Protocol Number TCR2-21-01

A PHASE 1/2 SINGLE ARM OPEN-LABEL CLINICAL TRIAL OF TC-510 IN PATIENTS WITH ADVANCED MESOTHELIN-EXPRESSING CANCER

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SIGNATURE OF AGREEMENT FOR PROTOCOL

A PHASE 1/2 SINGLE ARM OPEN-LABEL CLINICAL TRIAL OF TC-510 IN PATIENTS WITH ADVANCED MESOTHELIN-EXPRESSING CANCER

Protocol Number: TCR2-21-01
IND Number: 28244
Version: 2.0
Date: 29 March 2023

The Investigator has read and understood the protocol and by signing below agrees to conduct the study in compliance with all applicable United States (US) Food and Drug Administration (FDA) and local laws and regulations and Good Clinical Practice Guidelines.

Investigator Name:

_____

Signature	_____
Date	_____

Sponsor: TCR² Therapeutics

Signature	_____
Date	_____
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LIST OF ABBREVIATIONS

Abbreviation	Term
ACT	adoptive T cell therapy
ADC	antibody drug conjugates
ADCC	antibody-dependent cell-mediated cytotoxicity
AESI	adverse events of special interest
ALL	acute lymphoblastic leukemia
ALK	anaplastic lymphoma kinase
ANC	absolute neutrophil count
BRCA1/2	Breast Cancer genes 1 and 2
BSA	Body Surface Area
CAR	chimeric antigen receptor
CBER	Center for Biologics Evaluation and Research
CD	cluster of differentiation
CI	confidence interval
CMV	cytomegalovirus
CNS	central nervous system
CPF	Cell Processing Facility
CR	complete response
CRES	CAR-T-cell-related encephalopathy syndrome
CRF	case report form
CRO	Contract Research Organization
CRP	C-reactive protein
CRS	cytokine release syndrome
CSF	cerebrospinal fluid
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DCR	disease control rate
DL	dose level
DLBCL	diffuse large B-cell lymphoma
DLT	dose-limiting toxicity
bDNA	branched DNA
DNA	deoxyribonucleic acid
DoR	duration of response
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDC	electronic data capture

Abbreviation	Term
EEG	electroencephalogram
EGFR	epidermal growth factor receptor
EMA	European Medicines Agency
EOT	end of treatment
EQ-5D-3L	EuroQOL Group EQ-5D 3 Level Version
FCBP	female patients of childbearing potential
gavo-cel	Gavocabtagene autoleucel (formerly TC-210 T cells)
G-CSF	granulocyte colony stimulating factor
GCP	Good Clinical Practice
GFR	glomerular filtration rate
GM-CSF	granulocyte-macrophage colony-stimulating factor
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HTLV	human T-lymphotropic virus
IB	Investigator's Brochure
iCCA	intrahepatic cholangiocarcinoma
ICF	informed consent form
ICH	International Council on Harmonisation
ICU	intensive-care unit
IEC	Independent Ethics Committee
IFN	interferon
IHC	immunohistochemistry
IL	interleukin
INR	international normalized ratio
IO	Immuno-oncology agents
IP	intraperitoneal
IRB	Institutional Review Board
ISF	investigator site file
ITAM	immunoreceptor tyrosine-based activation motif
ITT	intent-to-treat
iv	intravenous(ly)
IVD	in vitro diagnostic
KM	Kaplan-Meier
LTFU	long-term follow-up
LTR	long terminal repeat
mAb	monoclonal antibody

Abbreviation	Term
MedDRA	Medical Dictionary for Regulatory Activities
Meso	mesothelioma
MH1	humanized llama-derived single-domain anti-mesothelin antibody
MHC	major histocompatibility complex
mITT	modified intent-to-treat
MMR	mismatch repair
MPM	malignant pleural/peritoneal mesothelioma
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
msec	millisecond
MSI	microsatellite instability
MSLN	The gene encoding mesothelin
MSLN	mesothelin
MSTO	MSTO-211H cell line
MTD	maximum tolerated dose
MUGA	multiple-gated acquisition
NA	not available
NCI	National Cancer Institute
NIH	National Institutes of Health
nM	nanomolar
NSCLC	non-small cell lung cancer
NSG	non-obese diabetic severe combined immunodeficient gamma mice
NT	non-transduced
ORR	overall response rate
OS	overall survival
PARP	poly (ADP-ribose) polymerase
PBMC	peripheral blood mononuclear cell
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PD-1	programmed death receptor-1
PFS	progression-free survival
PI	Principal Investigator
PgR	progesterone receptor
PR	partial response
PRO	patient-reported outcome
aPTT	activated partial thromboplastin time
PTT	partial thromboplastin time

Abbreviation	Term
qPCR	quantitative polymerase chain reaction
RCL	replication competent lentivirus
RCR	replication competent retrovirus
REB	Research Ethics Board
RECIST	Response Evaluation Criteria in Solid Tumors
RIT	recombinant immunotoxins
RNA	ribonucleic acid
RP2D	recommended phase 2 dose
RT-PCR	reverse transcription polymerase chain reaction
SAGE	serial analyses of gene expression
SAE	serious adverse event
scFv	single chain variable fragment
SCID-X1	X-linked Severe Combined Immunodeficiency
SD	stable disease
SGPT	serum glutamic pyruvic transaminase
SOE	schedule of events
SOP	standard operating procedure
SPM	study procedures manual
SRT	safety review team
TCR	T-cell receptor
TEAE	treatment-emergent adverse event
TIL	tumor infiltrating lymphocyte
TKI	tyrosine kinase inhibitor
TNBC	Triple negative breast cancer
TNF-α	tumor necrosis factor alpha
TNF	tumor necrosis factor
TRuC	T-cell receptor fusion construct
TTR	time to response
ULN	upper limit of normal
VEGFR	vascular endothelial growth factor receptor
VSV-g	vesicular stomatitis virus, glycoprotein

2 PROTOCOL SYNOPSIS

Name of Company: TCR^2 Therapeutics
Name of Finished Product: TC-510
Title: A Phase 1/2 Single Arm Open-Label Clinical Trial of TC-510 in Patients with Advanced Mesothelin-Expressing Cancer
Protocol Number: TCR2-21-01
Introduction and Study Rationale Mesothelin (MSLN) is cell-surface glycoprotein with an unclear biological function. Of note, MSLN knockout mice grow and reproduce normally and have no detectable phenotype. However, mesothelin may be a key factor in malignancy as aberrant mesothelin expression plays an active role in both malignant transformation and tumor aggressiveness by promoting cancer cell proliferation, invasion, and metastasis. MSLN is expressed at low levels on serosal cells of the pleura, peritoneum, and pericardium but it is overexpressed in a wide range of solid tumors, including epithelioid mesothelioma (95%), pancreatic adenocarcinoma (85%), serous ovarian carcinoma (75%), among others, where it is frequently associated with poorer prognosis. Given its high expression on tumors and low expression on normal tissue, mesothelin is an attractive target for immunotherapy. Gavocabtagene autoleucel (gavo-cel, TC-210) is a T cell receptor fusion construct (TRuC) T cell therapy obtained by genetically engineering T cells to express a single-domain antibody that recognizes human MSLN fused to the CD3ε subunit which, upon expression, is incorporated into the endogenous T cell receptor (TCR) complex. Preliminary data from a phase 1/2 clinical trial with gavo-cel in patients with mesothelin-expressing solid cancers have shown promising clinical activity and a manageable toxicity profile. However, some patients fail to respond whereas others relapse after having achieved a response to gavo-cel. T cell exhaustion mediated by PD-L1 upregulation may contribute to those clinical outcomes. The expression of PD-L1 on cancer cells can be divided into constitutive expression and inducible expression depending on the extrinsic or intrinsic stimuli. IFN-γ secreted by tumor-reactive T cells, signaling through the transcription factor STAT1, is the single major cytokine that induces PD-L1 protein expression. Therapy with engineered T cell therapies such as gavo-cel is associated with T cell activation and proliferation leading to cytokine secretion, including elevations of IFN-γ levels. Thus, PD-L1 expression on cancer cells induced by IFN-γ inflammatory signaling may result in suppression of T cell activation and exhaustion of engineered T cells in the tumor microenvironment. TC-510 is a novel T cell therapy that results from engineering gavo-cel to express a PD-1:CD28 switch receptor, which is expressed on the surface of the T cell, independently from the TCR. The PD-1:CD28 switch receptor comprises the PD-1 extracellular domain fused to the CD28 intracellular domain via a transmembrane domain. Thus, the switch is designed to produce a costimulatory signal upon engagement with PD-L1 on cancer cells. In animal models, TC-510 is superior to gavo-cel with regards to expansion, persistence, and in vivo activity.

Trial Objectives

Phase 1 Objectives

Primary

- To evaluate the safety of autologous genetically modified TC-510 in patients with MSLN-expressing metastatic or unresectable solid tumors.
- Establish the recommended phase 2 dose (RP2D) according to dose-limiting toxicity (DLT) of defined adverse events.

Secondary

- To determine the overall response rate (ORR) (complete response [CR] + partial response [PR]) according to Response Evaluation Criteria in Solid Tumors (RECIST) v 1.1 and duration of response (DoR).
- To determine the disease control rate (DCR), defined as a composite of ORR and stable disease (SD) lasting at least 8 weeks.
- To evaluate the survival benefit of autologous genetically modified TC-510 as assessed by progression-free survival (PFS) and overall survival (OS)
- To assess humoral immunogenicity to TC-510

Phase 2 Objectives

Primary

- To evaluate the efficacy of autologous genetically modified TC-510 in patients with MSLN-expressing metastatic or unresectable solid tumors.
- Endpoints: ORR (CR + PR) and DCR (ORR + SD ≥ 8 weeks) according to RECIST v 1.1.

Secondary

- To evaluate the efficacy of autologous genetically modified TC-510 in patients with MSLN-expressing unresectable, metastatic, or recurrent cancers as assessed by TTR, DoR, PFS, and OS.
- To further evaluate the safety of autologous genetically modified TC-510.
- To assess whether patients experience a response upon subsequent TC-510 infusion(s)

Exploratory Objectives (Phase 1 and 2)

- To evaluate TC-510 expansion, persistence, and cytokine production in vivo.
- To evaluate key TC-510 product attributes pre- and post-infusion (e.g., phenotype, functionality).
- To evaluate TC-510 infiltration in tumor tissue.
- To evaluate the correlation of metabolic activity of target and non-target lesions post TC-510 infusion and clinical activity.
- To evaluate immune markers in the tumor microenvironment (e.g., PD-L1 expression) before and after TC-510 infusion and their correlation with toxicity and response.
- To evaluate changes in health-related quality of life following treatment with TC-510 (only Phase 2)
- To evaluate the response to TC-510 by iRECIST.

Study Design

This first-in-human clinical trial is a phase 1/2 open-label study to evaluate the safety and efficacy of autologous genetically engineered TC-510 in patients with advanced MSLN-expressing cancers. Patients will be screened for leukapheresis eligibility, and if deemed eligible, will undergo a large-volume leukapheresis at the enrolling institution to obtain cells for the manufacture of autologous TC-510. Patients' peripheral blood mononuclear cells will be processed at the enrolling institution and frozen leukocytes will then be shipped to a central site for further processing. Then, the TC-510 (transduced T cells) will be formulated, cryopreserved, and shipped back to the treating institution. If patients then meet treatment eligibility criteria, they will proceed to receive lymphodepleting chemotherapy with cyclophosphamide and fludarabine followed by TC-510 infusion.

The study will proceed in 2 sequential phases:

Phase 1

- The key objective of the Phase 1 portion of the study will be the determination of the RP2D based on the observed toxicity profile upon TC-510 infusion and the evaluation of DLTs.
- Five TC-510 dose levels (50×10^6, 100×10^6, 130×10^6, 160×10^6, and 200×10^6) will be tested following a lymphodepleting chemotherapy regimen. If excessive toxicity is observed at DL0, de-escalation may occur to DL-1.
- TC-510 may be administered via a fractionated regimen at any point in the study if deemed appropriate for safety or efficacy reasoning by either the safety review team (SRT) or the Sponsor's Medical Monitor.
- Dose escalation will proceed according to a modified 3+3 design.
- A planned stagger of 14 days will be instituted between the first patient infused at each dose level and the 2nd patient.
- Patients 2 and 3 in any given cohort may be infused simultaneously.
- Once the RP2D is identified, up to 10 additional patients may be treated at the RP2D in an expansion cohort to further delineate the toxicity profile of TC-510 prior to advancing to the Phase 2 portion of the study. TC-510 retreatment may be possible for patients in the expansion cohort at the RP2D.
- Approximately 25-30 patients will be treated in the Phase 1 portion of the study.

Phase 2

- The objective of this phase will be to further characterize the safety profile of TC-510 and to properly evaluate its efficacy.
- Patients will receive TC-510 at the RP2D and will be stratified according to their cancer diagnosis in 7 cohorts: malignant pleural/peritoneal mesothelioma (MPM), serous ovarian adenocarcinoma, pancreatic adenocarcinoma, colorectal cancer, triple negative breast cancer, non-small cell lung cancer (NSCLC), and cholangiocarcinoma.
- A total of 20 patients will be treated with single agent TC-510 in each one of those cohorts. Up to approximately 140 patients will be treated in the phase 2 portion of the study.
- Patients achieving a best response of SD by 8 weeks post-infusion and those with progression after an initial repones to TC-510 infusion will be allowed to be considered for TC-510 retreatment at the

RP2D following a lymphodepleting chemotherapy regimen provided they re-meet the treatment eligibility criteria.

If the number of TC-510 transduced T cells is less than the minimum dose allowed at any given level, manufacturing of additional transduced T cells from excess banked leukapheresis product will be undertaken to achieve a total dose that meets the target dose requirement. In the event that no banked leukapheresis product is available, a second leukapheresis may be performed.

If, despite the latter, a TC-510 dose fails to meet the minimum dose requirement, patients may still be eligible to receive TC-510 and participate in this trial. These patients will be evaluated in a separate patient cohort. However, an additional patient whose TC-510 dose meets the minimum cell dose requirement will be added to the cohort.

Leukapheresis Inclusion/Exclusion Criteria

Patients will be assessed for and must meet leukapheresis eligibility criteria for leukapheresis at the Leukapheresis Eligibility visit.

Leukapheresis Inclusion Criteria

A patient must meet the following inclusion criteria to be eligible to undergo leukapheresis:

1. Patient (or legally authorized representative) has voluntarily agreed to participate by giving written informed consent in accordance with International Council on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines and applicable local regulations.
2. Patient is ≥ 18 years of age at the time the Informed Consent is signed.
3. Patient has a pathologically confirmed diagnosis of either epithelioid MPM, Serous Ovarian Adenocarcinoma (Serous Ovarian, Fallopian Tube or Primary Peritoneal cancers will be permitted), Pancreatic Adenocarcinoma, Colorectal Cancer, Triple Negative Breast Cancer, NSCLC, or Cholangiocarcinoma
   - For patients with MPM, a pathology report documenting epithelioid histology should be provided. In the event epithelioid histology cannot be clearly confirmed via pathology report, the patient may proceed after discussion with the sponsor medical monitor or after confirming adequate MSLN expression
   - For patients with triple negative breast cancer, treatment sites must provide proof of ER-negative and PR-negative status (in both cases defined as < 1%). Her2-negative status is defined as 0, 1+, or 2+ by immunohistochemistry (IHC). If IHC 2+, a negative in situ hybridization (FISH, CISH, or SISH) test is required by local laboratory testing.
4. For patients with epithelioid MPM, confirmation of MSLN expression is not required prior to enrollment. For Ovarian, Pancreatic, Colorectal, TNBC, NSCLC, and Cholangiocarcinoma only: Patient's tumor expresses MSLN on ≥ 50% of tumor cells with 1+, 2+, and/or 3+ intensity by immunohistochemistry at a TCR^2 Therapeutics designated central laboratory.

- A banked tumor biopsy is allowed at pre-screening if obtained within 5 years of pre-screening consent, otherwise a fresh tumor biopsy should be obtained.
- If the patient is pre-screened for MSLN expression at the TCR^2 Therapeutics central laboratory under clinical protocol TCR2-18-01, the results can be used to determine eligibility to proceed for apheresis.

5. Patient has advanced (i.e., metastatic or unresectable) cancer. Unresectable refers to a tumor lesion in which clear surgical excision margins cannot be obtained without leading to significant functional compromise.
6. Prior to TC-510 infusion, patients with MPM, Serous Ovarian Adenocarcinoma, TNBC, Colorectal, and NSCLC must have received at least 1 but no more than 5 systemic therapies for metastatic or unresectable disease. Patients with Pancreatic and Cholangiocarcinoma who are treatment naïve may be eligible for TC-510 therapy if they have elected not to pursue front-line therapy. Regardless of tumor type, patients must not exceed 5 prior lines of therapy (excluding bridging therapy and surgical procedures) or except where otherwise specified below:

   A. MPM
      - Patients must have received standard frontline therapy (e.g., platinum-based chemotherapy or checkpoint inhibitor therapy).

   B. Pancreatic Adenocarcinoma
      - Patients must have received frontline therapy with fluorouracil-based (e.g., FOLFIRINOX) or gemcitabine-based (e.g. gemcitabine ± Nab-paclitaxel) regimens for advanced disease or have elected not to pursue front-line therapy

   C. Serous Ovarian Adenocarcinoma
      - Patients must have received a platinum-based regimen.
      - Or if they are carriers of a BRCA1/2 mutation they must have received at least a PARP inhibitor unless the treating physician determines that it is in the patient's best interest to enroll in the TC-510 trial, deems the patient unsuitable, or the patient elects not to receive PARP inhibitor therapy at that time. PARP inhibitors may be given as maintenance therapy with/without bevacizumab following a prior chemotherapy regimen and be considered a single line of therapy. To be considered maintenance therapy, the interval between prior chemotherapy completion and the initiation of the PARP inhibitor with/without bevacizumab must be less than 8 weeks with no evidence of disease progression during that interval.

   D. Triple Negative Breast Cancer (TNBC)
      - Must have received at least 1 prior systemic anti-cancer therapy, including a PARP inhibitor for advanced TNBC with a germline mutation in BRCA1/2, and the combination of pembrolizumab with chemotherapy for advanced TNBC with combined positive score (CPS) ≥ 10.

   E. Colorectal Cancer

- Must have progressed after at least 1 prior standard systemic anti-cancer therapy (e.g., oxaliplatin or irinotecan-based regimens ± bevacizumab), including cetuximab or panitumumab in patients with wild type KRAS or immune checkpoint inhibitors in patients with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) colorectal cancer.

F. NSCLC

- Patients must have pathologically confirmed (by histology) diagnosis of NSCLC, which is currently stage 3B or stage 4 disease.
- A patient with non-squamous NSCLC must have been tested for relevant EGFR mutations, ALK translocation or other actionable genomic aberrations (eg, ROS rearrangement, BRAF V600E mutation) for which FDA-approved targeted therapy is available and, if positive, the patient should have received at least 1 such therapy prior to study enrollment.
- Patients with the *EGFR T790M* mutation must have received the FDA-approved tyrosine kinase inhibitor osimertinib.
- For patients without an actionable mutation, the patient must have received a currently approved frontline regimen (eg, immune checkpoint inhibitor-based therapy).

G. Cholangiocarcinoma

- Patients must have received at least 1 standard systemic regimen for unresectable or metastatic disease (eg, gemcitabine-or 5-FU-containing regimens) or they must have elected not to pursue frontline standard of care therapy.

7. Patient has an Eastern Cooperative Oncology Group performance status 0 or 1.
8. Subject is fit for leukapheresis and has adequate venous access for the cell collection.
9. Subjects must have an absolute lymphocyte count (ALC) >350/μL prior to leukapheresis. Patients with an ALC < 350/μL would still be eligible to proceed with leukapheresis if they have a CD3 count of ≥150/μL. If the CD3 is <150/μL consultation with the Sponsor's Medical Monitor is required.

Leukapheresis Exclusion Criteria

A patient meeting any of the following exclusion criteria is not eligible to undergo leukapheresis:

1. Inability to follow the procedures of the study (e.g., due to language problems, psychological disorders, dementia, confusional state).
2. Patient has received or plans to receive the following therapy/treatment prior to leukapheresis:
   - Cytotoxic chemotherapy within 3 weeks of leukapheresis
   - Corticosteroids: therapeutic doses of steroids must be stopped > 72 hours prior to leukapheresis. Use of inhaled steroids or topical cutaneous steroids is not exclusionary.

Corticosteroid therapy at a pharmacologic dose (> 5 mg/day of prednisone or equivalent doses of other corticosteroids) and other immunosuppressive drugs should be avoided until 3 months after TC-510 administration, unless medically indicated to treat new toxicity. Physiological replacement doses of steroids (up to 5 mg/day of prednisone equivalent, or higher if warranted by the patient's BMI) may be allowed.

