsive status epilepticus, refer to further management as in Grade 3 or 4 ICANS	• Dexamethasone 10 mg IV for 1 dose (or methylprednisolone equivalent) and reassess in 6 hours or earlier if clinically indicated ◦ If associated with concurrent CRS, add tocilizumab[1]
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 10 mg IV every 12 hours (or methylprednisolone equivalent) ◦ If associated with concurrent CRS, add tocilizumab[1] • Once ICANS improves to Grade 1 or less, taper and/or stop corticosteroids depending on clinical situation

ICANS Grade	Sign or symptom	Management		
		Diagnostic Work-up	**Supportive Care**	**Anti-IEC Therapies**
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) ◦ Add a meningitis-encephalitis panel from CSF in patients with neurologic symptoms that persist or worsen after ICANS therapy and/or if symptoms start after corticosteroids	• Supportive care as in Grade 1 ICANS • Consider ICU transfer • If there are new abnormal findings on brain imaging[1] not related to primary malignancy, control hypertension with the goal of maintaining mean arterial pressure (MAP) within 20-25 mmHg 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)	• Dexamethasone 10 mg IV every 6 hours (or methylprednisolone equivalent) ◦ If associated with concurrent CRS, add tocilizumab[2] • If Grade 3 encephalopathy is persistent for > 24 hours, increase dexamethasone to 20 mg IV every 6 hours (or methylprednisolone equivalent) • Once ICANS improves to Grade 1 or less, taper and/or stop corticosteroids depending on clinical situation
	Seizure	• Neurological work-up as in Grade 1 ICANS • EEG if clinically indicated (*e.g.,* ongoing seizures, depressed level of consciousness) • Rule out other potential causes of seizure (i.e., beta-lactams, etc.)	• Transfer to ICU • Supportive care as in Grade 1 ICANS • For focal or generalized convulsive seizures, or non-convulsive seizures, treat as per Appendix K	• Dexamethasone 20 mg IV every 6 hours (or methylprednisolone equivalent) ◦ If associated with concurrent CRS, add tocilizumab[2] • 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 • Supportive care as in Grade 1 ICANS	• If focal edema is in brain stem or thalamus, methylprednisolone 1,000 mg/day in divided doses IV for 3 days followed by taper depending on clinical situation ◦ If associated with concurrent CRS, add tocilizumab[2] • If focal edema is in other areas of brain, methylprednisolone 1,000 mg/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[2]

[1]Abnormal findings on imaging where correction of hypertension, uremia, and/or coagulopathy should be performed include changes suggestive of typical or atypical posterior reversible encephalopathy syndrome (PRES), temporal lobe and limbic system encephalitis (autoimmune or infection), acute disseminated encephalomyelitis, emboli, vasculitis, strokes, and/or seizure-related changes
[2]See Table 24 for Dosing of IL-6 Antagonists and Alternative Agents

ICANS Grade	Sign or symptom	Management		
		Diagnostic Work-Up	**Supportive Care**	**Anti-IEC Therapies**
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 • Supportive care as in Grade 1 ICANS • Consider mechanical ventilation for airway protection • If there are new abnormal findings on brain imaging[1] not related to primary malignancy, control hypertension with the goal of maintaining MAP within 20-25 mmHg 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)	• Methylprednisolone 1,000 mg/day in divided doses IV for 3 days followed by taper as clinically indicated; if associated with concurrent CRS, add tocilizumab[2] • Continue corticosteroids until improvement to less than or equal to Grade 1 ICANS and then taper and stop corticosteroids depending on clinical situation • If Grade 4 ICANS is refractory for > 24 hours or if patient is deteriorating rapidly, consider additional therapies (see Table 24)including activation of safety switches if applicable
	Seizure	• Neurological work-up as in Grade 1 ICANS • Rule out other potential causes of seizure (i.e., beta-lactams, etc.)	• Transfer to ICU • Supportive care as in Grade 1 ICANS • For focal or generalized convulsive or non-convulsive seizure or convulsive status epilepticus, treat as in Appendix K • For convulsive status epilepticus, treat as in Appendix L	• Methylprednisolone 1,000 mg/day in divided doses IV for 3 days followed by taper as clinically indicated; if associated with concurrent CRS, add tocilizumab[2] • If Grade 4 ICANS is refractory for > 24 hours or if patient is deteriorating rapidly, consider additional therapies (see Table 24) including activation of safety switches if applicable
	Motor Weakness	• Neurological work-up as in Grade 1 ICANS • MRI with and without contrast of the spine	• Transfer to ICU • Supportive care as in Grade 1 ICANS	• Methylprednisolone 1,000 mg/day in divided doses IV for 3 days followed by taper as clinically indicated; if associated with concurrent CRS, add tocilizumab[2] • If Grade 4 ICANS is refractory for > 24 hours or if patient is deteriorating rapidly, consider additional therapies (see Table 24) including activation of safety switches if applicable
	Diffuse cerebral edema or raised intracranial pressure	•Neurological work-up as in Grade 1 ICANS •Consider repeat neuro-imaging as in focal cerebral edema from Grade 3 ICANS	• Transfer to ICU • Supportive care as in Grade 1 ICANS • For diffuse cerebral edema or signs of raised intracranial pressure, treat as in Table 25	• Methylprednisolone 1,000 mg/day in divided doses IV for 3 days followed by taper as clinically indicated; if associated with concurrent CRS, add tocilizumab[2] • If Grade 4 ICANS is refractory for > 24 hours or if patient is deteriorating rapidly, consider additional therapies (see Table 24) including activation of safety switches if applicable