- Immunosuppression: any other immunosuppressive medication must be stopped ≥ 4 weeks prior to leukapheresis, including (but not limited to) calcineurin inhibitors, methotrexate or other chemotherapy drugs, mycophenolate, steroids (see above), rapamycin, thalidomide, or immunosuppressive antibodies such as rituximab, anti-tumor necrosis factor, anti-interleukin (IL) 6 or anti-IL6R.
- Use of an anti-cancer vaccine within 2 months in the absence of tumor response. The patient should be excluded if their disease is responding to an experimental vaccine given within 6 months.
- Any previous gene therapy using an integrating vector.
- Small molecule tyrosine kinase inhibitors (eg, epidermal growth factor receptor [EGFR] inhibitors), PARP inhibitors (e.g., olaparib, rucaparib, niraparib), or KRAS G12C inhibitors (e.g., sotorasib, adagrasib) within 72 hours.
- Any previous allogeneic hematopoietic stem cell transplant.
- Investigational treatment or clinical trial within 4 weeks or 5 half-lives of investigational product, whichever is shorter.
- Coronavirus disease 2019 (Covid-19; SARS-CoV-2) vaccine dose within 4 weeks from estimated date of leukapheresis, unless approved by TCR^2 Therapeutics Medical Monitor.

3. Central nervous system (CNS) disease/brain metastases:
   - Patients with leptomeningeal disease, carcinomatous meningitis, or symptomatic CNS metastases: Patients are eligible if they have completed their treatment, have recovered from the acute effects of radiation therapy or surgery prior to study entry, and a) have no evidence of brain metastases post treatment or b) are asymptomatic, have discontinued corticosteroid treatment or anti-seizure medications for these metastases for at least 4 weeks and have radiographically stable CNS metastases without associated edema or shift for at least 3 months prior to study entry (note: prophylactic anti-seizure medications are acceptable; up to 5 mg per day of prednisone or equivalent will be allowed, or higher if warranted by the patient's body mass index [BMI]).
4. Patient has any other prior or concurrent malignancy with the following exceptions:
   - Adequately treated basal cell or squamous cell carcinoma (adequate wound healing is required prior to study entry).
   - In situ carcinoma of the cervix or breast, treated curatively and without evidence of recurrence for at least 12 months prior to the study.

- Treated non-melanoma skin cancer.
- Stage 0 or 1 melanoma completely resected at least 12 months prior to the study.
- Successfully treated organ-confined prostate cancer with no evidence of progressive disease based on prostate specific antigen (PSA) levels and not on active therapy.
- A primary malignancy which has been completely resected and in complete remission for ≥ 5 years.
- Other malignancies deemed unlikely to be of clinical significance during TC-510 therapy by the Principal Investigator and as approved by the Sponsor.

5. Patient has active infection with HIV, hepatitis B virus, hepatitis C virus (HCV), or human T-lymphotropic virus (HTLV) as defined below:
   - Positive serology for HIV, HTLV-1, or HTLV-2.
   - Active hepatitis B infection as demonstrated by test for hepatitis B surface antigen. Patients who are hepatitis B surface antigen negative but are hepatitis B core antibody positive must have undetectable hepatitis B deoxyribonucleic acid (DNA) and receive prophylaxis against viral reactivation.
   - Active hepatitis C infection as demonstrated by hepatitis C ribonucleic acid (RNA) test. Patients who are HCV antibody positive will be screened for HCV RNA by any reverse transcription polymerase chain reaction (PCR) or branched DNA assay. If HCV antibody is positive, eligibility will be determined based on a negative screening RNA value.
6. Patient with pulse oximetry < 90% on room air or with one of the following will be excluded.
   - Radiographic evidence of underlying interstitial lung disease
   - Active interstitial lung disease/pneumonitis or a history of interstitial lung disease/pneumonitis requiring therapy with systemic corticosteroids.
7. History of autoimmune or immune mediated disease such as multiple sclerosis, lupus, rheumatoid arthritis, inflammatory bowel disease, or small vessel vasculitis.

Treatment Inclusion/Exclusion Criteria

A patient must meet the following treatment inclusion criteria to be eligible to proceed with first protocol defined therapy (e.g., lymphodepletion). Treatment eligibility will be formally assessed at Baseline. For retreatment with TC-510, the following criteria also apply.

Treatment Inclusion Criteria

A patient must meet the following inclusion criteria to be eligible to receive therapy on this study:

1. Patient (or legally authorized representative) has voluntarily agreed to participate by giving written informed consent in accordance with ICH GCP guidelines and applicable local regulations.

2. Patient has agreed to abide by all protocol required procedures including study-related assessments, and management by the treating institution for the duration of the study and long-term follow-up (LTFU).
3. Patient is ≥ 18 years of age at the time the Informed Consent is signed.
4. Patient has a pathologically confirmed diagnosis of either epithelioid MPM, Serous Ovarian Adenocarcinoma (Serous Ovarian, Fallopian Tube or Primary Peritoneal cancers will be permitted), Pancreatic Adenocarcinoma, Colorectal Cancer, Triple Negative Breast Cancer, NSCLC, or Cholangiocarcinoma.
   - For patients with MPM, a pathology report documenting epithelioid histology should be provided. In the event epithelioid histology cannot be clearly confirmed via pathology report, the patient may proceed after discussion with the sponsor medical monitor or after confirming adequate MSLN expression.
   - For patients with triple negative breast cancer, treatment sites must provide proof of ER-negative and PR-negative status (in both cases defined as < 1%). Her2-negative status is defined as 0, 1+, or 2+ by immunohistochemistry (IHC). If IHC 2+, a negative in situ hybridization (FISH, CISH, or SISH) test is required by local laboratory testing.
5. For patients with epithelioid MPM, confirmation of MSLN expression is not required prior to treatment. For Ovarian, Pancreatic, Colorectal, TNBC, NSCLC, and Cholangiocarcinoma only: Patient's tumor expresses MSLN on ≥ 50% of tumor cells with 1+, 2+, and/or 3+ intensity by immunohistochemistry at a TCR^2 Therapeutics designated central laboratory.
   - **Biopsy Timing:** For all patients, MSLN testing must be performed on a biopsy sample obtained within 6 months of the planned first protocol defined therapy. The pre-screening biopsy may fulfill the Baseline biopsy requirement if collected within 6 months of the start of treatment (provided there is adequate tissue for correlative testing). Otherwise, a new biopsy sample from within 6 months of the first protocol defined therapy should be submitted for MSLN testing. If deemed unsafe by the PI and/or the lesion is inaccessible, the Sponsor may allow the patient to proceed without the Baseline biopsy.
6. Patient has advanced (i.e., metastatic or unresectable) cancer. Unresectable refers to a tumor lesion in which clear surgical excision margins cannot be obtained without leading to significant functional compromise.
7. Patient has at least 1 lesion that meets evaluable and measurable criteria confirmed RECIST v 1.1. Patients who have received prior local therapy (including but not limited to embolization, chemoembolization, radiofrequency ablation, or radiation therapy) are eligible provided measurable disease falls outside of the treatment field or within the field and has shown ≥ 20% growth in size since post-treatment assessment.
8. The patient must not have required a paracentesis or thoracentesis within the preceding 4 weeks of TC-510 infusion nor be projected to require a paracentesis or thoracentesis within the next 8 weeks. Patients with catheters in place for frequent drainage may be allowed. Specific cases are to be discussed with the Medical Monitor and Sponsor.

9. Prior to TC-510 infusion, patients with MPM, Serous Ovarian Adenocarcinoma, TNBC, Colorectal, and NSCLC must have received at least 1 but no more than 5 systemic therapies for metastatic or unresectable disease. Patients with Pancreatic and Cholangiocarcinoma who are treatment naïve may be eligible for TC-510 therapy if they have elected not to pursue front-line therapy. Regardless of tumor type, patients must not exceed 5 prior lines of therapy (excluding bridging therapy and surgical procedures) or except where otherwise specified below:

   A. MPM
      - Patients must have received standard frontline therapy (e.g., platinum-based chemotherapy or checkpoint inhibitor therapy).

   B. Pancreatic Adenocarcinoma
      - Patients must have received frontline therapy with fluorouracil-based (e.g., FOLFIRINOX) or gemcitabine-based (e.g. gemcitabine ± Nab-paclitaxel) regimens for advanced disease or have elected not to pursue front-line therapy.

   C. Serous Ovarian Adenocarcinoma
      - Patients must have received a platinum-based regimen.
      - Or if they are carriers of a BRCA1/2 mutation they must have received at least a PARP inhibitor unless the treating physician determines that it is in the patient's best interest to enroll in the TC-510 trial, deems the patient unsuitable, or the patient elects not to receive PARP inhibitor therapy at that time. PARP inhibitors may be given as maintenance therapy with/without bevacizumab following a prior chemotherapy regimen and be considered a single line of therapy. To be considered maintenance therapy, the interval between prior chemotherapy completion and the initiation of the PARP inhibitor with/without bevacizumab must be less than 8 weeks with no evidence of disease progression during that interval.

   D. Triple Negative Breast Cancer
      - Must have received at least 1 prior systemic anti-cancer therapy, including a PARP inhibitor for advanced TNBC with a germline mutation in BRCA1/2, and the combination of pembrolizumab with chemotherapy for advanced TNBC with combined positive score (CPS) ≥ 10.

   E. Colorectal Cancer
      - Must have progressed after at least 1 prior standard systemic anti-cancer therapy (e.g., oxaliplatin or irinotecan-based regimens ± bevacizumab), including cetuximab or panitumumab in patients with wild type KRAS or immune checkpoint inhibitors in patients with MSI-H or dMMR colorectal cancer.

   F. NSCLC

      - Patients must have pathologically confirmed (by histology) diagnosis of NSCLC, which is currently stage 3B or stage 4 disease.

      - A patient with non-squamous NSCLC must have been tested for relevant EGFR mutations, ALK translocation or other actionable genomic aberrations (eg, ROS

rearrangement, BRAF V600E mutation) for which FDA-approved targeted therapy is available and, if positive the patient should have received at least 1 such therapy prior to study enrollment.

- Patients with the *EGFR T790M* mutation must have received the FDA-approved tyrosine kinase inhibitor osimertinib.

G. Cholangiocarcinoma

- Patients must have received at least 1 standard systemic regimen for unresectable or metastatic disease (eg, gemcitabine-or 5-FU-containing regimens) or they must have elected not to pursue frontline standard of care therapy.
- Patients must have measurable disease, defined as at least 1 lesion that can be accurately measured in at least 1 dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as $\geq$ 20 mm ($\geq$2 cm) with conventional techniques or as $\geq$ 10 mm ($\geq$1 cm) with computed tomography (CT) scan or magnetic resonance imaging (MRI).

10. Patient has an Eastern Cooperative Oncology Group performance status 0 or 1.
11. All patients must have undergone a rapid influenza diagnostic test and/or a respiratory viral panel (including coronavirus disease 2019 (Covid-19; SARS-CoV-2) as per Institutional guidelines within 14 days prior to the first protocol defined therapy. If the patient is positive for influenza, oseltamivir phosphate or zanamivir should be administered for 10 days (see Tamiflu® or Relenza® package insert for dosing). The patient must complete their 10-day treatment course prior to receiving TC-510.
    - For patients residing in the US, Canada, Europe and Japan, influenza testing is required during the months of October through May (inclusive).
    - For patients residing in the southern hemisphere such as Australia, influenza testing is required during the months of April through November (inclusive).
    - For patients with significant international travel, both calendar intervals above may need to be considered.

    In the event a patient tests positive for coronavirus disease 2019 (Covid-19; SARS-CoV-2), TC-510 infusion should be delayed until the patient is asymptomatic and deemed fit for infusion by the treating physician.
12. Patient has a left ventricular ejection fraction ≥ 45% as measured by resting echocardiogram/MUGA, with no clinically significant pericardial effusion.
13. FPCP must have a negative urine or serum pregnancy test (FPCP is defined as premenopausal and not surgically sterilized). FPCP must agree to use effective birth control or to abstain from heterosexual activity throughout the study, starting on the day of first dose of lymphodepleting chemotherapy through 12 months post TC-510 infusion. Effective contraceptive methods include intra-uterine device, oral or injectable hormonal contraception, or 2 adequate barrier methods (e.g., diaphragm with spermicide, cervical cap with spermicide,

or female condom with spermicide). Spermicides alone are not an adequate method of contraception.

Or

Male patients must be surgically sterile or agree to use a double barrier contraception method or abstain from heterosexual activity with a female of childbearing potential starting at the first dose of protocol-defined treatment and for 4 months thereafter or longer (if indicated in the country specific monograph/label for cyclophosphamide).

14. Patient must have adequate organ function as indicated by the laboratory values in the following table.

System	Laboratory Value
Hematological	
Absolute Neutrophil count (ANC)	≥ 1×10^9/L (without G-CSF support within 7 days prior to the first protocol defined therapy)
Absolute Lymphocyte count (ALC)	≥ 0.3×10^9/L
Platelets	≥ 100×10^9/L
Hemoglobin	≥ 90 g/L (without transfusion support within 7 days prior to the first protocol defined therapy)
Coagulation	
Partial Thromboplastin Time (PTT)	≤ 1.5 × upper limit of normal (ULN)
Prothrombin time (PT)	≤ 1.5× upper limit of normal (ULN)
Renal	
Calculated or measured creatinine clearance[a]	≥ 40 mL/minute
Hepatic	
Serum total bilirubin	≤ 2 × ULN (unless subject has documented Gilbert's Syndrome with direct bilirubin < 35% of total bilirubin or unless secondary to bile duct obstruction by tumor)
Alanine aminotransferase (ALT)	≤ 2.5 × ULN or ≤ 5 × ULN if documented liver metastasis
Aspartate aminotransferase (AST)	≤ 2.5 × ULN or ≤ 5 × ULN if documented liver metastasis

[a] = Creatinine clearance will be calculated using the Cockcroft-Gault formula at Baseline:

$$\text{Cr clearance} = \frac{(140 - \text{age}) \times \text{weight kg}}{72 \times \text{serum creatinine mg/dl}} (\times 0.85 \text{ in females})$$

Treatment Exclusion Criteria

A patient meeting any of the following exclusion criteria is not eligible for participation in the treatment portion of this study:

1. Inability to follow the procedures of the study (e.g., due to language problems, psychological disorders, dementia, confusional state).
2. Known or suspected non-compliance, drug, or alcohol abuse.
3. Participation in another study with investigational drug within the 28 days or 5 half-lives of the drug, whichever is shorter, preceding and during the present study.

4. Patient is pregnant (or intends to become pregnant during the course of the study) or breastfeeding.
5. Patient has received or plans to receive the following therapy/treatment prior to the first protocol-defined therapy (unless otherwise specified):
   - Cytotoxic chemotherapy within 3 weeks of TC-510 infusion.
   - Corticosteroids: therapeutic doses of steroids must be stopped at least 2 weeks prior to TC-510 infusion. Use of inhaled steroids or topical cutaneous steroids is not exclusionary. Corticosteroid therapy at a pharmacologic dose (> 5 mg/day of prednisone or equivalent doses of other corticosteroids) and other immunosuppressive drugs should be avoided until 3 months after TC-510 administration, unless medically indicated to treat new toxicity. Physiological replacement doses of steroids (up to 5 mg/day of prednisone equivalent, or higher if warranted by the patient's BMI) may be allowed.
   - Any other immunosuppressive medication must be stopped ≥ 4 weeks prior to first protocol-defined treatment, including (but not limited to) calcineurin inhibitors, methotrexate or other chemotherapy drugs, mycophenolate, steroids (see above), rapamycin, thalidomide, or immunosuppressive antibodies such as rituximab, anti-tumor necrosis factor, IL 6 or anti-IL6R.
   - Anti-PD-1 monoclonal antibody therapy must be stopped ≥ 4 weeks prior to first protocol-defined treatment.
   - Use of an anti-cancer vaccine within 2 months in the absence of tumor response. The patient should be excluded if their disease is responding to an experimental vaccine given within 6 months.
   - Any previous gene therapy using an integrating vector (except for TC-510 in the case of retreatment).
   - Small molecule tyrosine kinase inhibitors (e.g., EGFR inhibitors), PARP inhibitors (e.g., olaparib, rucaparib, niraparib), or KRAS G12C inhibitors (e.g., sotorasib, adagrasib) within 72 hours.
   - Any previous allogeneic hematopoietic stem cell transplant.
   - Investigational treatment or clinical trial within 4 weeks or 5 half-lives of investigational product, whichever is shorter.
   - Radiotherapy to the target lesions within 4 weeks prior to lymphodepleting chemotherapy. A non-target lesion with unequivocal progression may be considered a target lesion regardless of time from last radiotherapy dose. NOTE: There is no washout period for palliative radiation to non-target lesions.
   - Hepatic radiation, chemoembolization, and/or radiofrequency ablation within 4 weeks.
   - Immune therapy (e.g., monoclonal antibody therapy, checkpoint inhibitors) within 4 weeks.

- Coronavirus disease 2019 (Covid-19; SARS-CoV-2) vaccine dose within 4 weeks of first protocol defined treatment, unless approved by TCR^2 Therapeutic's Medical Monitor.

6. Toxicity from previous anti-cancer therapy that has not recovered to ≤ grade 1 (except for non-clinically significant toxicities (e.g., alopecia, vitiligo). Patients with grade 2 toxicities that are deemed stable or irreversible (e.g., peripheral neuropathy) may be eligible.
7. History of allergic reactions attributed to compounds of similar chemical or biologic composition to fludarabine, cyclophosphamide, or other agents used in the study.
8. History of autoimmune or immune mediated disease such as multiple sclerosis, lupus, rheumatoid arthritis, inflammatory bowel disease, or small vessel vasculitis.
9. Major surgery (other than diagnostic surgery) within 4 weeks prior to first protocol defined therapy, minor surgery including diagnostic surgery within 2 weeks (14 days) excluding central intravenous port placements and needle aspirate/core biopsies. Radio frequency ablation or transcatheter arterial chemoembolization within 6 weeks prior to first protocol defined therapy.
10. CNS disease/brain metastases:
    - Patients with leptomeningeal disease, carcinomatous meningitis, or symptomatic CNS metastases: patients are eligible if they have completed their treatment, have recovered from the acute effects of radiation therapy or surgery prior to study entry, and a) have no evidence of brain metastases post treatment or b) are asymptomatic, have discontinued corticosteroid treatment or anti-seizure medications for these metastases for at least 4 weeks and have radiographically stable CNS metastases without associated edema or shift for at least 3 months prior to study entry (note: prophylactic anti-seizure medications are acceptable; up to 5 mg/day of prednisone or equivalent will be allowed; higher doses may be allowed if warranted due to patient BMI).
11. Patient has any other prior or concurrent malignancy with the following exceptions:
    - Adequately treated basal cell or squamous cell carcinoma (adequate wound healing is required prior to study entry).
    - In situ carcinoma of the cervix or breast, treated curatively and without evidence of recurrence for at least 12 months prior to enrollment.
    - Treated non-melanoma skin cancer.
    - Stage 0 or 1 melanoma completely resected at least 12 months prior to enrollment.
    - Successfully treated organ-confined prostate cancer with no evidence of progressive disease based on PSA levels and are not on active therapy.
    - A primary malignancy which has been completely resected and in complete remission for ≥ 5 years.
    - Malignancies deemed unlikely to be of clinical significance during TC-510 therapy by the Principal Investigator and as approved by the Sponsor.

12. Patient has an electrocardiogram showing a clinically significant abnormality at screening or showing a QTc interval > 450 msec in males and > 470 msec in females (> 480 msec for patients with bundle branch block). Either Fridericia's or Bazett's formula may be used to correct the QT interval.
13. Patient has uncontrolled intercurrent illness including, but not limited to:
    - Ongoing or active infection; eg, sepsis, prolonged unresolved bacterial cholangitis with destruction of bile duct branches (eg, after endoprosthesis insertion), or 2 or more cholangitis in the last 6 months.
    - Clinically significant cardiac disease defined by congestive heart failure New York Heart Association class 3 or class 4.
    - Uncontrolled clinically significant arrhythmia.
    - Acute coronary syndrome (angina or myocardial infarction), stroke, or peripheral vascular event in the last 6 months.
    - Serious thrombotic event in the last 6 months.
    - Interstitial lung disease (patients with existing pneumonitis as a result of radiation are not excluded; however, patients must not be oxygen dependent as demonstrated by oxygen saturation < 92 % on room air).
    - Liver cirrhosis or primary sclerosing cholangitis.
14. Patient has active infection with HIV, hepatitis B virus, HCV, or HTLV as defined below:
    - Positive serology for HIV, HTLV-1, or HTLV-2.
    - Active hepatitis B infection as demonstrated by test for hepatitis B surface antigen. Patients who are hepatitis B surface antigen negative but are hepatitis B core antibody positive must have undetectable hepatitis B DNA and receive prophylaxis against viral reactivation.
    - Active hepatitis C infection as demonstrated by hepatitis C RNA test. Patients who are HCV antibody positive will be screened for HCV RNA by any reverse transcription PCR or branched DNA assay. If HCV antibody is positive, eligibility will be determined based on a negative screening RNA value.

TC-510 Dose and Treatment Schedule

Phase 1

Each patient will receive a single dose of TC-510. At each dose level (DL), TC-510 will be given to at least 3 patients following lymphodepleting chemotherapy (fludarabine 30 mg/m^2/d on days -7 through -4 and cyclophosphamide 600 mg/m^2/d on days -6 through -4). Patients will be enrolled at the following dose levels to determine the RP2D:

Dose Level	**Transduced T cells on day 0**
DL -1	25×10^6
DL 0 (initial dose level)	**50×10^6**
DL 1	100×10^6
DL 2	130×10^6
DL 3	160×10^6
DL 4	200×10^6

- A variation on the target dose of 15% (i.e., ± 15%) will be allowed at each dose level.
- In the event of excessive toxicity at the initial dose level (DL0), the study will resume at DL-1 (25×10^6 transduced T cells on day 0).
- Dose escalation will be directed by the SRT upon review of the safety data.
- Once identified, up to 10 patients may be subsequently treated at the RP2D in an expansion cohort to further delineate the toxicity profile of TC-510 prior to advancing to the Phase 2 portion of the study. TC-510 retreatment may be permissible for patients in the RP2D expansion cohort, given that all appropriate criteria are met.

Phase 2

Patients will be stratified according to their cancer diagnosis into 7 cohorts: MPM, serous ovarian cancer adenocarcinoma, pancreatic adenocarcinoma, colorectal cancer, triple negative breast cancer, non-small cell lung cancer, cholangiocarcinoma. Within each cohort, patients will receive a single TC-510 infusion at the RP2D determined in the phase 1 portion of the study. Patients meeting the defined criteria for retreatment as described in Section 6.0 and Section 9.4 will be candidates to redosing with TC-510 at the RP2D (or at a lower dose) following lymphodepleting chemotherapy.

Definition of DLT

A DLT is defined as any of the following TC-510-related events with onset within the first 28 days following TC-510 infusion:

- Any Common Terminology Criteria for Adverse Events (CTCAE) grade 5 organ toxicity.
- Any CTCAE grade 4 organ toxicity (cardiac, dermatologic, gastrointestinal, hepatic, pulmonary, renal/genitourinary, or neurologic) with an attribution of definitely or probably related to the TC-510 infusion that does not improve to grade ≤ 2 within 4 weeks.

- Any grade 3 or greater autoimmune toxicity related to TC-510.
- Grade 4 cytokine release syndrome (CRS) that does not improve to ≤ 3 within 72 hours. If grade 4 CRS resolves to grade 3 within 72 hours, then the grade 3 CRS must further resolve to grade ≤ 2 within the following 11 days so that the time between grade 4 CRS and its resolution to grade ≤ 2 does not exceed 14 days.
- Grade 4 neurotoxicity that does not improve to ≤ 3 within 72 hours. If grade 4 neurotoxicity resolves to grade 3 within 72 hours, then the grade 3 neurotoxicity must further resolve to grade ≤ 2 within the following 11 days so that the time between grade 4 neurotoxicity and its resolution to grade ≤ 2 does not exceed 14 days.

The following adverse events will NOT be considered DLTs:

- Grade 1 to 4 toxicities expected with lymphodepleting chemotherapy (e.g., cytopenias).
- Fever grade ≤ 3.
- Myelosuppression (includes bleeding in the setting of platelet count less than 50×10^9/L and documented bacterial infections in the setting of neutropenia), defined as lymphopenia, decreased hemoglobin, neutropenia and/or thrombocytopenia.
- Immediate hypersensitivity reactions occurring with 2 hours of TC-510 infusion (related to cell infusion) that are not reversible to a grade 2 or less within 24 hours of TC-510 administration with standard therapy.

Criteria for Evaluation

Safety

This will be the first-in-human trial of TC-510 and therefore, no clinical safety information with this therapeutic is currently available. However, TC-510 represents the third TRuC T cell product to be studied in subjects with cancer, the first 2 are gavo-cel and TC-110. TC-510 results from engineering gavo-cel T cells to have them express a PD-1:CD28 switch. A series of common toxicities have been identified across a growing number of trials involving autologous T cells genetically engineered to express either chimeric antigen receptors or T cell receptors targeting tumor antigens. The most important of such toxicities are CRS, neurotoxicity, graft-versus-host disease (GvHD), and myelosuppression. CRS events have also been reported in patients receiving gavo-cel therapy but they have been generally manageable upon institution of standard CRS management measures (e.g., corticosteroids, tocilizumab). As of January 10, 2023, no GvHD events and only 2 low grade self-limited neurotoxicity events have been reported among patients treated with gavo-cel. A detailed guidance on appropriate monitoring and management of these toxicities is included in Section 8 of this protocol.

Most adoptive T cell therapy trials involve the use of lymphodepleting chemotherapy prior to T cell infusion. Most frequently, lymphodepletion chemotherapy involves the combination of cyclophosphamide and fludarabine, which are expected to be associated with toxicities such as cytopenias.

To manage the risk of CRS and other potential TC-510 mediated toxicities, TCR^2 will implement specific adverse event (AE) pages in the electronic case report form (eCRF) to carefully document

the events and to enable evaluation and identification of potential risk factors. To help Investigators manage TC-510 mediated toxicities, TCR^2 has developed treatment guidelines based on published literature which are included in the protocol. TCR^2 has also developed standard protocol guidance on the management of myelosuppression, including a recommendation to consult with physicians with expertise in bone marrow transplant, infectious diseases, and the management of aplastic anemia.

The theoretical risks of replication competent lentivirus (RCL) and insertional oncogenesis will be monitored in accordance with FDA and European Medicines Agency (EMEA) guidance in the long-term follow-up study protocol.

Statistical Methods

The co-primary clinical endpoints for efficacy are ORR, defined as the proportion of patients with a CR or PR via independently reviewed RECIST v 1.1 relative to the total number of patients in the analysis population, and DCR, defined as the ORR plus the proportion of patients with SD for at least 8 weeks via independently reviewed RECIST v 1.1 relative to the total number of patients in the analysis population.

Study Populations

- Intent-to-treat (ITT) population: all patients who have signed the informed consent form and have undergone leukapheresis.
- Modified intent-to-treat (mITT) population: all patients enrolled and treated with the planned dose of study drug.
- DLT Evaluable population: (phase 1 only) patients treated in the phase 1 portion of the study who received study drug and were followed for at least 28 days post-administration or who have a DLT within 28 days of the first study drug dose.
- Safety Set: all patients treated with any dose of study drug.

The primary analysis population for safety and efficacy will be the mITT defined as all patients who received a TC-510 infusion.

No specific statistical hypotheses are being evaluated with respect to the primary, secondary, or exploratory objectives and endpoints. The primary focus will be on determining the safety profile and a preliminary efficacy profile of TC-510, as well as on establishing the kinetics of TC-510 following infusion. All analyses will be descriptive and exploratory. Descriptive statistics on continuous data will include means, medians, standard deviations, and ranges. Categorical data will be summarized using frequency counts and percentages. Graphical summaries of the data may be presented. Assessments will be displayed by cohort and time as well as across cohorts for specific parameters. Time to event endpoints will be summarized and displayed graphically using Kaplan-Meier (KM) methodology to estimate the median, and the 25th and 75th percentiles. Two-sided 80% confidence intervals will be produced. Overall survival may be assessed at fixed time points such as 1 year and 2 years using KM methods.

3 BACKGROUND

3.1 Adoptive Cell Therapy Background

3.2 Mesothelin

MSLN is a 40-kDa glycosyl-phosphatidyl inositol-linked membrane protein differentiation antigen, whose expression is mostly restricted to mesothelial cells lining the pleura, pericardium, and peritoneum in healthy individuals (Chang, 1996; Hassan, 2008). Mesothelin is overexpressed in multiple cancers, including more than 90% of MPMs and pancreatic adenocarcinomas, approximately 70% of ovarian cancers, and approximately 50% of non-small cell lung cancers (NSCLCs), among others (Argani, 2001; Hassan, 2008; Hassan, 2005; Ordóñez, 2003). The precise physiological function of MSLN is not completely understood, but it has been postulated to promote metastasis through its binding to mucin 16 (Chen, 2013). Given its overexpression in tumors and low levels of expression in a restricted number of normal tissues, MSLN is an attractive target for immunotherapy. Indeed, several MSLN-specific approaches are currently being tested in clinical trials for patients with MSLN-positive cancers. Therapeutic modalities include antibodies, recombinant immunotoxins, and chimeric antigen receptor (CAR) T cells.

3.2.1 Mesothelin Expression in Cancer

The expression of MSLN in cancer has been broadly studied. Serial analyses of gene expression (SAGE) (http://www.ncbi.nlm.nih.gov/projects/SAGE/), conducted at the National Institutes of Health (NIH), have shown high messenger ribonucleic acid (mRNA) expression of MSLN in NSCLC, pancreatic cancer, MPM, ovarian cancer, cholangiocarcinoma, and other adenocarcinomas (Hassan, 2008). Expression profiles have been further supported by IHC studies performed on biopsy tissues taken from patients with multiple tumor types (Inaguma, 2017). While the IHC staining can vary depending on the antibody clone that is used, most IHC analyses indicate that 90% of ovarian cancer and > 75% of MPM or pancreatic cancer biopsies are immunoreactive to anti-MSLN antibodies. Mesothelin expression and prevalence in various tumor types has been reviewed by Inaguma (2017) (see Table 1).

Table 1. MSLN Expression in Different Human Cancers as Assessed by the Anti-Mesothelin Antibody 5B2

		MSLN Expression (5B2 Anti-MSLN Antibody)					
	Total	Luminal/Membrane			Total		
Diagnosis	**No.**	**No.**	**%**	**Range**	**No.**	**%**	**Range**
Tumors with adenocarcinoma-like differentiation							
Ovary, serous carcinoma	79	71	89.9	5-100 (90)	73	92.4	5-100 (90)

Table 1. MSLN Expression in Different Human Cancers as Assessed by the Anti-Mesothelin Antibody 5B2

	Total	MSLN Expression (5B2 Anti-MSLN Antibody)					
		Luminal/Membrane			Total		
Diagnosis	**No.**	**No.**	**%**	**Range**	**No.**	**%**	**Range**
Pancreas, invasive ductal carcinoma	132	93	70.5	5-100 (50)	100	75.8	5-100 (80)
Uterus, endometrioid adenocarcinoma	82	52	63.4	5-100 (20)	62	75.6	5-100 (30)
Colorectum, adenocarcinoma	188	91	48.4	5-100 (10)	108	57.4	5-100 (30)
Lung, adenocarcinoma	76	36	47.4	5-100 (30)	50	65.8	5-100 (80)
Liver, cholangiocellular carcinoma	39	16	41	10-100 (60)	16	41	20-100 (95)
Stomach, adenocarcinoma	81	31	38.3	5-90 (20)	60	74.1	5-100 (80)
Mammary gland, invasive ductal carcinoma	119	13	10.9	5-60 (10)	13	10.9	5-100 (60)
Prostate gland, adenocarcinoma	107	0	0	—	1	0.9	90 (—)
Mammary gland, lobular carcinoma	82	0	0	—	0	0	—
Tumors with squamous cell differentiation							
Thymic carcinoma	17	6	35.3	5-100 (93)	13	76.5	10-100 (100)
Lung, squamous cell carcinoma	72	20	27.8	5-90 (10)	33	45.8	5-100 (20)
Uterine cervix, squamous cell carcinoma	21	3	14.3	20-40 (20)	6	28.6	20-100 (90)
Thymoma	33	0	0	—	0	0	—
Other epithelial tumors							
Malignant mesothelioma	143	107	75	5-100 (90)	111	77.6	5-100 (100)
Epithelioid	115	93	80.9	5-100 (90)	97	84.3	5-100 (100)
Biphasic	17	11	64.7	20-100 (70)	11	64.7	20-100 (100)
Sarcomatoid	10	2	20	80 (80)	2	20	90-100 (95)
Urinary tract, urothelial carcinoma	85	11	12.9	5-100 (20)	18	21.2	10-100 (35)
Kidney, clear cell renal cell carcinoma	206	9	4.4	5-100 (20)	15	7.3	5-100 (50)

MSLN = mesothelin; — = not applicable.

3.3 Target Disease Background and Study Rationale

Several analyses have demonstrated a high prevalence of strong MSLN expression in multiple solid tumors. Thus, MSLN-expressing cancers are amenable to targeted anti-MSLN approaches. Cancers such as MPM, serous ovarian adenocarcinoma, triple negative breast cancer, and colorectal cancer are amongst those with the highest levels of membranous MSLN expression and will be the patient population of this clinical trial.

3.3.1 Malignant Pleural/Peritoneal Mesothelioma

MPM represents about 80% of mesothelioma cases. Malignant pleural/peritoneal mesothelioma is a regional and highly aggressive tumor that arises from the mesothelium of the pleural and peritoneal surface. Rarely, other serosal membranes of the human body are also coated with mesothelium, such as the pericardium (pericardial mesothelioma), are affected. The incidence of MPM has increased significantly and it is estimated that 40,000 people die each year worldwide due to asbestos-related MPM. Different types of MPM have been identified including epithelioid (50%-70% of cases), biphasic (30%), and sarcomatoid (10%-20%) with increasingly aggressive behavior and worse prognosis. In addition to a high incidence (25%-60%) of somatic *BAP1* mutations, MPM is also associated with frequent alterations in other major tumor suppressor genes, such as *p16/Cdkn2a, p19/Arf*, *p19/Cdkn2b*, and *NF2*. Effective treatment options for patients with MPM are very limited. The standard of care recommended for MPM is palliative chemotherapy with a doublet of platinum salt and an anti-folate. Unfortunately, objective response rates are 17% to 40% and the median overall survival (OS) of patients with MPM is 12 to 19 months when systemic chemotherapy is used with or without anti-angiogenic agents or targeted therapy. Anti-CTLA-4 antibody failed to show a survival advantage as second-line therapy in MPM. Anti-programmed death receptor-1 (PD-1) and anti-PD-L1 antibodies (e.g., pembrolizumab, nivolumab, avelumab) are currently being tested in several trials in MPM. Early phase trials with anti-PD-1 or anti-PD-L1 antibodies have shown partial response rates up to 28% and disease control rates up to 76% with median duration of response of 12 months, but confirmatory data are required to validate these agents as the second line treatment of choice in MPM.

3.3.2 Ovarian Cancer

Ovarian cancers can be classified in several subtypes according to their histopathology, which also determines their therapy. Epithelial ovarian cancer comprises 90% of all ovarian malignancies, with other pathologic subtypes such as germ cell and sex-cord stromal tumors being much rarer. It is estimated that 22,240 new diagnoses and 14,070 deaths from ovarian cancer will occur in 2018 in the US (Seer, 2018). Ovarian cancer is characterized by late-stage presentation (more than 70% of cases), bulky metastatic tumor burden, and frequent recurrence of eventual chemoresistant disease, which result in cure rates below 15% among patients with stage 3/4 disease. The 2 canonical types of drugs used to treat ovarian cancer—taxane and platinum-based agents—have not been replaced in the past 20 years, although the optimum timing of treatment (neoadjuvant versus adjuvant) and the best route of administration (intravenous versus intraperitoneal) remain unknown.

Recurrent ovarian cancer is not curable. The objectives of therapy are symptom palliation and extension of life. Patients with platinum-sensitive ovarian cancer should be treated with a platinum-based agent. Those progressing after platinum retreatment and those with platinum-resistant disease, non-platinum combination and targeted therapies are available. The initial clinical efficacy of novel therapeutics, such as PARP inhibitors and immune-checkpoint inhibitors, has ushered in a new wave of drug development in

ovarian cancer. The synthetic lethality of breast cancer susceptibility gene mutated (i.e., deficient) ovarian cancer cells exposed to the PARP inhibitor olaparib resulted in a median PFS of 7 months and median OS of 16.6 months. Efficacy with checkpoint inhibitors in patients with advanced recurrent ovarian cancer has been modest thus far. Best ORR has been 15% with nivolumab, 12% with pembrolizumab, and 10% with ipilimumab (Hamanishi et al, 2015; Varga et al, 2015).

3.3.3 Pancreatic Cancer

Pancreatic ductal adenocarcinoma is the seventh leading cause of cancer death worldwide and has the highest incidence-to-mortality ratio of any solid tumor. The median 5-year survival among patients with pancreatic cancer is only 9%, highlighting the urgent need to develop novel therapeutic strategies for this deadly disease. The mainstay of current treatment for metastatic pancreatic ductal adenocarcinoma is combination chemotherapy. There are 2 regimens that are first-line treatment options for patients with metastatic disease with good performance status: FOLFIRINOX (fluorouracil, leucovorin, irinotecan, and oxaliplatin) and gemcitabine combined with nanoparticle albumin-bound paclitaxel (nab-paclitaxel). The EGFR inhibitor erlotinib was tested in combination with gemcitabine for the first-line treatment of unselected patients with metastatic pancreatic ductal adenocarcinoma and showed a statistically but not clinically significant increased OS (6.2 months vs 5.9 months), leading to its FDA approval. However, as other regimens were shown to have more promising results, this combination is rarely utilized. The only approved second-line therapy is a combination of fluorouracil and protein-encapsulated irinotecan for patients who received a gemcitabine-based therapy in the first line. In practice, however, patients with good performance status are often offered the alternative first-line regimen that they did not receive initially. Other actionable mutations found to be enriched in small patient subsets with KRAS wild-type pancreatic cancer, including anaplastic lymphoma kinase (ALK) amplifications (0.16%), BRAF mutations (2.2%), NRG1 fusions (0.5%), and NTRK gene fusions (0.3%).

3.3.4 Triple Negative Breast Cancer

TNBC fails to express the estrogen receptor (ER), the progesterone receptor (PgR), and the human epidermal growth factor receptor-2 (HER-2) and represents approximately 15% of all breast cancers. Historically, the treatment of TNBC has been limited to chemotherapy given the lack of standard therapeutic targets. Early stage TNBC has a high response rate to chemotherapy, but relapse is common and tends to occur rapidly. Once TNBC becomes metastatic, it is incurable. Single agent chemotherapy with an anthracycline, a taxane, or a platinum agent are the most active regiments in the frontline setting in metastatic TNBC, with response rates in 20%-30% of cases and a median OS of 10-13 months. Combination chemotherapy regimens can increase ORRs, but they are also associated with more toxicity without clear evidence of improvement on OS. Transcriptomic analyses have more precisely classified breast cancers into distinct molecular subtypes, including normal breast-like, luminal A and luminal B (ER + and/or PgR+), HER-2 enriched, claudin-low, and basal-like. Most cases of TNBC (50%-75%) are basal-like, whereas those with low levels of ER and PgR expression

(1%-10%) are more likely to be luminal (46%) or HER-2-enriched (29%) by gene expression. Most claudin-low breast tumors fail to express ER, PgR, and HER-2 by IHC and have metaplastic/medullary differentiation, elevated expression of immune-related genes, stem cell and mesenchymal features, and active transforming growth factor-beta signaling.

Further analyses have defined additional subtypes, including 2 basal-like (BL1 and BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem- like (MSL), luminal androgen receptor (LAR) and unstable (UNS) subtypes, based on tumor cell intrinsic gene expression patterns. Recent progress has identified 2 groups of patients with metastatic TNBC for whom biomarker-driven targeted therapy has been approved by the FDA. First, patients with germline BRCA1/2 mutations (20% of TNBC cases), for whom the PARP inhibitors olaparib (ORR 59.9%, PFS 7 months, OS 17.1 months in patients with 2 or fewer prior lines of therapy) and talazoparib (ORR 62.6%, PFS 8.6 months in patients with 3 or fewer prior lines of therapy) are approved after progression on chemotherapy, and second, patients with tumors that express PD-L1 on immune cells occupying at least 1% of the tumor area, for whom immunotherapy with the PD-L1 inhibitor atezolizumab in combination with *nab*-paclitaxel (ORR 59%, PFS 7.5 months, OS 25 months in treatment naïve patients) is approved.

3.3.5 Colorectal Cancer

Colorectal cancer (CRC) is the second greatest cancer threat to life in Western countries. Metastatic colorectal cancer carries poor prognosis, and current therapeutic regimes convey limited improvements in survival and high rates of detrimental side effects in patients that may not stand to benefit. Little progress has been made regarding targeted therapies for patients with metastatic colorectal cancer (mCRC). The CALGB/SWOG 80405, Fire-3, and PEAK randomized controlled clinical trials have established the vascular endothelial growth factor receptor (VEGFR) inhibitor bevacizumab and the EGFR inhibitors cetuximab and panitumumab as frontline therapies for patients with mCRC when combined with FOLFOX/FOLFOXIRI chemotherapy. However, the improvement in outcomes standard regimens has been modest, with PFS in FIRE-3 improved from 5.8 months to 9.7 months with the addition of cetuximab in patients with wild-type RAS CRC. Recently, the Phase III Keynote-177 study compared the clinical activity of pembrolizumab to that of standard 5FU-based chemotherapy (with or without bevacizumab or cetuximab) among 307 patients with newly diagnosed Mismatch Repair Deficient (dMMR) /MSI-H CRC. Pembrolizumab was superior to chemotherapy with PFS of 16.6 months compared with 8.2 months. The median OS had not yet been reached in the pembrolizumab group but was 36.7 months in the chemotherapy group. An overall response rate was 43.8% in the pembrolizumab group, compared with 33.1% in the chemotherapy group. These data have established checkpoint inhibitor therapy as standard of care among patients with newly diagnosed MMR deficient/MSI-high CRC. However, the benefit of this approach is very limited among late-stage patients refractory to chemotherapeutic regimens.