[1]Abnormal findings on imaging where correction of hypertension, uremia, and/or coagulopathy should be performed include changes suggestive of typical or atypical posterior reversible encephalopathy syndrome (PRES), temporal lobe and limbic system encephalitis (autoimmune or infection), acute disseminated encephalomyelitis, emboli, vasculitis, strokes, and/or seizure-related changes. [2] See Table 24 for dosing of IL-6

Table 24: Recommendations for Use of IL-6 Antagonists and Alternative Agents for Management of CRS and ICANS

Drug	Recommended Dose for CRS and/or ICANS	Maximum Dose	Mechanism of Action	Comments
Tocilizumab	8 mg/kg IV	Maximum 800 mg per dose	IL-6 receptor antagonist	• Maximum of 4 doses total over the entire course of CRS andICANS • Dose may be repeated every 8 hours for up to three doses in a 24-hour period
Siltuximab	11 mg/kg IV once	-	IL-6 antibody	• Recommended primarily for patients who are intolerant to tocilizumab • No more than 1 dose in a 3-week period
Anakinra	100 mg subcutaneously daily for 7 days	-	IL-1 receptor antagonist	• Renal dose adjustment may be needed for creatinine clearance < 30 mL/minute
Cyclophosphamide	1,500 mg/m^2 IV for one dose	-	Alkylating agent	• Give with mesna 1500 mg/m^2 IV over 24 hours for one dose
Anti-thymocyte globulin (rabbit)	1-2 mg/kg IV daily for 3 days	-	Immunosuppressant	• Hypersensitivity reactions can occur; premedicate with diphenhydramine and scheduled dose of corticosteroid • Infuse over a minimum of 6 hours
Safety switches	-	-	-	• If the IEC product contains a safety switch (*e.g.,* iCapsase-9 or EGFRt-positive), the corresponding drug to eliminate those cells can be considered in doses according to manufacturer Examples include rimiducid to eliminate iCaspase-9 or cetuximab to eliminate EGFRt-positive cells

APPENDIX K. MANAGEMENT OF FOCAL OR GENERALIZED CONVULSIVE OR NON-CONVULSIVE SEIZURES

- Consult Neurology
- For focal and generalized convulsive seizures, lorazepam 1-2 mg IV and repeat as needed (to a maximum cumulative dose of 4 mg)
- For electrographical seizures, including non-convulsive status epilepticus, lorazepam 0.5 mg IV and repeat every 5 minutes as needed (to a maximum cumulative dose of 2 mg)
- Levetiracetam 500-1,500 mg IV bolus (in addition to maintenance dose)
- Replete with magnesium as needed to maintain magnesium level > 2 mg/dL
- Thiamine 100 mg IV every 8 hours for 5 days
- If non-convulsive seizures persist, transfer to ICU and add phenobarbital loading dose of 60 mg 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 1,000-1,500 mg IV every 12 hours
    - Phenobarbital 30 mg IV every 12 hours (~0.5 mg/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 15-40 mcg/mL

APPENDIX L. MANAGEMENT OF CONVULSIVE STATUS EPILEPTICUS

- Assess circulation, airway, breathing (CAB) / consider airway protection / check blood glucose
- Transfer to ICU
- Consult Neurology
- Lorazepam 0.1 mg/kg (maximum 4 mg/dose) given at a maximum rate of 2 mg/minute; may repeat in 5 to 10 minutes
- Levetiracetam 500-1,500 mg IV bolus (in addition to maintenance dose)
- Replete with magnesium as needed to maintain magnesium > 2 mg/dL
- Thiamine 100 mg IV every 8 hours for 5 days
- If seizures persist, add phenobarbital loading dose of 15 mg/kg IV (monitor for respiratory depression, bradycardia and hypotension)
- If refractory, consider additional therapies (see Appendix K) including activation of safety switches if applicable
- Maintenance doses after resolution of convulsive status epilepticus
  - Levetiracetam 1,000-1,500 mg IV every 12 hours
  - Phenobarbital 0.5 mg/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 15-40 mcg/mL
- Continuous EEG monitoring if seizures are refractory to treatment

Table 25: Management of Diffuse Cerebral Edema and/or Raised Intracranial Pressure

For papilledema without diffuse cerebral edema or other signs of raised intracranial pressure	• Acetazolamide 1,000 mg IV followed by 250-1,000 mg IV every 12 hours (monitor renal function and acid/base balance once or twice daily and adjust dose accordingly) • Dexamethasone 20 mg 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 1,000 mg/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 28-30 mmHg, but maintained for no longer than 24 hours • Hyperosmolar therapy with either mannitol (20 g/dL solution) **or** hypertonic saline (3% or 23.4% as detailed below) ◦ Mannitol: initial dose 0.5-1 g/kg IV; maintenance dose 0.25-1 g/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 250 mL 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 herniation): dose to be administered by physician; initial dose of 30 mL IV; repeat after 15 minutes, if needed • If patient has ommaya reservoir, drain CSF to target OP < 20 mmHg • Control hypertension with the goal of maintaining mean arterial pressure (MAP) within 20-25 mmHg 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 (see Table 24) including activation of safety switches if applicable • 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