3.3.6 Non-small Cell Lung Cancer

Non-small Cell Lung Cancer remains the leading cause of cancer-related mortality worldwide, accounting for approximately 18% of all cancer deaths. Despite treatment with platinum- and

taxane-based chemotherapy, patients with metastatic NSCLC have a median survival of approximately 10 months, and a 5-year survival rate of approximately 15%. Despite the increased number of treatment options available for patients with non-squamous histology NSCLC, there has been little OS improvement from several new agents, including pemetrexed, erlotinib, and bevacizumab, beyond very small subpopulations. Therapeutic options for epidermal growth factor receptor (*EGFR*) wild-type non-squamous NSCLC are particularly limited after failure of front-line chemotherapy. Overall, this group of patients only has an OS of about 8 months after progression from platinum agents. Once resistance to tyrosine kinase inhibitors (TKIs) occurs, the patients whose tumors harbor *EGFR* mutations or anaplastic lymphoma kinase (*ALK*) translocations will have a rapid disease progression. Therefore, NSCLC remains a disease with high unmet medical need. T cell checkpoint regulators such as CTLA-4 and PD-1 (CD279) down-regulate T cell activation and proliferation upon engagement by their cognate ligands. T cell checkpoint inhibitors induce antitumor activity by breaking immune tolerance to tumor cell antigens. Programmed death receptor-1 and PD-L1 inhibitors are effective against metastatic NSCLC lacking sensitizing *EGFR* or *ALK* mutations. Pembrolizumab (Keytruda, Merck), nivolumab (Opdivo, Bristol-Myers Squibb), and atezolizumab (Tecentriq, Genentech) are approved as second-line therapy.

3.3.7 Cholangiocarcinoma

Cholangiocarcinomas are biliary epithelial tumors of the intrahepatic, perihilar, and distal biliary tree. Intrahepatic cholangiocarcinomas (iCCAs) (20% of cases) arise above the second-order bile ducts, whereas the cystic duct is the anatomical point of distinction between perihilar cholangiocarcinomas (pCCAs) (50%-60%), and distal cholangiocarcinomas (dCCAs) (20%-30%). Most patients have advanced-stage disease at presentation due to its aggressiveness and difficulty in early diagnosis. While surgery is the preferred therapy, only 35% cases have early disease amenable to surgical resection with curative intent. For unresectable cholangiocarcinoma, the available standard-of-care chemotherapy (gemcitabine and cisplatin) renders a median OS < 1 year, partly due to the desmoplastic stroma that fosters cancer cell survival and poses a barrier to the delivery of chemotherapy. Recurrent mutations in IDH1, IDH2, FGFR1, FGFR2, FGFR3, EPHA2, and BAP1 are found predominantly in iCCAs, whereas ARID1B, ELF3, PBRM1, PRKACA, and PRKACB mutations occur preferentially in pCCA/dCCA. Some of the latter represent actionable mutations whose therapeutic potential is currently being investigated in clinical trials. At present, clinical data on immunotherapy in cholangiocarcinoma are limited. PD-L1 expression has been reported in 9% to 72% of specimens, and on 46% to 63% of immune cells within the tumor microenvironment.

3.4 Study Rationale

Novel therapies are needed for the majority of advanced solid cancers as they relapse and become treatment refractory. Immune checkpoint inhibitor therapy has revolutionized the treatment of solid tumors but unfortunately only a fraction of patients will truly benefit from these agents, and for patients with specific tumor types such as colorectal cancer, pancreatic ductal adenocarcinoma, serous ovarian adenocarcinoma, and triple negative breast cancer, the benefit provided by these agents has been negligible. However, other immunotherapeutic approaches such as adoptive T cell therapies (ACT), have generated promising preliminary data in solid tumors. Several modalities of ACT such as CAR T therapies and TCR T cell therapies have shown the potential of T cells to treat cancer. Autologous engineered ACT is produced by collecting a patient's T cells via leukapheresis, followed by an ex vivo process that includes T cell activation, transduction with a transgene-containing viral vector (typically retrovirus or lentivirus containing a synthetic T cell receptor), and expansion of the synthetic T cell receptor-expressing T cells prior to infusion back into the same patient. The ability to genetically engineer autologous T cells and employ them to induce cancer regression has been demonstrated in multiple clinical trials using CAR T cell approaches in patients with relapsed or refractory hematological malignancies expressing either CD19 or the B-cell maturation antigen such as non-Hodgkin's lymphoma, ALL, chronic lymphocytic leukemia (CLL), and multiple myeloma. Tumor infiltrating lymphocyte (TIL) therapy and TCR-T cell approaches have shown clinically active for the treatment of patients with a variety of solid tumors. Recently, a TRuC T cell therapy (gavocabtagene autoleucel, also known as gavo-cel) directed against the tumor antigen mesothelin has shown promising preliminary efficacy in a phase 1 study for patients with mesothelin positive mesothelioma, ovarian cancer, cholangiocarcinoma, or non-small cell lung cancer.

3.4.1 Mesothelin-Targeting TRuC T Cell Therapy

The phase 1 portion of the clinical trial (TCR2-18-01) was designed to evaluate the safety profile of gavocabtagene autoleucel (gavo-cel; TC-210 T cells). To that end, patients were assigned to receive gavo-cel at 4 increasing doses ($5 \times 10^7/m^2$, $1 \times 10^8/m^2$, $5 \times 10^8/m^2$, and $1 \times 10^9/m^2$). Within each dose cohort, gavo-cel was first tested as a single agent, and then, in the absence of any safety signal, the same gavo-cel dose was tested following lymphodepletion. The presence or absence of lymphodepletion defined distinct dose levels, even when employing the same gavo-cel dose. Dose levels without lymphodepletion enrolled 1 patient whereas dose levels employing lymphodepletion enrolled 3 patients. Dose escalation was conducted following a modified 3+3 design with the main objective of defining the gavo-cel recommended Phase 2 dose.

Patients with 4 different indications were enrolled to the phase 1 portion of the study: malignant mesothelioma, serous ovarian cancer, non-small cell lung cancer, and cholangiocarcinoma. One key inclusion criterion for the trial required that tumors expressed mesothelin on at least 50% of the viable cancer cells and such expression must be 2+ and/or 3+ as assessed by

immunohistochemistry at a central laboratory. In addition, all patients must have failed all FDA approved options for their respective indications, unless otherwise specified.

All patients received a single gavo-cel infusion on day 0 (with the exception of the patients that received gavo-cel at DL3.5A via fractionation, as described below) and subsequently were closely followed for safety. Response assessments following RECISTv1.1 criteria were conducted by CT scans that were centrally and independently reviewed at 1, 2, 3, 6, 9, and 12 months during the first-year post infusion.

As of January 10, 2022, 23 patients (17 MPM, 5 ovarian, 1 cholangiocarcinoma) had received a single intravenous dose of gavo-cel at $5 \times 10^7/m^2$ either alone (DL 0, n=1) or after lymphodepletion (LD) (DL1, n=6), at $1 \times 10^8/m^2$ either alone (DL2, n=1) or after LD (DL3, n=6), at $5 \times 10^8/m^2$ either alone (DL4, n=1) or after LD (DL5, n=3), or at $3 \times 10^8/m^2$ via fractionated dosing following LD (DL3.5A, n=5).

The median number of prior therapies was 7 (range, 1-13), including immune checkpoint inhibitors in 15 (65%) patients and anti-mesothelin agents in 6 (26%) patients. Bridging therapy was required by 16 (70%) patients.

Twenty-three patients were evaluable for safety. Hematological toxicity was very common and a consequence of employing lymphodepleting chemotherapy prior to gavo-cel infusion in most patients. Grade 3 or higher neutropenia, lymphopenia, or thrombocytopenia was reported in 83%, 96%, and 26% of patients, respectively.

CRS was reported in 18 (78%) patients being grade 1 or 2 in 11 (48%) patients and grade 3 or 4 in 7 (30%) patients. The median number of days to onset of CRS was 2 (range, 0-7). Only 1 (4%) patient, treated at DL5, experienced a neurotoxic event, which was grade 2 and transient. Of the 18 patients with CRS, 12 (67%) patients received tocilizumab and 9 (50%) patients received corticosteroids for the management of cytokine release related symptoms.

Two dose limiting toxicities were reported during dose escalation. The first one was a grade 3 pneumonitis event experienced by a patient with mesothelioma treated at DL1, in the context of grade 3 cytokine release syndrome. Both complications resolved with tocilizumab and corticosteroid therapy. Several weeks after the resolution of these adverse events, this patient developed grade 5 fungal sepsis. The SRT deemed this event to be unrelated to gavo-cel but recommended to expand the DL1 cohort from 3 patients to 6 patients. Subsequently 4 other pneumonitis events were reported (1 at DL5 and 3 at DL3.5A).

The second DLT was a grade 5 bronchioalveolar hemorrhage event, which was reported on a patient with mesothelioma treated at DL5. This patient had previously developed CRS-related disseminated intravascular coagulation and was placed on low molecular heparin therapy. Because the other 2 patients treated at DL5 also developed severe CRS, the SRT declared $5 \times 10^8/m^2$ the Maximum Tolerated Dose (MTD). Subsequently patients were treated at DL3.5A ($3 \times 10^8/m^2$ following lymphodepletion, via fractionated dosing which consisted of patients receiving 33% of the total dose on gavo-cel on day 0 and 67% of the total dose of gavo-cel on

days 3-7), a dose level intermediate between DL3 and DL5. The development of pneumonitis in 3 out of the 5 patients treated at DL3.5A led the Safety Review Team to declare DL3 ($1 \times 10^8/m^2$ following lymphodepletion) as the RP2D. A total of 6 patients have received therapy at the RP2D as of January 10, 2022. Of them, 4 (67%) developed CRS which was grade 1 in 1 (17%) patient, grade 2 in 2 (33%) patients and grade 4 in 1 (17%) patient. The grade 4 CRS, which was deemed to be grade 4 due to worsening hypoxia and bilateral effusions, improved to grade 2 in less than 24 hours upon intrapleural catheter placement. No neurotoxicity or pneumonitis events were reported amongst patients treated at the RP2D.

3.4.2 TC-510

The limited efficacy of CARs in solid tumors is thought to be due to a number of factors including T cell exhaustion and lack of persistence. Chimeric antigen receptors are physically removed from the native TCR and signal only through one of the 6 TCR subunits (i.e., CD3ζ chain). The failure to initiate and harness a complete TCR response is arguably a primary underlying factor preventing CAR T cell success in solid tumor indications. In order to bypass this limitation, we have developed a novel T cell engineering platform based on T cell receptor fusion constructs (TRuCs) including a PD-1/CD28 switch. TC-510 is a novel cell therapy, consisting of genetically engineered T cells that express a humanized single-domain antibody that recognizes human MSLN fused to the cluster of differentiation (CD) 3ε subunit and a PD-1xCD28 switch receptor which, upon expression, is incorporated into the endogenous TCR complex. There, it redirects the T cells to recognize and eliminate MSLN-positive tumor cells. Furthermore, the addition of the PD-1xCD28 switch receptor helps with overcoming PD-L1/PD-L2 mediated immunosuppression and turns this inhibitory function into a costimulatory signal. We herein show preclinical evidence underscoring the efficacy of TRuC T cells re-programmed to target MSLN including a PD-1/CD28 switch. TC-510 show robust anti-tumor activity in vitro and in vivo in cancer models.

3.4.3 Structure of TC-510 T-Cell Receptor Fusion Construct Complex

TC-510 is comprised of genetically modified antigen specific autologous T cells that have been reprogrammed to target cells that express MSLN. As in gavo-cel, TC-510 TRuC consists of a binding domain (a humanized llama-derived single-domain anti-MSLN antibody referred to as MH1), a 15 amino acid flexible glycine/serine spacer (G4S)3 and the human CD3ε subunit integrated into the TCR complex (Figure 1). Unlike CAR T cells, which also contain co-stimulatory signaling domains, gavo-cel TRuC complex harnesses the natural immunoreceptor tyrosine-based activation motif (ITAM) domains of the TCR complex.

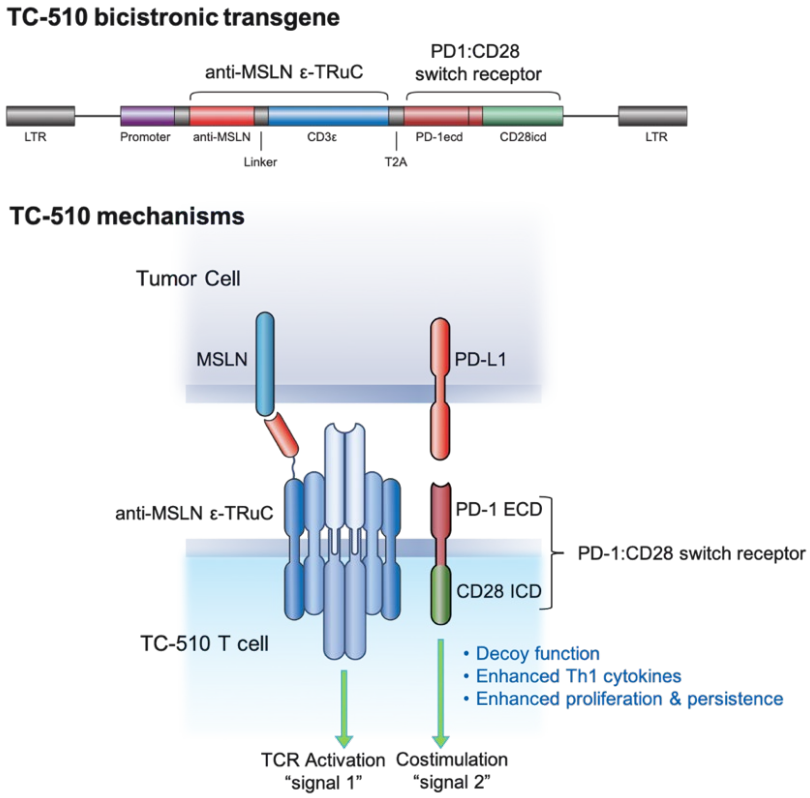

Figure 1. Schematic Representation of the TC-510 T-Cell Receptor Fusion Construct Complex

3.4.4 TC-510 Generation Process Overview

T-510 T cell is an autologous engineered T cell therapy that uses a patient's own leukocytes collected via leukapheresis, starting material. The leukocytes are then frozen and shipped to the processing facility. After thawing non-T cell subpopulations are removed to generate a highly pure T cell population, which then undergoes ex vivo expansion and transduction with a lentiviral vector that encodes for the Gavo-cel and PD1/CD28 transgenes. The transduced T cells are subsequently further expanded and then washed and cryopreserved and shipped to the patient's treating center for administration.

3.4.5 Summary of Results From Preclinical Studies

Detailed descriptions of the complete non-clinical investigation are provided in the Investigator Brochure (IB), section 3. Key non-clinical findings are outlined below:

- Good transduction efficiency with high expression of both the anti-MSLN TRuC and the PD1xCD28 switch receptor observed with TC-510.

- Potent cytotoxicity in vitro with higher production of cytokines and the key cytolytic factor, perforin, in comparison to TC-210.
- Increased cytokine production in comparison to TC-210 in an in vitro assay designed to simulate CRS conditions.
- Enhanced expansion/proliferation and retention of cytotoxic capacity upon repeated in vitro restimulation with MSLN and PD-L1 expressing target cells in comparison to TC-210.
- Potent, dose-dependent anti-tumor activity with complete tumor regressions in MSLN-expressing mesothelioma models with low and high expression of PD-L1.
- Increased intratumoral and peripheral expansion compared to TC-210 with higher, transient increases in serum cytokine levels.
- Good tolerability of TC-510 in vivo with similar tissue biodistribution patterns as TC-210 and no adverse histopathological findings.
- In vitro and in vivo, the activity of the PD-1xCD28 switch receptor is dependent upon TRuC activation, suggesting that the enhanced activity of TC-510 will be limited to tumor microenvironments where both MSLN and PD-L1 are highly expressed.

4 INVESTIGATIONAL PLAN

4.1 Overall Study Objectives

This is a phase 1/2 open-label study to evaluate the safety and efficacy of autologous genetically engineered TC-510 in patients with MSLN-expressing cancers.

The primary objective of the phase 1 portion of the study is to evaluate the safety of TC-510 and establish the RP2D.

The primary objective of the phase 2 portion of the study is to evaluate the efficacy of TC-510 administered at the RP2D, as measured by the co-primary endpoints ORR and DCR in patients with MPM, Serous Ovarian Adenocarcinoma, Pancreatic Adenocarcinoma, Colorectal Cancer, TNBC, NSCLC, and Cholangiocarcinoma.

4.1.1 Phase 1 Objectives

Primary

- To evaluate the safety of autologous genetically modified TC-510 in patients with MSLN-expressing metastatic or unresectable solid tumors.
- Establish the RP2D according to DLT of defined AEs.

Secondary

- To determine the ORR (CR + PR) according to RECIST v 1.1 and DoR.
- To determine the DCR, defined as a composite of ORR and SD lasting at least 8 weeks.
- To evaluate the survival benefit of autologous genetically modified TC-510 as assessed by PFS and OS.
- To assess humoral immunogenicity to TC-510

Phase 2 Objectives

Primary

- To evaluate the efficacy of TC-510 in patients with MSLN-expressing metastatic or unresectable solid tumors.
  - Endpoints: ORR (CR + PR) and DCR (ORR + SD ≥ 8 weeks) according to RECIST v 1.1.

Secondary

- To evaluate the efficacy of autologous genetically modified TC-510 in patients with MSLN-expressing unresectable, metastatic, or recurrent cancers as assessed by TTR, DoR, PFS, and OS.
- To further evaluate the safety of autologous genetically modified TC-510.
- To assess whether patients experience a response upon subsequent TC-510 infusion(s).

Exploratory Objectives (Phase 1 and 2)

- To evaluate TC-510 expansion, persistence, and cytokine production in vivo.
- To evaluate key TC-510 product attributes pre- and post-infusion (e.g., phenotype, functionality).
- To evaluate TC-510 infiltration in tumor tissue.
- To evaluate the correlation of metabolic activity of target and non-target lesions post TC-510 infusion and clinical activity.
- To evaluate immune markers in the tumor microenvironment (e.g., PD-L1 expression) before and after TC-510 infusion and their correlation with toxicity and response.
- To evaluate the changes in health-related quality of life following treatment with TC-510 (only Phase 2).
- To evaluate the response to TC-510 by iRECIST

5 OVERALL STUDY DESIGN

5.1 Overall Study Design

This first-in-human clinical trial is a phase 1/2 open-label study to evaluate the safety and efficacy of autologous genetically engineered TC-510 in patients with advanced MSLN -expressing cancers. The study will be completed when the last patient treated with TC-510 has been followed for approximately 5 years or when the study is closed at the request of the Sponsor. After completing follow-up on this study, patients will be offered to transition to a dedicated LTFU protocol and monitored for gene therapy-related delayed adverse events for 15 years (from most recent TC-510 infusion), in accordance with FDA regulatory requirements for gene therapy clinical trials.

Patients will be screened for general health, performance status, diagnosis, disease stage, and MSLN expression (if applicable).

- Patients must be aged ≥ 18 years with a diagnosis of MPM, serous ovarian adenocarcinoma, pancreatic adenocarcinoma, colorectal cancer, triple negative breast cancer, NSCLC, or cholangiocarcinoma. Patients will sign a Pre-screening informed consent form (ICF) to undergo testing to determine adequate presence of MSLN expression on the tumor confirmed by central histology assessment (if applicable).
- Patients that have screened positive for adequate MSLN expression or for whom pre-screening MSLN testing is not required (i.e. patients with epithelioid MPM), will have the remaining leukapheresis eligibility criteria (as defined in section 6) determined during the Leukapheresis Eligibility visit.
- Patients meeting all leukapheresis eligibility will undergo a large-volume leukapheresis at the enrolling institution to obtain cells for the manufacture of autologous TC-510. Patient's peripheral blood mononuclear cells (PBMC) will be collected and processed at the site and frozen leukocytes will be shipped centrally for further processing. Then, TC-510 transduced T cells will be formulated, cryopreserved, and shipped back to the enrolling institution for infusion.
- The phase 1 portion of the study will evaluate varying doses of TC-510 preceded by lymphodepleting chemotherapy. The lymphodepleting chemotherapy regimen will consist of fludarabine for 4 days (day -7 to day -4) and cyclophosphamide for 3 days (day -6 through day -4) (refer to Figure 3).
- It is estimated that 25-30 patients will be treated during the dose-escalation phase.
- A modified 3 + 3 dose escalation strategy will be used to identify the RP2D.
- Prior to the administration of protocol-defined therapy, all patients will sign the Treatment ICF, treatment eligibility criteria will be confirmed, and a baseline tumor assessment obtained (if necessary).

- For the phase 1 portion of the study, TC-510 may be administered via a fractionated regimen at any point in the study if deemed appropriate for safety or efficacy reasons by either the SRT or the Sponsor's Medical Monitor. The TC-510 dose would be fractionated such that one-third (approximately 33%) of the TC-510 dose will be administered on day 0 and, if well tolerated, the remaining two-thirds (approximately 67%) of the dose will be administered on day 7. In the event the initial one-third (33%) dose elicits ≥ grade 3 CRS and/or ≥ grade 2 neurotoxicity, the infusion of the second dose should be delayed until the CRS and/or neurotoxicity regresses to ≤ grade 1, or otherwise until treatment is deemed safe by both the treating physician and the Sponsor's Medical Monitor. A delay of the second infusion by more than 7 days must be approved by the Sponsor. In the event of significant toxicity observed after the first infusion of TC-510, the Sponsor's Medical Monitor and the Investigator, may determine that the cell dose should be fractionated such that one-third (approximately 33%) of the dose will be administered on day 0 and, if well tolerated, a second-third (approximately 33%) will be administered 7 days later, and if that fraction is well tolerated, the third and final fraction (approximately 33%) will be administered 7 days after the second fraction was received. The same delay or hold rules as described above will apply to this fractionation method. In the phase 2 portion of the study, TC-510 will be administered at the RP2D.
- Both the lymphodepleting chemotherapy as well as the TC-510 infusion may either be given as an outpatient treatment or patients may be hospitalized at the discretion of the Investigator. For the phase 1 portion of the study, all patients should be observed overnight following administration of TC-510. For the Phase 2 portion of the study, the Sponsor recommends overnight observation following TC-510 infusion.

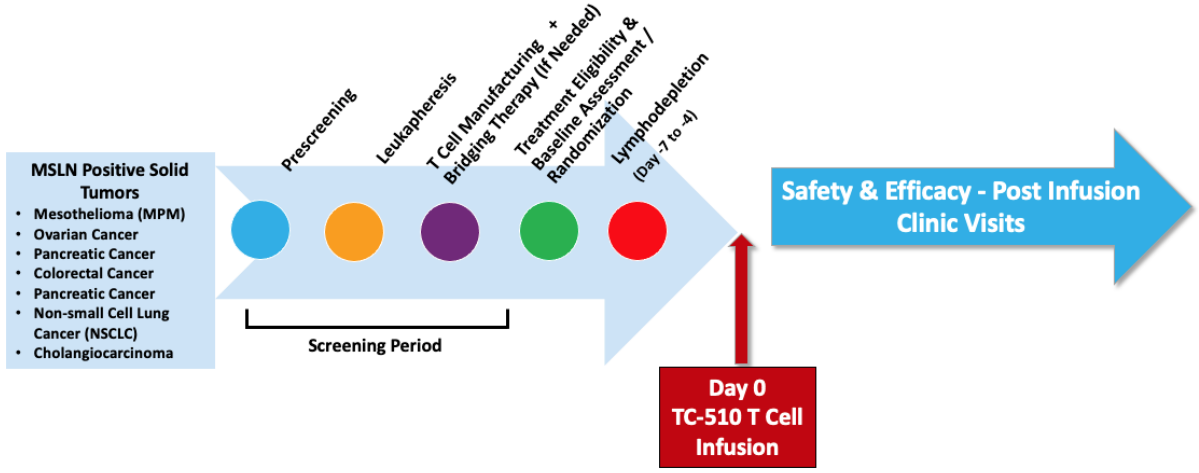

Figure 2. Schematic Diagram of Study Design

MPM = malignant pleural/peritoneal mesothelioma; MSLN = mesothelin.

The study will have 2 well-differentiated phases, phase 1 and phase 2. Independent of the phase of the study, each patient will follow the same study treatment schedule and procedural

requirements (unless specified otherwise). Each patient will proceed through the following study periods:

- Pre-screening/Leukapheresis
- Baseline assessment (Treatment Eligibility)
- Lymphodepleting chemotherapy
- TC-510 infusion
- Post-infusion follow-up

Phase 1

- Patients receiving TC-510 in this dose escalation phase must have one of the following cancer diagnoses: MPM, Serous Ovarian Adenocarcinoma, Pancreatic Adenocarcinoma, Colorectal Cancer, TNBC, NSCLC, or Cholangiocarcinoma.
- This dose escalation phase will proceed with varying cell doses to determine the RP2D: (50×10^6, 100×10^6, 130×10^6, 160×10^6, and 200×10^6). All doses mentioned throughout the protocol denote transduced TC-510. A variation on the target dose of 15% (i.e., ± 15%) will be allowed at each dose level.
- Each patient enrolled to the dose-escalation phase of the study will receive TC-510 as either a single infusion or in a fractionated regimen.
- Prior to escalation to the next dose level, the SRT, comprised of the Study Sponsor Medical Monitor and at least 2 Study Investigators, will review the safety data and make recommendations on further study conduct of phase 1, as outlined in section 13.
- In the event the RP2D is determined for 1 or more specific indications, dose escalation may continue to determine the indication-specific RP2D for other indications where TC-510 may be tolerated at higher doses. Once the RP2D for a specific indication is determined, enrollment of the Phase 2 specific arm for such indication may commence. Protocol stagger, safety observation and dose escalation rules will continue to apply to patients within the indication-specific dose escalation cohort(s) until the indication-specific RP2D is determined.

Phase 2

- This phase will evaluate the preliminary antitumor activity (efficacy) and better characterize safety of TC-510 at the selected RP2D.
- Patients will receive TC-510 at the RP2D and will be enrolled according to their cancer diagnosis to 7 distinct cohorts of up to 20 patients each: MPM, Serous Ovarian Adenocarcinoma, Pancreatic Adenocarcinoma, Colorectal Cancer, Triple Negative Breast Cancer, Non-small Cell Lung Cancer, or Cholangiocarcinoma.
- Overall, the phase 2 portion of the study will treat 140 patients.

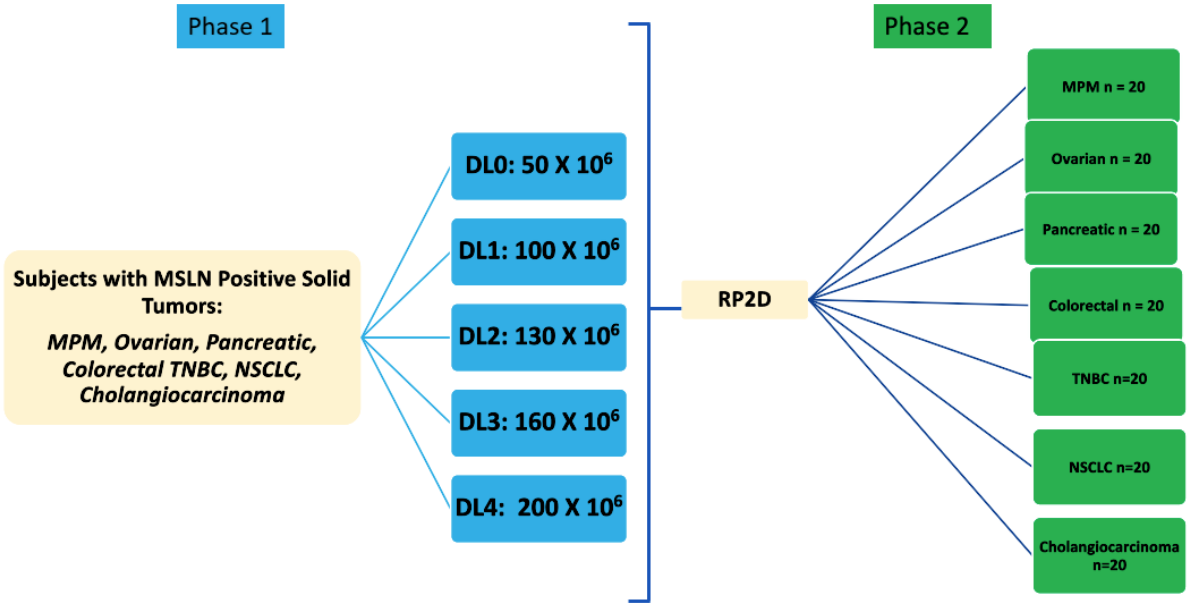

Figure 3. Patient Cohorts in the TCR2-21-01 Phase 1/2 Clinical Trial

MPM = malignant pleural/peritoneal mesothelioma; MSLN = mesothelin; NSCLC = non-small cell lung cancer; RP2D = recommended phase 2 dose; TNBC = Triple Negative Breast Cancer.

5.2 Replacement of Patients

Patients who withdraw from the study prior to initiation of lymphodepleting chemotherapy may be replaced.

If the transduced T cell dose is less than the protocol specified dose, manufacturing of additional transduced T cells from excess banked leukapheresis product will be undertaken to achieve a total TC-510 dose in the target range. If no banked leukapheresis product is available, a second leukapheresis may be performed. If, after a second leukapheresis and manufacturing attempt, cell dose fails to meet the minimum dose requirement, patients may still be eligible to receive TC-510 and participate on this trial. However, an additional patient whose cell dose meets the minimum cell dose requirement will be added to the cohort.

In the event a patient meets the MSLN threshold with archival tissue at pre-screening but does not meet the MSLN threshold at Baseline with fresh tissue biopsy, the patient may still be eligible to receive TC-510 if approved by the Sponsor. If such patient receives TC-510, these patients will be replaced in the cohort by a patient who meets all the inclusion/exclusion criteria.

In phase 2, if a patient is confirmed to have at least 1 lesion that meets evaluable and measurable criteria defined by RECIST v1.1 at pre-screening, and then due to bridging therapy or other circumstances does not meet the requirement via a central reader at Baseline, the patient may still receive TC-510. If such patient receives TC-510, these patients will be replaced in the cohort by

a patient who does meet all of the inclusion/exclusion criteria and evaluated as a separate patient cohort.

Patients that originally consented, and had undergone leukapheresis, during the phase 1 portion of the study can undergo treatment in phase 2 if they are eligible. Patients that are not eligible for phase 2, but have cell product available, may still be treated with TC-510. If such patient receives TC-510, these patients will be replaced in the cohort by a patient who meets all inclusion/exclusion criteria, and they evaluated as a separate patient cohort.

5.3 Study Duration and Completion

For each individual patient, the length of study participation includes a screening period that would account for the time from signing the pre-screening ICF to leukapheresis, a 4-day conditioning chemotherapy treatment period (as applicable), a TC-510 treatment period (which may include an in-hospital period), and a post-treatment assessment period lasting a maximum of 108 weeks, and long-term follow-up for up to approximately 5 years post-infusion. Thus, for patients who complete the entire protocol from the date of pre-screening informed consent through the completion of up to approximately 5 years of long-term follow-up post TC-510 infusion, the duration of the study may be approximately 5 years and 2 months. However, individual study duration will vary depending on a patient's pre-screening requirements, potential retreatments (phase 1 RP2D expansion cohort and phase 2 only), and survival.

The study will be completed when the last patient that was treated with TC-510 has been followed for approximately 5 years, or at the request of the Sponsor.

Patients will be followed on this study for approximately 5 years and will be offered to transition to a dedicated expanded LTFU protocol and monitored for gene therapy-related delayed adverse events for 15 years (from most recent TC-510 infusion), in accordance with FDA regulatory requirements for gene therapy clinical trials.

The primary analyses will be conducted when all patients in the phase 1 portion of the study have been followed for safety for at least 28 days post TC-510 infusion and when all patients enrolled in the phase 2 portion of the study have completed the week 12 disease response assessment, are lost to follow up, withdraw from the study, or die, whichever occurs first.

5.4 Patient Screening and Enrollment

All patients or legally appointed representatives/caregivers must personally sign and date the Institutional Review Board (IRB) approved consent form(s) prior to initiating any study specific procedures or activities that are not part of the patients' routine care.

Each patient who signs an informed consent to enter the pre-screening period will receive a unique patient identification number to be used as the primary identifier throughout the course of the study. The 10-digit Patient identification (ID) number format 2101-###-###, will include the first 4 digits as study identifier, the next 3 digits will designate the number assigned to the

Investigator (the site/investigator number) followed by a 3-digit sequential patient number. This number will be used to identify the patient throughout the study and must be used on all study documentation related to the patient. Patient ID numbers are assigned sequentially at each participating site and will not be assigned to more than one patient. Patients are considered to be enrolled on the study upon completion of a successful apheresis procedure. Should a patient not meet treatment eligibility criteria or be discontinued from the study, the patient screening number cannot be reassigned to any other patient. For patients that proceed to the baseline/treatment eligibility confirmation, they should complete all tests to confirm eligibility within a 14 day time period (with the exception of the baseline biopsy, scans, and ECHO as noted in Appendix A). In the event that the patient requires re-testing, a 7-day extension will be allowed for the patient to complete re-testing for eligibility confirmation. The same process will be followed for retreatment patients.

6 PATIENT ELIGIBILITY

Patients will be assessed for and must meet leukapheresis eligibility criteria for leukapheresis at the Leukapheresis Eligibility visit. Following a successful leukapheresis, patients will then be assessed for treatment eligibility criteria at Baseline. The site will provide documentation of eligibility at the leukapheresis eligibility and baseline timepoints prior to proceeding with subsequent procedures. When considering a potential patient's participation on the study, the investigator should also reference the Treatment Inclusion/Exclusion criteria outlined in section 6.1 and section 6.2.

6.1 Leukapheresis Inclusion/Exclusion Criteria

Patients will be assessed for and must meet leukapheresis eligibility criteria for leukapheresis at the Leukapheresis Eligibility visit.

Leukapheresis Inclusion Criteria

A patient must meet the following inclusion criteria to be eligible to undergo leukapheresis:

1. Patient (or legally authorized representative) has voluntarily agreed to participate by giving written informed consent in accordance with ICH GCP guidelines and applicable local regulations.
2. Patient is ≥ 18 years of age at the time the Informed Consent is signed.
3. Patient has a pathologically confirmed diagnosis of either epithelioid MPM, Serous Ovarian Adenocarcinoma (patients with Serous Ovarian, Fallopian Tube or Primary Peritoneal cancers will be permitted), Pancreatic Adenocarcinoma, Colorectal Cancer, TNBC, NSCLC, or Cholangiocarcinoma.
   - For patients with MPM, a pathology report documenting epithelioid histology should be provided. In the event epithelioid histology cannot be clearly confirmed via pathology

report, the patient may proceed after discussion with the sponsor medical monitor or after confirming adequate MSLN expression.

- For patients with triple negative breast cancer, treatment sites must provide proof of ER-negative and PR-negative status (in both cases defined as < 1%). Her2-negative status is defined as 0, 1+, or 2+ by immunohistochemistry (IHC). If IHC 2+, a negative in situ hybridization (FISH, CISH, or SISH) test is required by local laboratory testing.

4. For patients with epithelioid MPM, confirmation of MSLN expression is not required prior to enrollment. For Ovarian, Pancreatic, Colorectal, TNBC, NSCLC, and Cholangiocarcinoma only: Patient's tumor expresses MSLN on ≥ 50% of tumor cells with 1+, 2+, and/or 3+ intensity by immunohistochemistry at a TCR^2 Therapeutics designated central laboratory.
   - A banked tumor biopsy is allowed at pre-screening if obtained within the prior 5 years, otherwise a fresh tumor biopsy should be obtained.
   - If the patient is pre-screened for MSLN expression at the TCR^2 Therapeutics central laboratory under clinical protocol TCR2-18-01, the results can be used to determine eligibility to proceed for apheresis.
5. Patient has advanced (i.e., metastatic or unresectable) cancer. Unresectable refers to a tumor lesion in which clear surgical excision margins cannot be obtained without leading to significant functional compromise.

6. Prior to TC-510 infusion, patients with MPM, Serous Ovarian Adenocarcinoma, TNBC, Colorectal, and NSCLC must have received at least 1 but no more than 5 systemic therapies for metastatic or unresectable disease. Patients with Pancreatic and Cholangiocarcinoma who are treatment naïve may be eligible for TC-510 therapy if they have elected not to pursue front-line therapy. Regardless of tumor type, patients must not exceed 5 prior lines of therapy (excluding bridging therapy and surgical procedures) or except where otherwise specified below:

   A. MPM

      - Patients must have received standard frontline therapy (e.g., platinum-based chemotherapy or checkpoint inhibitor therapy).

   B. Pancreatic Adenocarcinoma

      - Patients must have received frontline therapy with fluorouracilbased (e.g., FOLFIRINOX) or gemcitabine-based (e.g. gemcitabine ± Nabpaclitaxel) regimens for advanced disease or have elected not to pursue standard front-line therapy.

   C. Serous Ovarian Adenocarcinoma

      - Patients must have received a platinum-based regimen.
      - Or if they are carriers of a BRCA1/2 mutation they must have received at least a PARP inhibitor unless the treating physician determines that this is in the patient's best interest to enroll in the TC-510 trial, deems the patient unsuitable, or the patient elects not to receive PARP inhibitor therapy at that time. PARP inhibitors may be given as maintenance therapy with/without bevacizumab following a prior chemotherapy regimen and be considered a single line of therapy. To be considered maintenance therapy, the interval between prior chemotherapy completion and the initiation of the PARP inhibitor with/without bevacizumab must be less 8 weeks with no evidence of disease progression during that interval.

   D. Triple Negative Breast Cancer

      - Must have received at least 1 prior systemic anti-cancer therapy, including a PARP inhibitor for advanced TNBC with a germline mutation in BRCA1/2, and the combination of pembrolizumab with chemotherapy for advanced TNBC with combined positive score (CPS) ≥ 10.

   E. Colorectal Cancer

      - Must have progressed after at least 1 prior standard systemic anti-cancer therapy (e.g., oxaliplatin or irinotecan-based regimens ± bevacizumab), including cetuximab or panitumumab in patients with wild type KRAS or immune checkpoint inhibitors in patients with MSI-H or dMMR colorectal cancer.

   F. Non-small Cell Lung Cancer

      - Patients must have a pathologically confirmed (by histology) diagnosis for NSCLC, which is currently stage 3B or stage 4 disease.

- A patient with non-squamous NSCLC must have been tested for relevant EGFR mutations, ALK translocation or other actionable genomic aberrations (eg, ROS rearrangement, BRAF V600E mutation) for which FDA-approved targeted therapy is available and, if positive, the patient should have received at least 1 such therapy prior to study enrollment.
- Patients with *EGFR T790M* mutation must have received the FDA-approved tyrosine kinase inhibitor osimertinib.
- For patients without an actionable mutation, the patient must have received a currently approved frontline regimen (eg, immune checkpoint inhibitor-based therapy).

G. Cholangiocarcinoma

- Patients must have received at least standard systemic regimen for unresectable or metastatic disease (eg, gemcitabine- or 5-FU-containing regimens) or they must have elected not to pursue frontline standard of care therapy.

7. Patient has an Eastern Cooperative Oncology Group performance status 0 or 1.
8. Subject is fit for leukapheresis and has adequate venous access for the cell collection.
9. Subjects must have an absolute lymphocyte count (ALC) >350/μL prior to leukapheresis. Patients with an ALC < 350/μL would still be eligible to proceed with leukapheresis if they have a CD3 count of ≥150/μL. If the CD3 is <150/μL consultation with the Sponsor's Medical Monitor is required.

Leukapheresis Exclusion Criteria

A patient meeting any of the following exclusion criteria is not eligible to undergo leukapheresis:

1. Inability to follow the procedures of the study (e.g., due to language problems, psychological disorders, dementia, confusional state).
2. Patient has received or plans to receive the following therapy/treatment prior to leukapheresis:
   - Cytotoxic chemotherapy within 3 weeks of leukapheresis.
   - Corticosteroids: therapeutic doses of steroids must be stopped > 72 hours prior to leukapheresis. Use of inhaled steroids or topical cutaneous steroids is not exclusionary. Corticosteroid therapy at a pharmacologic dose (> 5 mg/day of prednisone or equivalent doses of other corticosteroids) and other immunosuppressive drugs should be avoided until 3 months after TC-510 administration, unless medically indicated to treat new toxicity. Physiological replacement doses of steroids (up to 5 mg/day of prednisone equivalent, or higher if warranted by the patient's BMI) may be allowed.
   - Immunosuppression: any other immunosuppressive medication must be stopped ≥ 4 weeks prior to leukapheresis, including (but not limited to) calcineurin inhibitors,

methotrexate or other chemotherapy drugs, mycophenolate, steroids (see above), rapamycin, thalidomide, or immunosuppressive antibodies such as rituximab, anti-tumor necrosis factor, anti-interleukin (IL) 6 or anti-IL6R.

- Use of an anti-cancer vaccine within 2 months in the absence of tumor response. The patient should be excluded if their disease is responding to an experimental vaccine given within 6 months.
- Any previous gene therapy using an integrating vector.
- Small molecule tyrosine kinase inhibitors (e.g., EGFR inhibitors), PARP inhibitors (e.g., olaparib, rucaparib, niraparib), or KRAS G12C inhibitors (e.g., sotorasib, adagrasib) within 72 hours.
- Any previous allogeneic hematopoietic stem cell transplant.
- Investigational treatment or clinical trial within 4 weeks or 5 half-lives of investigational product, whichever is shorter.
- Coronavirus disease 2019 (Covid-19; SARS-CoV-2) vaccine dose within 4 weeks from estimated date of leukapheresis, unless approved by TCR^2 Therapeutics Medical Monitor.

3. CNS disease/brain metastases:
   - Patients with leptomeningeal disease, carcinomatous meningitis, or symptomatic CNS metastases: patients are eligible if they have completed their treatment, have recovered from the acute effects of radiation therapy or surgery prior to study entry, and a) have no evidence of brain metastases post treatment or b) are asymptomatic, have discontinued corticosteroid treatment or anti-seizure medications for these metastases for at least 4 weeks and have radiographically stable CNS metastases without associated edema or shift for at least 3 months prior to study entry (note: prophylactic anti-seizure medications are acceptable; up to 5 mg per day of prednisone or equivalent will be allowed, or higher if warranted by the patient's BMI).
4. Patient has any other prior or concurrent malignancy with the following exceptions:
   - Adequately treated basal cell or squamous cell carcinoma (adequate wound healing is required prior to study entry).
   - In situ carcinoma of the cervix or breast, treated curatively and without evidence of recurrence for at least 12 months prior to the study.
   - Treated non-melanoma skin cancer.
   - Stage 0 or 1 melanoma completely resected at least 12 months prior to the study.
   - Successfully treated organ-confined prostate cancer with no evidence of progressive disease based on PSA levels and are not on active therapy.
   - A primary malignancy which has been completely resected and in complete remission for ≥ 5 years.

- Other malignancies deemed unlikely to be of clinical significance during TC-510 therapy by the Principal Investigator and as approved by the Sponsor.

5. Patient has active infection with HIV, hepatitis B virus, HCV, or HTLV as defined below:
   - Positive serology for HIV, HTLV-1, or HTLV-2.
   - Active hepatitis B infection as demonstrated by test for hepatitis B surface antigen. Patients who are hepatitis B surface antigen negative but are hepatitis B core antibody positive must have undetectable hepatitis B DNA and receive prophylaxis against viral reactivation.
   - Active hepatitis C infection as demonstrated by hepatitis C RNA test. Patients who are HCV antibody positive will be screened for HCV RNA by any reverse transcription PCR or branched DNA assay. If HCV antibody is positive, eligibility will be determined based on a negative screening RNA value.
6. Patient with pulse oximetry < 90% on room air or has one of the following will be excluded:
   - Radiographic evidence of underlying interstitial lung disease.
   - Active interstitial lung disease/pneumonitis or a history of interstitial lung disease/pneumonitis requiring therapy with systemic corticosteroids.
7. History of autoimmune or immune mediated disease such as multiple sclerosis, lupus, rheumatoid arthritis, inflammatory bowel disease, or small vessel vasculitis

6.2 Treatment Inclusion/Exclusion Criteria

A patient must meet the following treatment inclusion criteria to be eligible to proceed with first protocol defined therapy (e.g., lymphodepletion). Treatment eligibility will be formally assessed at Baseline. For retreatment with TC-510, the following criteria also apply.

Treatment Inclusion Criteria

A patient must meet the following inclusion criteria to be eligible to receive therapy on this study:

1. Patient (or legally authorized representative) has voluntarily agreed to participate by giving written informed consent in accordance with ICH GCP guidelines and applicable local regulations.
2. Patient has agreed to abide by all protocol required procedures including study-related assessments, and management by the treating institution for the duration of the study.
3. Patient is ≥ 18 years of age at the time the Informed Consent is signed.
4. Patient has a pathologically confirmed diagnosis of either epithelioid MPM, Serous Ovarian Adenocarcinoma (patients with Serous Ovarian, Fallopian Tube or Primary Peritoneal cancers will be permitted), Pancreatic Adenocarcinoma, Colorectal Cancer, TNBC, NSCLC, Cholangiocarcinoma.

- For patients with MPM, a pathology report documenting epithelioid histology should be provided. In the event epithelioid histology cannot be clearly confirmed via pathology report, the patient may proceed after discussion with the sponsor medical monitor or after confirming adequate MSLN expression.
- For patients with TNBC, treatment sites must provide proof of ER-negative and PR-negative status (in both cases defined as < 1%). Her2-negative status is defined as 0, 1+, or 2+ by IHC. If IHC 2+, a negative in situ hybridization (FISH, CISH, or SISH) test is required by local laboratory testing.

5. For patients with epithelioid MPM, confirmation of MSLN expression is not required prior to treatment. For Ovarian, Pancreatic, Colorectal, TNBC, NSCLC, and Cholangiocarcinoma only: Patient's tumor expresses MSLN on ≥ 50% of tumor cells with 1+, 2+, and/or 3+ intensity by immunohistochemistry at a TCR^2 Therapeutics designated central laboratory:
   - **Biopsy Timing:** For all patients, MSLN testing must be performed on a biopsy sample obtained within 6 months of the planned first protocol defined therapy. The pre-screening biopsy may fulfill the Baseline biopsy requirement if collected within 6 months of the start of treatment (provided there is adequate tissue for correlative testing). Otherwise, a new biopsy sample from within 6 months of the first protocol defined therapy should be submitted for MSLN testing. If deemed unsafe by the PI and/or the lesion is inaccessible, the Sponsor may allow the patient to proceed without the Baseline biopsy.
6. Patient has advanced (i.e., metastatic or unresectable) cancer. Unresectable refers to a tumor lesion in which clear surgical excision margins cannot be obtained without leading to significant functional compromise.
7. Patient has at least 1 lesion that meets evaluable and measurable criteria confirmed RECIST v 1.1. Patients who have received prior local therapy (including but not limited to embolization, chemoembolization, radiofrequency ablation, or radiation therapy) are eligible provided measurable disease falls outside of the treatment field or within the field and has shown ≥ 20% growth in size since post-treatment assessment.
8. The patient must not have required a paracentesis or thoracentesis within the preceding 4 weeks of TC-510 infusion nor be projected to require a paracentesis or thoracentesis within the next 8 weeks. Patients with catheters in place for frequent drainage may be allowed. Specific cases are to be discussed with the Medical Monitor and Sponsor.
9. Prior to TC-510 infusion, patients with MPM, Serous Ovarian Adenocarcinoma, TNBC, Colorectal, and NSCLC must have received at least 1 but no more than 5 systemic therapies for metastatic or unresectable disease. Patients with Pancreatic and Cholangiocarcinoma who are treatment naïve may be eligible for TC-510 therapy if they have elected not to pursue front-line therapy. Regardless of tumor type, patients must not exceed 5 prior lines of therapy (excluding bridging therapy and surgical procedures) or except where otherwise specified below:

   A. MPM

- Patients must have received standard frontline therapy (e.g., platinum-based chemotherapy or checkpoint inhibitor therapy).

B. Pancreatic Adenocarcinoma

- Patients must have received frontline therapy with fluorouracilbased (e.g., FOLFIRINOX) or gemcitabine-based (e.g. gemcitabine ± Nabpaclitaxel) regimens for advanced disease or have elected not to pursue standard front-line therapy.

C. Serous Ovarian Adenocarcinoma

- Patients must have received a platinum-based regimen.
- Or if they are carriers of a BRCA1/2 mutation they must have received at least a PARP inhibitor unless the treating physician determines that this is in the patient's best interest to enroll in the TC-510 trial, deems the patient unsuitable, or the patient elects not to receive PARP inhibitor therapy at that time. PARP inhibitors may be given as maintenance therapy with/without bevacizumab following a prior chemotherapy regimen and be considered a single line of therapy. To be considered maintenance therapy, the interval between prior chemotherapy completion and the initiation of the PARP inhibitor with/without bevacizumab must be less 8 weeks with no evidence of disease progression during that interval.

D. Triple Negative Breast Cancer

- Must have received at least 1 prior systemic anti-cancer therapy, including a PARP inhibitor for advanced TNBC with a germline mutation in BRCA1/2, and the combination of pembrolizumab with chemotherapy for advanced TNBC with combined positive score (CPS) ≥ 10.

E. Colorectal Cancer

- Must have progressed after at least 1 prior standard systemic anti-cancer therapy (e.g., oxaliplatin or irinotecan-based regimens ± bevacizumab), including cetuximab or panitumumab in patients with wild type KRAS or immune checkpoint inhibitors in patients with MSI-H or dMMR colorectal cancer.

F. Non-small Cell Lung Cancer

- Patients must have a pathologically confirmed (by histology) diagnosis of NSCLC, which is currently stage 3B or stage 4 disease.
- A patient with non-squamous NSCLC must have been tested for relevant EGFR mutations, ALK translocation or other actionable genomic aberrations (eg, ROS rearrangement, BRAF V600E mutation) for which FDA-approved targeted therapy is available and, if positive, the patient should have received at least 1 such therapy prior to study enrollment.
- Patients with *EGFR T790M* mutation must have received the FDA-approved tyrosine kinase inhibitor osimertinib.

- For patients without an actionable mutation, the patient must have received a currently approved frontline regimen (eg, immune checkpoint inhibitor-based therapy).

G. Cholangiocarcinoma

- Patients must have received at least 1 standard systemic regimen for unresectable or metastatic disease (eg, gemcitabine-or 5-FU-containing regimens) or they must have elected not to pursue frontline standard of care therapy.
- Patients must have measurable disease, defined as at least 1 lesion that can be accurately measured in at least 1 dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as $\geq$ 20 mm ($\geq$2 cm) with conventional techniques or as $\geq$ 10 mm ($\geq$1 cm) with computed tomography (CT) scan or magnetic resonance imaging (MRI).

10. Patient has an Eastern Cooperative Oncology Group performance status 0 or 1.
11. All patients must have undergone a rapid influenza diagnostic test and/or a respiratory viral panel (including coronavirus disease 2019 (Covid-19; SARS-CoV-2) as per Institutional guidelines within 14 days prior to the first protocol defined therapy. If the patient is positive for influenza, oseltamivir phosphate or zanamivir should be administered for 10 days (see Tamiflu® or Relenza® package insert for dosing). The patient must complete their 10--day treatment course prior to receiving TC-510.

    - For patients residing in the US, Canada, Europe and Japan, influenza testing is required during the months of October through May (inclusive).
    - For patients residing in the southern hemisphere such as Australia, influenza testing is required during the months of April through November (inclusive).
    - For patients with significant international travel, both calendar intervals above may need to be considered.

    In the event a patient tests positive for coronavirus disease 2019 (Covid-19; SARS-CoV-2), TC-510 infusion should be delayed until the patient is asymptomatic and deemed fit for infusion by the treating physician.
12. Patient has a left ventricular ejection fraction ≥ 45% as measured by resting echocardiogram/MUGA, with no clinically significant pericardial effusion.
13. FPCP must have a negative urine or serum pregnancy test (FPCP is defined as premenopausal and not surgically sterilized). FPCP must agree to use effective birth control or to abstain from heterosexual activity throughout the study, starting on the day of first dose of lymphodepleting chemotherapy through 12 months post TC-510 infusion. Effective contraceptive methods include intra-uterine device, oral or injectable hormonal contraception, or 2 adequate barrier methods (e.g., diaphragm with spermicide, cervical cap with spermicide, or female condom with spermicide). Spermicides alone are not an adequate method of contraception.

    Or

Male patients must be surgically sterile or agree to use a double barrier contraception method or abstain from heterosexual activity with a female of childbearing potential starting at the first dose of protocol-defined treatment and for 4 months thereafter or longer (if indicated in the country specific monograph/label for cyclophosphamide).

14. Patient must have adequate organ function as indicated by the laboratory values in the following table.

System	Laboratory Value
Hematological	
Absolute Neutrophil count (ANC)	≥ 1×10^9/L (without G-CSF support within 7 days prior to the first protocol defined therapy)
Absolute Lymphocyte count (ALC)	≥ 0.3×10^9/L
Platelets	≥ 100×10^9/L
Hemoglobin	≥ 90 g/L (without transfusion support within 7 days prior to the first protocol defined therapy)
Coagulation	
Partial Thromboplastin Time (PTT)	≤ 1.5 × upper limit of normal (ULN)
Prothrombin time (PT)	≤ 1.5× upper limit of normal (ULN)
Renal	
Calculated or measured creatinine clearance[a]	≥ 40 mL/minute
Hepatic	
Serum total bilirubin	≤ 2 × ULN (unless subject has documented Gilbert's Syndrome with direct bilirubin <35% of total bilirubin or unless secondary to bile duct obstruction by tumor)
Alanine aminotransferase (ALT)	≤ 2.5 × ULN or ≤ 5 × ULN if documented liver metastasis
Aspartate aminotransferase (AST)	≤ 2.5 × ULN or ≤ 5 × ULN if documented liver metastasis
[a] = Creatinine clearance will be calculated using the Cockcroft-Gault formula at Baseline: $\text{Cr clearance} = \frac{(140 - \text{age}) \times \text{weight kg}}{72 \times \text{serum creatinine mg/dl}} (\times 0.85 \text{ in females})$	

Treatment Exclusion Criteria

A patient meeting any of the following exclusion criteria is not eligible for participation in the treatment portion of this study:

1. Inability to follow the procedures of the study (e.g., due to language problems, psychological disorders, dementia, confusional state).
2. Known or suspected non-compliance, drug, or alcohol abuse.
3. Participation in another study with investigational drug within the 28 days or 5 half-lives of the drug, whichever is shorter, preceding and during the present study.
4. Patient is pregnant (or intends to become pregnant during the course of the study) or breastfeeding.
5. Patient has received or plans to receive the following therapy/treatment prior to the first protocol-defined therapy (unless otherwise specified):
   - Cytotoxic chemotherapy within 3 weeks of TC-510 infusion.

- Corticosteroids: therapeutic doses of steroids must be stopped at least 2 weeks prior to TC-510 infusion. Use of inhaled steroids or topical steroids is not exclusionary. Corticosteroid therapy at a pharmacologic dose (> 5 mg/day of prednisone or equivalent doses of other corticosteroids) and other immunosuppressive drugs should be avoided until 3 months after TC-510 administration, unless medically indicated to treat new toxicity. Physiological replacement doses of steroids (up to 5 mg/day of prednisone equivalent, or higher if warranted by the patient's BMI) may be allowed.
- Any other immunosuppressive medication must be stopped ≥ 4 weeks prior to first protocol-defined treatment, including (but not limited to) calcineurin inhibitors, methotrexate or other chemotherapy drugs, mycophenolate, steroids (see above), rapamycin, thalidomide, or immunosuppressive antibodies such as rituximab, anti-tumor necrosis factor, anti-interleukin (IL) 6 or anti-IL6R.
- Anti-PD-1 monoclonal antibody therapy must be stopped ≥ 4 weeks prior to first protocol-defined treatment.
- Use of an anti-cancer vaccine within 2 months in the absence of tumor response. The patient should be excluded if their disease is responding to an experimental vaccine given within 6 months.
- Any previous gene therapy using an integrating vector (except for TC-510 in the case of retreatment).
- Small molecule tyrosine kinase inhibitors (e.g., EGFR inhibitors), PARP inhibitors (e.g., olaparib, rucaparib, niraparib), or KRAS G12C inhibitors (e.g., sotorasib, adagrasib) within 72 hours.
- Any previous allogeneic hematopoietic stem cell transplant.
- Investigational treatment or clinical trial within 4 weeks or 5 half-lives of investigational product, whichever is shorter.
- Radiotherapy to the target lesions within 4 weeks prior to lymphodepleting chemotherapy. A non-target lesion with unequivocal progression may be considered a target lesion regardless of time from last radiotherapy dose. NOTE: There is no washout period for palliative radiation to non-target lesions.
- Hepatic radiation, chemoembolization, and/or radiofrequency ablation within 4 weeks.
- Immune therapy (e.g., monoclonal antibody therapy, checkpoint inhibitors) within 4 weeks.
- Coronavirus disease 2019 (Covid-19; SARS-CoV-2) vaccine dose within 4 weeks of first protocol defined treatment, unless approved by TCR^2 Therapeutic's Medical Monitor.

6. Toxicity from previous anti-cancer therapy that has not recovered to ≤ grade 1 (except for non-clinically significant toxicities, e.g., alopecia, vitiligo). Patients with grade 2 toxicities that are deemed stable or irreversible (e.g., peripheral neuropathy) may be eligible.

7. History of allergic reactions attributed to compounds of similar chemical or biologic composition to fludarabine, cyclophosphamide, or other agents used in the study.
8. History of autoimmune or immune mediated disease such as multiple sclerosis, lupus, rheumatoid arthritis, inflammatory bowel disease, or small vessel vasculitis.
9. Major surgery (other than diagnostic surgery) within 4 weeks prior to first protocol defined therapy, minor surgery including diagnostic surgery within 2 weeks (14 days) excluding central intravenous port placements and needle aspirate/core biopsies. Radio frequency ablation or transcatheter arterial chemoembolization within 6 weeks prior to first protocol defined therapy.
10. CNS disease/brain metastases:
    - Patients with leptomeningeal disease, carcinomatous meningitis, or symptomatic CNS metastases: patients are eligible if they have completed their treatment, have recovered from the acute effects of radiation therapy or surgery prior to study entry, and a) have no evidence of brain metastases post treatment or b) are asymptomatic, have discontinued corticosteroid treatment or anti-seizure medications for these metastases for at least 4 weeks and have radiographically stable CNS metastases without associated edema or shift for at least 3 months prior to study entry (note: prophylactic anti-seizure medications are acceptable; up to 5 mg/day of prednisone or equivalent will be allowed; higher doses may be allowed if warranted due to patient BMI).
11. Patient has any other prior or concurrent malignancy with the following exceptions:
    - Adequately treated basal cell or squamous cell carcinoma (adequate wound healing is required prior to study entry).
    - In situ carcinoma of the cervix or breast, treated curatively and without evidence of recurrence for at least 12 months prior to enrollment.
    - Treated non-melanoma skin cancer.
    - Stage 0 or 1 melanoma completely resected at least 12 months prior to the study.
    - Successfully treated organ-confined prostate cancer with no evidence of progressive disease based on PSA levels and are not on active therapy.
    - A primary malignancy which has been completely resected and in complete remission for ≥ 5 years.
    - Malignancies deemed unlikely to be of clinical significance during TC-510 therapy by the Principal Investigator and as approved by the Sponsor.
12. Patient has an electrocardiogram showing a clinically significant abnormality at screening or showing a QTc interval > 450 msec in males and > 470 msec in females (> 480 msec for patients with bundle branch block). Either Fridericia's or Bazett's formula may be used to correct the QT interval.
13. Patient has uncontrolled intercurrent illness including, but not limited to:

- Ongoing or active infection; eg, sepsis, prolonged unresolved bacterial cholangitis with destruction of bile duct branches (eg, after endoprosthesis insertion), or 2 or more cholangitis in the last 6 months.
- Clinically significant cardiac disease defined by congestive heart failure New York Heart Association class 3 or class 4.
- Clinically significant cardiac disease defined by congestive heart failure New York Heart Association class 3 or class 4.
- Uncontrolled clinically significant arrhythmia.
- Acute coronary syndrome (angina or myocardial infarction), stroke, or peripheral vascular event in the last 6 months.
- Serious thrombotic event in the last 6 months
- Interstitial lung disease (patients with existing pneumonitis as a result of radiation are not excluded; however, patients must not be oxygen dependent as demonstrated by oxygen saturation < 92% on room air).
- Liver cirrhosis or primary sclerosing cholangitis

14. Patient has active infection with HIV, hepatitis B virus, HCV, or HTLV as defined below:
    - Positive serology for HIV, HTLV-1, or HTLV-2.
    - Active hepatitis B infection as demonstrated by test for hepatitis B surface antigen. Patients who are hepatitis B surface antigen negative but are hepatitis B core antibody positive must have undetectable hepatitis B DNA and receive prophylaxis against viral reactivation.
    - Active hepatitis C infection as demonstrated by hepatitis C RNA test. Patients who are HCV antibody positive will be screened for HCV RNA by any reverse transcription PCR or branched DNA assay. If HCV antibody is positive, eligibility will be determined based on a negative screening RNA value.

6.3 Concomitant Treatments

6.3.1 Study Treatment and Concomitant Therapy

During the course of the study, investigators may prescribe any concomitant medications or treatment deemed necessary to provide adequate supportive care except those medications listed in the excluded medication section 6.5.2. All concurrent therapies, including medications and supportive therapy (e.g., intubation, dialysis, and blood products), should be recorded from the date the patient is enrolled into the study (i.e., completion of a successful leukapheresis) through 3 months after receiving TC-510. After 3 months post TC-510 infusion, only targeted concomitant medication will be collected, including immunosuppressive drugs, anti-infective drugs, vaccinations, and any therapy for the treatment of the patient's malignancy for 1 year beyond disease progression. After the period of 1 year post-progression has ended, the following

targeted concomitant medications will be collected for the remainder of the patient's time on trial, up to approximately 5 years: mutagenic treatments including cytotoxic drugs, radiation therapy and antineoplastic therapy (including stem cell transplant). Specific concomitant medication collection requirements and instructions are included in the case report form (CRF) completion guidelines.

6.3.2 Prohibited Concomitant Medications

In general, medications that might interfere with the evaluation of the investigational product should not be used unless absolutely necessary. Medications in this category include (but are not limited to):

- Immunosuppressive agents.
- Corticosteroid anti-inflammatory agents including prednisone, dexamethasone, solumedrol, and cyclosporine. Immunosuppressive doses of systemic corticosteroids. Participants are permitted the use of topical, ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Adrenal replacement steroid doses > 10 mg daily prednisone are permitted. A brief (less than 3 weeks) course of corticosteroids for prophylaxis (e.g., contrast dye allergy) or for treatment of non-autoimmune conditions (e.g., delayed-type hypersensitivity reaction caused by a contact allergen) is permitted.
- Any concurrent systemic anti-neoplastic therapy (i.e., chemotherapy, hormonal therapy, immunotherapy, radiation, or standard or investigational agents for treatment of the disease under study), except as needed for treatment of disease progression.
- Any complementary medications (e.g., herbal supplements or traditional Chinese medicines) intended to treat the disease under study. Such medications are permitted if they are used as supportive care.
- Any live/attenuated vaccine (e.g., varicella, zoster, yellow fever, rotavirus, oral polio and MMR) during treatment and until 100 days post last dose.

If permissibility of a specific medication/treatment is in question, please contact the TCR^2 Therapeutics Medical Monitor on the title page.

6.4 Study Restrictions

6.4.1 Contraception

There are no data regarding the safety of TC-510 during pregnancy or lactation in humans. Female patients who are pregnant, intending to become pregnant, or breast feeding are excluded from this study.

Female and male patients of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively. The required duration of contraception is described below:

- Female patients of childbearing potential must agree to use an effective method of contraception starting at the first dose of chemotherapy for at least 12 months thereafter, for 4 months after the TC-510 gene modified cells are no longer detected in the blood.
- Male patients must agree to use an effective method of contraception starting at the first dose of chemotherapy and for 4 months thereafter or longer (if indicated in the country specific monograph/label for cyclophosphamide).

Female patient of childbearing potential is defined as premenopausal and not surgically sterilized.

Either 1 highly effective method of contraception or use of 2 adequate barrier methods is required. Highly effective methods of contraception include intra-uterine device, injectable hormonal contraception, oral contraception, or adequate barrier methods (e.g., diaphragm with spermicide, cervical cap with spermicide, or female condom with spermicide [spermicides alone is not an adequate method of contraception]).

Abstinence (relative to heterosexual activity) can be used as the sole method of contraception if it is consistently employed as the patient's preferred and usual lifestyle and if considered acceptable by local regulatory agencies and IRBs. Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc) and withdrawal are not acceptable methods of contraception.

6.5 Discontinuation of Patients from Treatment

Patients have the right to withdraw from the study at any time and for any reason without prejudice to their future medical care by the physician or at the institution.

However, the Investigator must make every reasonable effort to keep each patient on study for the whole duration of the trial. In cases where the patient is deemed "lost to follow-up," the Investigator or designee must make every effort to regain contact with the patient; e.g., 3 documented attempts, 1 of which must be a certified letter to the patient's last known mailing address or local equivalent methods. These contact attempts should be documented in the patient's medical records. Should the patient continue to be unreachable, only then will he/she be considered to have withdrawn from the study with the primary reason as "lost to follow-up."

Withdrawal of full consent for a study means that the patient does not wish to receive further protocol required therapy or undergo procedures and the patient does not wish to continue further study follow-up. Patient data collected up to withdrawal of consent will be retained and included in the analysis of the study, and where permitted, publicly available data (death records) can be included after withdrawal of consent (FDA *Guidance for Industry: Guidance for Sponsors, Clinical Investigators, and IRBs Data Retention When Patients Withdraw from FDA-Regulated Clinical Trials* [October 2008]). The Investigator is to discuss with the patient appropriate procedures for withdrawal from the study.

If a patient withdraws consent, all final end-of-study assessments should be performed, if possible, on the day the decision is made to take the patient off-study or as soon as possible thereafter. All the results of the evaluations and observations, together with a description of the reasons for study withdrawal, must be recorded in the medical records and CRFs.

As part of the study, sites may be asked to conduct searches of public records, such as those establishing survival status, if available, to obtain survival data for any patient for whom the survival status is not known. Sites may be also asked to retrieve autopsy reports to confirm status of disease at the time of death.

The Investigator and/or Sponsor can also decide to withdraw a patient from the investigational product and/or other protocol-required therapies, protocol procedures, or the study as a whole or at any time prior to study completion.

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

- Clinical or laboratory adverse event, either serious or non-serious, at the discretion of the Investigator
- Patient request/non-compliance
- Pregnancy
- Product not available
- Lost to follow-up
- Death
- Decision by Sponsor
- Patient withdrawal of consent from further follow-up
- Investigator decision

6.6 Post-Infusion Follow-Up

Post-infusion follow-up will be broken up into 2 categories: Active Follow-up and Long-term Follow-up. Patients will be followed for approximately 2 years post-infusion in Active Follow-up. When a patient completes approximately 2 years (Week 108) of Active Follow-up or in the event a patient is removed from Active Follow-up prior to reaching 2 years (Week 108) for any reason (eg, radiographically confirmed or clinically assessed disease progression, adverse events, investigator decision, patient decision), they will be transitioned to Long-term Follow-up and will be followed per Appendix A: PART 2—Long-term Follow-up Schedule of Events for approximately 5 years post the most recent TC-510 infusion.

The 2 types of protocol defined post-infusion follow-up are outlined below:

Active Follow-up: Includes all patients being actively followed post-infusion with visits, efficacy assessments (scans), blood draws, and all other assessments as specified in Appendix A: Part 1—Schedule of Events for Study TCR2-21-01

Long Term Follow-up (LTFU): Includes all patients who have come off Active Follow-up for any reason as noted above, as well as patients who have completed Active Follow-up by completing Week 108. Patients in LTFU will be followed for approximately 5 years post the most recent TC-510 infusion and assessments will be performed according to Appendix A: PART 2 – Long-term Follow-up Schedule of Events.

Patients treated with TC-510 will be followed on this study for up to approximately 5 years and will be offered to transition to a dedicated LTFU protocol and monitored for 15 years from time of the most recent TC-510 infusion for observation of delayed adverse events in accordance FDA and European Medicines Agency requirements for gene therapy clinical trials *(FDA Guidance 'Long Term Follow-Up After Administration of Human Gene Therapy Products Guidance for Industry. U.S Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research,' [January2020])*

During all post-infusion follow-up, patients will be monitored for the emergence of any of the following new clinical conditions reported or observed and the action taken will be reported to the Sponsor:

- New malignancies (and anti-cancer therapies when available).
- New incidence or exacerbation of a pre-existing neurological disorder.
- New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder.
- Opportunistic and/or serious infections.
- Unanticipated illness or hospitalization deemed related to gene modified cell therapy.

Reporting criteria for adverse events related to gene therapy that occur in post-infusion follow up are described in section 10.2.

7 STUDY TREATMENT AND INVESTIGATIONAL PRODUCT

The investigational product for this study is named TC-510. The conditioning chemotherapy regimen to be administered in this study will consist of a combination of fludarabine and cyclophosphamide. Study treatment refers to all protocol required therapies.

7.1 Study Treatment: Leukapheresis and TC-510 Manufacturing

TC-510 is an engineered autologous ACT. Patients who complete pre-screening procedures and who meet leukapheresis eligibility criteria as defined in section 6 will be eligible to undergo leukapheresis to obtain starting material for the manufacture. A large-volume non-mobilized PBMC collection will be performed (12- to 15-liter apheresis) according to institutional standard

procedures and Investigational Product Manual (IPM) for collection of the starting material. The goal will be to collect approximately 5 to 10 × 10^9 total PBMCs (minimum collection goal 1.5 × 10^7 PMBC/kg). The leukapheresed cells will then be frozen and transported either the same day or overnight to the TCR^2 Therapeutics approved Cell Processing Facility (CPF) as described in the Investigational Product Manual (IPM). Sites should refer to the IPM for all details surrounding collection, apheresis product testing, cryopreservation, and shipping. In cases where the minimum number of PBMCs are not collected or the manufacturing of sufficient TC-510 is not successful, a second leukapheresis may be performed. Citrate anticoagulant should be used during the procedure and prophylaxis against the adverse effects of this anticoagulant (e.g., $CaCl_2$ infusions) may be employed at the Investigator's discretion.

Upon arrival at the CPF, each patient's leukapheresed product will be processed to enrich for the T cells containing PBMC fraction. T cells will be then stimulated to expand and transduced with a lentiviral vector to introduce the TC-510 transgene to obtain TC-510. Transduced T cells (i.e., TC-510) are then expanded and cryopreserved to generate the investigational product per CPF Standard Operating Procedures (SOPs). Once the TC-510 product has passed release tests, the CPF will ship the product back to the treating facility.

Patients must meet treatment eligibility at the Baseline visit). TC-510 will be administered first during the phase 1 portion of the study (i.e., dose-escalation phase) at the initial dose of 50 × 10^6 transduced T cells (i.e., DL0). The dose-escalation phase will evaluate varying TC-510 cell doses: (section 12). During the phase 2 portion of the study, patients will receive TC-510 at the RP2D. A dose range of ± 15% of the target dose may be administered.

TC-510 will be supplied cryopreserved in cryostorage bags. The product in the bag will be opaque, with cream to white color. The cryostorage bags containing TC-510 will arrive frozen in a liquid nitrogen dry shipper. The bags must be stored in vapor phase of liquid nitrogen and the product remains frozen until the patient is ready for treatment to assure viable live autologous cells are administered to the patient. Several inactive ingredients are added to the product to assure viability and stability of the live cells through the freezing, thawing, and infusion process. Each bag contains a patient-specific product, and the intended patient will be identified by patient ID number. The product should be thawed and administered to the patient as specified in the IPM. The product must not be thawed until the patient is ready for the infusion. In case of accidental overdose, treatment should be supportive. Corticosteroid therapy and/or tocilizumab may be considered if any dose is associated with severe toxicity. If any problems related to the use of TC-510 or any products that support the management of TC-510 (e.g., cryostorage bags, patient identification labels) required in this study are identified, please contact TCR^2 Therapeutics to file a complaint.

7.2 Bridging Therapy

During the time between leukapheresis and starting protocol-defined therapy, patients may receive therapy aimed at maintaining control over or stabilizing his or her disease. The therapy used during this time will be considered a "bridging" therapy aimed at bridging the gap between prior treatment and treatment with TC-510. The protocol will allow for patients to receive

appropriate therapies for the indication and disease status. The patients must be off-treatment for specific timeframes based on the product mechanism as described in the eligibility criteria.

7.3 Lymphodepleting Chemotherapy

Lymphodepleting chemotherapy will be supplied by the investigative site unless otherwise noted. Sites should refer to the current product label for guidance on packaging, storage, preparation, administration, and toxicity management associated with the administration of both agents.

Clinical sites are allowed to follow institutional guidelines for administration, hydration, and monitoring parameters pertaining to the chemotherapy agents described in this protocol. In the absence of institutional standards, suggested guidelines are detailed below.

Patients will receive a non-myeloablative chemotherapy regimen consisting of fludarabine 30 mg/m^2/day on days -7 through -4 (i.e., 4 doses) and cyclophosphamide 600 mg/m^2/day on days -6 through -4 (ie, 3 doses) (Table 2).

For retreatment (phase 1 RP2D expansion cohort and phase 2 only), lymphodepletion chemotherapy will be given only on days -7 through -5; there will be no chemotherapy given on day -4. The dosing will consist of fludarabine 30mg/m^2/day on days -7 through -5 (i.e., 3 doses) and cyclophosphamide 600mg/m^2/day on days -6 through -5 (i.e., 2 doses).

Two alternative lymphodepleting chemotherapy regimens exist in the event of fludarabine shortage. **Sponsor approval is required prior to proceeding with an alternative regimen**.

- Alternative 1: bendamustine 100 mg/m$^{2/}$day IV on days -5 and -4 (ie, 2 doses).
  - In the event of retreatment and if bendamustine must be used, the regimen should be abbreviated to 70 mg/m$^{2/}$day IV on days -5 and -4 (ie, 2 doses).
- Alternative 2: Cyclophosphamide 600 mg/m^2/day IV on days -6, -5, and -4 (ie, 3 doses) and oxaliplatin 130 mg/m^2 IV on day -6 (ie, 1 dose).

In the event the patient has an unforeseeable delay or missed dosing day for this lymphodepleting regimen, the missed dose will be administered, and the TC-510 dosing day will be moved back, maintaining the last dosing day at day -4 relative to TC-510 infusion (or day -5 for retreatment due to the abbreviated lymphodepleting chemotherapy regimen utilized in retreatment). The intent of this regimen is to induce lymphocyte depletion to promote an optimal environment for the expansion of TC-510 in vivo.

Table 2. TCR2-21-01 Treatment Schema

Day	Lymphodepleting Chemotherapy				Recommended Supportive Medication[d]
	Drug[a,b]	Dose	Route	Administration[c]	
-7	Fludarabine	30 mg/m^2	IV	In 50-100 mL 0.9% NaCl (concentration ≤ 1 mg/mL) over 30 minutes	**Hydration:** Instruct patients to drink plenty of liquids during, and for 24 hours following chemotherapy (approximately 2 liters/24 hours). Patients should be kept well-hydrated but closely monitored to prevent fluid overload. Ensure adequate antiemetic provision prior to commencing cyclophosphamide infusions. **Mesna:** Given according to Institutional guidelines or recommendations described in section 7.3. **G-CSF:** Start 24 hours post final cyclophosphamide until resolution of neutropenia if deemed necessary by the treating physician[f]. **Cell therapy premedication:** Approximately 30-60 minutes prior to the TC-510 infusion, patients will be premedicated against potential infusion reactions with antihistamines and acetaminophen.
-6	Fludarabine	30 mg/m^2	IV	In 50-100 mL 0.9% NaCl (concentration ≤ 1 mg/mL) over 30 minutes	
	Cyclophosphamide	600 mg/m^2		In 200-500 mL 0.9% NaCl over 1-2 hours	
-5	Fludarabine	30 mg/m^2	IV	In 50-100 mL 0.9% NaCl (concentration ≤ 1 mg/mL) over 30 minutes	
	Cyclophosphamide	600 mg/m^2		In 200-500 mL 0.9% NaCl over 1-2 hours	
-4	Fludarabine	30 mg/m^2	IV	In 50-100 mL 0.9% NaCl (concentration ≤ 1 mg/mL) over 30 minutes	
	Cyclophosphamide	600 mg/m^2		200-500 mL 0.9% NaCl over 1-2 hours	
-3	No treatment				
-2	No treatment				
-1	No treatment				
0	TC-510 infusion[e]				

G-CSF = granulocyte-colony stimulating factor; IV = intravenous.

[a] = Fludarabine dose will be adjusted in renal impairment as described in section 7.3.1.

[b] = On days -6 through -4 ("cyclophosphamide days"), 1 L of 0.9% NaCl will be given IV prior to lymphodepletion, followed by fludarabine and cyclophosphamide. An additional 1 L of 0.9% NaCL will be given IV at the completion of the cyclophosphamide infusion.

[c] = Instructions for administration of fludarabine and cyclophosphamide are given as guidance and may be modified according to institutional practice.

[d] = Doses of supportive medications listed are recommendations and may be adjusted per institutional practice.

[e] = The TC-510 infusion is described in section 7.1.

[f] = Long-acting (pegylated) G-CSF may be given instead of short acting G-CSF according to institutional standard practice or physician's preference. If pegylated G-CSF is administered, give one dose 24 hours after the last chemotherapy dose is administered.

7.3.1 Fludarabine

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

Refer to the most recent version of the package insert for specific details surrounding the administration of fludarabine.

The dose of fludarabine will be adjusted for patients with renal dysfunction as shown in Table 3. For patients ≥ 65 years of age, the measured creatinine clearance value at baseline must be used to adjust the fludarabine dose. Clinical sites may follow their institutional standard of care practices to round down doses of fludarabine to the closest vial as long as the final fludarabine dose is within 10% of the calculated planned dose (i.e, fludarabine 30 mg/m^2 or 20 mg/m^2 for patients with renal dysfunction). This dose adjustment is permitted only when required by the institution for fludarabine shortage. **Sponsor approval must be obtained for each patient prior to initiating this fludarabine dose adjustment.**

Table 3. Fludarabine Dose Adjustment for Renal Impairment

Creatinine Clearance	Fludarabine Dose
≥ 80 mL/minute	30 mg/m^2
40-79 mL/minute	20 mg/m^2

7.3.2 Cyclophosphamide and Mesna

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

Refer to the most recent version of the package insert for specific details surrounding the administration of cyclophosphamide.

Mesna will be given to cover the duration of cyclophosphamide chemotherapy according to institutional guidelines. The London Cancer 2014, guideline for high dose cyclophosphamide is provided as an example: (http://www.londoncancer.org/media/75898/140214-London-Cancer-Mesna-Guideline-v1.pdf).

7.3.3 Bendamustine

Bendamustine is a nitrogen mustard derivative alkylating agent and is active against resting as well as dividing cells. Bendamustine is used for the treatment of patients with Chronic Lymphocytic Leukemia (CLL) and indolent B-cell non-Hodgkin's lymphoma (NHL). Refer to the most recent version of the package insert for specific details surrounding the administration of bendamustine.

7.3.4 Oxaliplatin

Oxaliplatin is a metal salt derived alkylating agent and is approved to treat colon and colorectal cancers. Refer to the most recent version of the package insert for specific details surrounding the administration of oxaliplatin.

7.4 TC-510 Infusion

After the completion of lymphodepleting chemotherapy, the Investigator should ensure that the following safety criteria are met prior to TC-510 infusion:

- The patient completed all days of lymphodepleting chemotherapy
- No significant worsening of any clinical condition since the start of lymphodepleting chemotherapy
- No suspected or active systemic infection
- No onset of fever ≥ 38ºC / 100.4ºF since the last dose of lymphodepleting chemotherapy
- No requirement for supplemental O_2 to keep O_2 saturation on room air (SaO_2) > 92%
- No presence of progressive radiographic abnormalities (if chest X-ray was performed)
- The Investigator deems that it is safe for the patient to proceed with TC-510 infusion

If per the assessment of the Investigator, the patient does not meet any of the above criteria, the TC-510 infusion should be held until consultation with the Sponsor/Medical Monitor.

On day 0 of the study, patients participating in the phase 1 portion of the study will receive TC-510 within the dose range of 50×10^6 to 200×10^6 transduced cells by IV infusion. The recommended dose for patients participating in the phase 2 portion will be determined at the end of the dose-escalating phase 1.

Please follow all instructions listed in the IPM for proper preparation and infusion of the product. TC-510 is a patient-specific product. Upon receipt, verification that the product and patient-specific labels match the intended patient information is essential. Do not infuse the product if the information on the patient-specific label does not match the intended patient. Prior to infusion, 2 clinical personnel in the presence of the patient will independently verify and confirm that the information on the infusion bag label is correctly matched to the patient, in accordance with institutional practice for the administration of cell products.

Thirty to 60 minutes prior to cell infusion, patients will be premedicated against potential infusion reactions with antihistamines and acetaminophen (paracetamol) as per institutional practice. Steroids must not be administered as premedication for T cell infusion due to their lymphotoxic potential against the TC-510 product.

TC-510 must not be thawed until immediately prior to infusion. The product can be thawed either in a water bath at the patient's bedside or with a device such as GE ViaThaw in a centralized facility, according to institutional standard procedures. The cells must be infused without delay and, if thawed centrally, must be transported to the patient by appropriately trained clinical staff, to preserve the chain of custody. The product must not be washed or otherwise processed.

It is expected that the infusion will commence within approximately 10 minutes of thawing (or within 10 minutes of receipt if thawed centrally) and complete within 45 minutes of thawing (or receipt from centralized thawing facility) to minimize exposure of the TC-510 product to cryoprotectant. If the cells are provided in multiple bags, the additional bag(s) must not be thawed until half of the first bag has been infused without reaction.

If, after thawing, the infusion bag is noted to be damaged or leaking, both the Principal Investigator and Sponsor must be notified, and the product should not be infused.

TC-510 is to be administered using a dual spike infusion set by gravity over 15 to 30 minutes (in the absence of reaction) via non-filtered tubing. The bag should be gently agitated during infusion to avoid cell clumping. **Infusion pumps must not be used.**

For administration of TC-510, 100 to 250 mL of 0.9% NaCl should be connected to the second lumen of the infusion set, used to prime the line, and then the lumen should be closed. On completion of the infusion of a cell bag, the main line should be closed and approximately 50 mL NaCl should be transferred into the cell bag and then infused to minimize cell loss. This process should be repeated for each bag if multiple bags are provided.

On completion of the cell infusion, the set should be flushed using additional saline from the attached bag. In the event institutional practice requires a single spike infusion set, the line must be flushed with 0.9% NaCl once the infusion is complete. In the event of an adverse reaction to TC-510 infusion, the infusion rate should be reduced, and the reaction managed according to institutional standard procedures.

Corticosteroids should be avoided unless medically required.

In the event a patient develops a febrile episode following the infusion, appropriate cultures and medical management should be initiated, with attention to the initiation of empirical antibiotic treatment in the case of febrile neutropenia.

The infusion of the TC-510 product may be delayed by up to 5 days (or longer if agreed upon after consultation with the Sponsor's Medical Monitor) should a patient develop significant chemotherapy-related complications, if it is judged to be in the patient's best interest by the Investigator.

Cytopenias alone should not be a reason to delay TC-510 infusion unless complications (e.g., uncontrolled infection or bleeding) are present.

The volume of TC-510 product infused, the thaw start and stop time, and the infusion start and stop time of each bag (if applicable) will all be noted in the patient medical record and eCRF.

Vital signs will be recorded within 10 minutes prior to the infusion (±3 minutes) and at 5 minutes (± 3 minutes), 15 minutes (± 3 minutes), 30 minutes (± 5 minutes), 1 hour (± 5 minutes), 1.5 hours (± 5 minutes), 2 hours (± 5 minutes), and 4 hours (± 5 minutes) after the infusion has

started. For the phase 1 portion of the study, patients should be observed overnight following the infusion of TC-510.

The same infusion process will be followed for retreatment with TC-510 (phase 1 RP2D expansion cohort and phase 2 only).

7.4.1 Subsequent Therapy

Subsequent therapy for a patient's disease such as non-study specified chemotherapy, immunotherapy, targeted agents, as well as stem cell transplant and radiation therapy must be collected in the CRFs as per the CRF completion guidelines.

8 TOXICITY MANAGEMENT

8.1 Infection Prophylaxis

Patients should receive prophylaxis for infection with Pneumocystis jiroveci pneumonia, herpes virus, varicella zoster, and fungal infections according to National Comprehensive Cancer Network guidelines or standard institutional practice.

The Sponsor's recommendations for prophylaxis are as follows:

- Pneumocystis jiroveci pneumonia: daily single strength trimethoprim sulfamethoxazole, for 1 year.
- Herpes Simplex and Varicella Zoster: acyclovir (800 mg twice daily) or valacyclovir (500 mg twice daily) for 1 year.
- Cytomegalovirus (CMV): patients will be screened for CMV seropositivity at Baseline. If CMV viremia is detected at Baseline, treatment should be initiated prior to lymphodepleting chemotherapy. Patients will be monitored for CMV as detailed in the schedule of events. If CMV viremia is documented, an infectious disease specialist should be consulted and treatment initiated (if required) according to institutional practice. Recommended regimens include ganciclovir-based therapy if absolute neutrophil count (ANC) $\geq 1.0 \times 10^9$/L, and foscarnet if ANC $< 1.0 \times 10^9$/L.
- Testing for infectious disease markers is required only at the Leukapheresis Eligibility visit and does not need to be repeated at Baseline to satisfy the inclusion / exclusion criteria. Testing performed within 3 weeks of leukapheresis is acceptable. Patients who are hepatitis B surface antigen negative but are hepatitis B core antibody positive must have undetectable hepatitis B DNA. Patients who are HCV antibody positive will be screened for HCV RNA by any reverse transcription PCR (RT PCR) or branched DNA (bDNA) assay.

8.2 Tumor Lysis Syndrome

All patients with significant tumor burden and without a contraindication should receive tumor lysis syndrome prophylaxis (e.g., allopurinol) as per institutional guidelines prior to TC-510 infusion. Prophylaxis should be discontinued when the risk of tumor lysis has passed.

8.3 Cytokine Release Syndrome

Cytokine release syndrome has been described with therapies that activate T lymphocytes, such as bispecific T cell engagers (e.g., blinatumomab) and ACT (e.g., CAR T cell therapy). Cytokine release syndrome results from the massive release of cytokines from cells targeted by therapeutics, immune effector cells recruited to the tumor area, and patient's immune cells activated during this process. Cytokine release syndrome is associated with a variety of clinical signs and symptoms, which can be life-threatening, including cardiac, gastrointestinal, laboratory (disseminated intravascular coagulation, renal and hepatic abnormalities), neurological, respiratory, skin, vascular (hypotension), and constitutional (fever, rigors, headaches, malaise, fatigue, arthralgia, nausea, and vomiting). The goal of CRS management is to prevent life-threatening conditions while preserving the potential benefit of TC-510 induced antitumor activity.

To further harmonize CRS grading criteria, a consensus grading system was published and will be used to collect additional grading on CRS events (Table 4).

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

Note: Organ toxicities associated with CRS may be graded according to CTCAE v 5.0, but they do not influence CRS grading.
ASTCT = American Society for Transplantation and Cellular Therapy; BiPAP = bilevel positive airway pressure; CPAP = continuus positive airway pressure; CRS = cytokine release syndrome; CTCAE = Common Terminology Criteria for Adverse Events.

[a] = Fever is defined as temperature ≥ 38°C not attributable to any other cause. In patients who have CRS and then receive antipyretic or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In such cases, CRS grading is driven by hypotension and/or hypoxia.

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

[c] = Low-flow nasal cannula is defined as oxygen delivered at ≤ 6 L/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal canula is defined as oxygen delivered at > 6L/minute

A recommended CRS management algorithm according to grade is illustrated in Table 5. The algorithm has been further adapted from CTCAE for use with immunotherapy and should be implemented in accordance with institutional guidelines. The diagnosis of CRS is clinical, and it is supported by the exclusion of infection as well as the presence of increased cytokine levels and other biomarkers. If CRS is suspected, the following labs, in addition to assessment for infection, should be measured approximately every 24-48 hours until symptoms are improving or an alternative diagnosis is confirmed:

- Cytokines (central and local)
- C-reactive protein (CRP)
- Ferritin
- Coagulation parameters including prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), fibrinogen, D-dimer

Table 5. Management Guidelines for Cytokine Release Syndrome

Grade	Clinical Presentation for Grading Assessment	Management Guidelines
1	Constitutional symptoms not life-threatening (eg, fever, nausea, fatigue, headache, myalgia, malaise)	• Vigilant supportive care[a] • Assess for infection and treat[b]
2	Symptoms require and respond to moderate intervention (hypotension responds to fluids or one low dose pressor, hypoxia responds to < 40% O_2, and/or grade 2 organ toxicity)	• Monitor cardiac and other organ function • Vigilant supportive care[a] • Assess for infection and treat[b] • Treat hypotension with fluid and pressors • Administer O_2 for hypoxia • Consider tocilizumab 8mg/kg iv (maximum 800 mg) ± corticosteroids[c] in patients with extensive co-morbidities or of older age
3	Symptoms require and respond to aggressive intervention (hypotension requires multiple pressors or high-dose pressors, hypoxia requires ≥ 40% O_2, grade 3 organ toxicity or grade 4 transaminitis)	• Monitor patient very closely for cardiac and other organ dysfunction. Most likely will require monitoring in an ICU. • Vigilant supportive care[a] • Assess for infection and treat[b] • Treat hypotension with fluid and pressors. • Administer O_2 for hypoxia. • Administer tozilizumab 8mg/kg iv (maximum 800 mg) ± corticosteroids[c]
4	Life-threatening symptoms Grade 4 organ toxicity (excluding transaminitis)	• Manage patient in ICU • Intensive supportive care including mechanical ventilation, fluids, pressors, antibiotics, and other measures as required • Administer tocilizumab 8mg/kg iv (maximum 800 mg) ± corticosteroids[c]
5	Death	Not applicable

ICU = intensive care unit; IV = intravenous(ly); IL-1R = interleukin 1R; TNF-α = tumor necrosis factor alpha.
[a] = Supportive care includes monitor fluid balance, maintain adequate hydration, and blood pressure.
[b] = Assessment and treatment to include history and physical, blood, and urine cultures, imaging studies, administration of antimicrobial agents for concurrent bacterial infections, and for treatment of fever and neutropenia as per institutional practice; and antipyretics, analgesics as needed.
[c] = Corticosteroid dose: 2 mg/kg methylprednisolone as an initial dose, then 2 mg/kg per day (plan for rapid taper). Other anticytokine agents may be used, including TNF-α and IL-1R inhibitors, JAK2 inhibitors or dasatinib.

Patients with severe CRS may receive tocilizumab, corticosteroids, dasatinib, or other agents after discussion with Medical Monitor. Tocilizumab is a humanized anti-IL-6 receptor antibody that has been approved by US FDA for the management of CRS. Patients may receive a repeat dose if clinical signs and symptoms do not improve within 24 to 48 hours. Patients are considered responders if CRS resolves within 14 days of the first dose of tocilizumab, no more than 2 doses of tocilizumab are needed, and no drugs other than tocilizumab and corticosteroids are used for treatment.

Side effects attributed to chronic use of tocilizumab in rheumatologic disease include transaminitis, thrombocytopenia, elevated cholesterol and low-density lipoproteins, neutropenia, and increased infections, but acute infusion toxicities have not been reported in CRS use. Please refer to the most recent version of the product label for tocilizumab.

In the event of rapid onset grade 1 CRS or CRS ≥ grade 2, please contact the Sponsor's Medical Monitor to discuss the preferred management.

8.4 Fever and Neutropenia

Evaluation for a source of infection should be performed per institutional guidelines. Fever should be treated with acetaminophen and comfort measures. Corticosteroids should be avoided. Patients who are neutropenic and febrile should receive broad-spectrum antibiotics. Maintenance IV fluids (normal saline) is recommended in patients with high fevers, particularly if oral intake is poor and/or if the patient has tachycardia.

Neutropenia is a common effect of lymphodepleting chemotherapy. The administration of prophylactic G-CSF (e.g., filgrastim) is recommended (but not mandatory) in all patients for management of neutropenia according to published guidelines (e.g., American Society of Clinical Oncology guidelines). Granulocyte colony stimulating factor may be started 24 hours after the administration of lymphodepleting chemotherapy and continued until neutrophil recovery according to institutional practice or physician's preference. Long-acting (pegylated) G-CSF may be used as per institutional standard practice or physician's preference. Pegylated G-CSF should be given as 1 dose 24 hours after the final dose of cyclophosphamide. GM-CSF is not allowed to be used while on study.

8.5 Blood Product Support

The patient should receive platelets and packed red blood cells as needed as per institutional guidelines. All blood products should be irradiated. Hemoglobin should be kept > 8.0 gm/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 cells and decrease the risk of CMV infection.

8.6 Neurotoxicity

Neurotoxicity (e.g., encephalopathy, somnolence, delirium, seizures, aphasia) has been observed with different ACT modalities, particularly with anti-CD19 CAR T cell therapies, which has been termed CAR T cell-related encephalopathy syndrome (Table 6). Evaluation of any new onset neurotoxicity should include a neurological examination (e.g., Immune Effector Cell-Associated Encephalopathy [ICE] score), brain MRI, papilledema grading, and examination of the cerebrospinal fluid (CSF) as clinically indicated, to determine the ASTCT Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) Consensus Grading for Adults score.

Table 6. ASTCT ICANS Consensus Grading for Adults				
Symptom or Sign	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
ICE score[a]	7-9	3-6	0-2	0 (patient is unarousable and unable to perform ICE)
Depressed level of consciousness[b]	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
Seizures	NA	NA	Any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (> 5 minutes); or Repetitive clinical or electrical seizures without return to baseline in between
Motor findings[c]	NA	NA	NA	Deep focal motor weakness such as hemiparesis or paraparesis
Elevated ICP/ cerebral edema	NA	NA	Focal/local edema on neuroimaging[d]	Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad

ICANS grade is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause; for example, a patient with an ICE score of 3 who has a generalized seizure is classified as grade 3 ICANS.
N/A indicates not applicable.
[a] = ICE grading system:
- Orientation: Orientation to year, month, city, hospital: 4 points (1 point each)
- Naming: Name 3 objects (eg, clock, pen, button): 3 points (1 point each)
- Following commands: (eg, Show me 2 fingers or close your eyes and stick out your tongue): 1 point
- Writing: Ability to write a standard sentence (eg, Our national bird is the bald eagle): 1 point
- Attention: Count backwards from 100 by 10: 1 point

A patient with an ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, but a patient with an ICE score of 0 may be classified as grade 4 ICANS if unarousable.
[b] = Depressed level of consciousness should be attributable to no other cause (eg, no sedating medication).
[c] = Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading.
[d] = 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. Source: Lee et al, 2019.

Endotracheal intubation may be needed for airway protection in severe cases. Corticosteroids may be considered for any severe or life-threatening neurotoxicity and anti-seizure and sedatives may be considered as clinically indicated. Guidance for management of CAR T-cell-related encephalopathy syndrome is provided in Table 7.

Table 7. Management of Chimeric Antigen Receptor T-Cell-Related Encephalopathy Syndrome
Grade 1
• Vigilant supportive care; aspiration precautions; IV hydration. • Withhold oral intake of food, medicines, and 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.

Table 7. Management of Chimeric Antigen Receptor T-Cell-Related Encephalopathy Syndrome
• Low doses of lorazepam (0.25-0.5 mg IV every 8 hours) or haloperidol (0.5 mg IV every 6 hours) can be used, with careful monitoring, for agitated patients. • Neurology consultation. • Fundoscopic exam to assess for papilledema. • MRI of the brain with and without contrast; diagnostic lumbar puncture with measurement of opening pressure; MRI spine if the patient has focal peripheral neurological deficits; CT scan of the brain can be performed if MRI of the brain is not feasible. • Daily 30 minutes EEG until toxicity symptoms resolve; if no seizures are detected on EEG, continue levetiracetam 750 mg every 12 hours. • If EEG shows non-convulsive status epilepticus, treat institutional guidelines. • Consider anti-IL-6 therapy with tocilizumab 8 mg/kg IV (maximum 800 mg) or other anticytokine agents such as siltuximab 11 mg/kg IV, or dasatinib 100mg IV daily (may increase to 140 mg daily at the discretion of the investigator) if CRES is associated with concurrent CRS.
Grade 2
• Supportive care and neurological work-up as described for grade 1 CRES. • Tocilizumab 8 mg/kg (maximum 800 mg) IV other anticytokine agents such as siltuximab 11 mg/kg IV, or dasatinib 100 mg IV daily (may increase to 140 mg daily at the discretion of the investigator) if associated with concurrent CRS. • Dexamethasone 10 mg IV every 6 hours or methylprednisolone 1 mg/kg IV every 12 hours if refractory to anti-IL-6 therapy, or for CRES without concurrent CRS. • Consider transferring patient to ICU if CRES associated with grade ≥ 2 CRS.
Grade 3
• Supportive care and neurological work-up as indicated for grade 1 CRES. • ICU transfer is recommended. • Anti-IL-6 therapy if associated with concurrent CRS, as described for grade 2 CRES and if not administered previously. • Corticosteroids as outlined for grade 2 CRES if symptoms worsen despite anti-IL-6 therapy, or for CRES without concurrent CRS; continue corticosteroids until improvement to grade 1 CRES and then taper. • Stage 1 or 2 papilledema with CSF opening pressure < 20 mmHg should be treated as per algorithm described in Neelapu et al, 2018. • Consider repeat neuroimaging (CT or MRI) every 2-3 days if patient has persistent grade ≥ 3 CRES.
Grade 4
• Supportive care and neurological work-up as outlined for grade 1 CRES. • ICU monitoring; consider mechanical ventilation for airway protection. • Anti-IL-6 therapy and repeat neuroimaging as described for grade 3 CRES. • High-dose corticosteroids continued until improvement to grade 1 CRES and then taper; for example, methylprednisolone IV 1 g/day for 3 days, followed by rapid taper at 250 mg every 12 hours for 2 days, 125 mg every 12 hours for 2 days, and 60 mg every 12 hours for 2 days. • For convulsive status epilepticus, treat as per institutional guidelines. • Stage ≥ 3 papilledema, with a CSF opening pressure ≥ 20 mmHg or cerebral edema, should be treated as per algorithm in Neelapu, et al, 2018.

Table 7. Management of Chimeric Antigen Receptor T-Cell-Related Encephalopathy Syndrome
CRES = Cell-related Encephalopathy Syndrome; CRS = Cytokine Release Syndrome; CSF = cerebrospinal fluid; CT = computed tomography; EEG = electroencephalogram; ICU = intensive-care unit; IL-6 = interleukin 6; IV = intravenous; MRI = magnetic resonance imaging.

9 STUDY PROCEDURES AND SCHEDULE OF EVENTS

Research staff at investigative sites should refer to the schedule of events (SOE) for an outline of the procedures required. The visit schedule is calculated from TC-510 infusion on day 0.

An overview of study assessments/procedures is outlined in Appendix A: Schedule of Events. Refer to the CRF completion guidelines for data collection requirements and documentation of study procedures.

9.1 Screen Failures

A log documenting the Investigator's assessment of each prescreened patient with regard to the protocol inclusion and exclusion criteria is to be maintained by the Investigator.

Patients are considered enrolled on the study upon completion of a successful leukapheresis procedure. Enrolled patients that sign the Treatment ICF and are deemed ineligible for treatment with TC-510 will be considered a screen failure.

Once a patient is deemed a screen failure, no further data will be collected, unless a patient re-screens. Re-screening patients will keep their original patient identification number and will not be assigned a new number.

9.2 Remote Visits

The Sponsor will permit remote or virtual visits if there is an assessment by the Principal Investigator that remote visits are necessary for the safety of the trial participant and will not impact data integrity.

9.3 Clinical Assessments and Procedures

9.3.1 Demographics

Demographic data including year of birth, age, sex, race, and ethnicity will be collected at pre-screening.

9.3.2 Medical History

Relevant medical history will be recorded at screening in the patient's medical record and CRF.

9.3.3 Disease History

The following information will be recorded in the CRF: cancer diagnosis, date of initial diagnosis, location of disease at initial diagnosis, stage at initial diagnosis, type of histology, histological grade, results of any historical molecular testing performed (if available), date of diagnosis of metastatic disease, location of metastatic disease, stage at screening.

9.3.4 Physical Examination

Patients will undergo a physical examination at pre-screening and baseline visits and subsequently as specified in the SOE (Appendix A).

9.3.5 Vital Signs

Measurement of vital signs (temperature, pulse, pulse oximetry, respiratory rate, blood pressure, and weight) as specified in the SOE Appendix A.

9.3.6 Performance Status

Performance status will be measured using the Eastern Cooperative Oncology Group (ECOG) performance scale (Table 8) as per the SOE (Appendix A). It is recommended that each patient's ECOG performance status be assessed by the same person throughout the study.

Table 8. Eastern Cooperative Oncology Group Performance Status

Grade	ECOG Performance Status
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

ECOG = Eastern Cooperative Oncology Group.
Source: Oken et al, 1982.

9.3.7 Clinical Safety Assessments

Patients will be assessed for adverse events and graded according to the National Cancer Institute (NCI) CTCAE Version 5.0. CRS will be graded per the ASTCT consensus grading scale (Table 4). Any new onset of neurotoxicity shall include a neurological examination based on ICE score, and any event of ICANS will be graded according to the ASTCT ICANS Consensus Grading for Adults (Table 6).

All adverse events must be recorded in the CRF.

Details on assessing and reporting adverse events and serious adverse events (SAEs) are described in section 10 and section 11.

9.3.8 Laboratory Assessments

A listing of laboratory assessments to be performed by the local laboratory are contained within Appendix B).

Laboratory certifications and reference ranges for all testing facilities utilized throughout the study must be provided to TCR^2 Therapeutics before the study initiates.

Please refer to SOE (Appendix A) for additional information regarding the frequency of these assessments.

9.3.9 Cardiac Assessments

All cardiac assessments will be performed at the site.

The following assessments will be conducted in order to monitor patient safety:

- An echocardiogram or multiple-gated acquisition (MUGA) scan will be performed at the baseline visit to determine treatment eligibility as well as the day 10 post-infusion visit. Additional assessments may be performed if clinically indicated. The same method of cardiac evaluation must be used consistently for any follow-up assessments. Blood troponin levels should be tested on the days when echocardiogram/MUGA scans are performed.
- ECG (12-lead) will be performed following a minimum of 15 minutes of supine rest at the Baseline visit.
- Patients with tumor lesions abutting the pericardium will undergo continuous cardiac monitoring (telemetry) for 5 days post-infusion.

9.3.10 Tumor Response Assessments

Imaging scans of the chest, abdomen, and pelvis will be performed at Leukapheresis Eligibility, baseline, week 4, week 8, week 12, week 24, and every 12 weeks until confirmed disease progression, study completion, or withdrawal. Acceptable imaging modalities for this study include:

- **Diagnostic-quality CT scan** with oral and/or IV iodinated contrast of the chest and abdomen/pelvis (CT is the preferred modality for tumor assessments)
    - MRI of the abdomen/pelvis acquired before and after gadolinium contrast agent administration and a non-contrast enhanced CT of the chest, if a patient is contraindicated for contrast enhanced CT

- **PET Scans:** In addition to CT scans and/or MRIs, patients will undergo PET scans of the chest, abdomen, and pelvis at baseline, week 4, week 12, and as clinically indicated thereafter, as well as at time of disease progression, study completion, or withdrawal from the study. Please note, a combined PET/CT alone is not sufficient as the CT portion is not of diagnostic quality. Patients who undergo a PET/CT must still have a separate diagnostic-quality CT of the chest and abdomen/pelvis.

The same imaging modality and image-acquisition protocol (including the use of IV contrast) should be consistently used across all time points for individual patients to allow uniform comparison of lesions. Prior to starting the study, a detailed imaging acquisition protocol will be provided to the sites to describe the requirements for image acquisition and the standardized procedure for the transfer of image data to a central vendor.

Tumor assessments will be evaluated according to the RECIST v1.1.To allow time for an immune response to become apparent and to account for potential post-treatment transient inflammation of the tumor site ("pseudoprogression"), response assessments will not be carried out before 4 weeks post TC-510 infusion, unless there is unequivocal clinical evidence of deterioration. If disease progression is equivocal, confirmation of disease progression is required by a follow-up scan performed at least 4 weeks apart, unless there is an immediate medical need to initiate anti-cancer therapy before the confirmatory scan can be performed. Disease progression will not be declared until results from the confirmatory scan are available. If confirmed, the date of progression will be that of the initial scan where progression was first suspected (i.e., not the confirmatory scan). When progression is assessed by the investigator, the investigator should notify the Sponsor and upload scan images for central review. Disease progression will not be declared until results from the central review confirm disease progression. Study teams should wait to perform the EOA visit and take the patient off active follow-up until progression is confirmed centrally, unless there is an immediate medical need to initiate anticancer therapy. Patients whose progression is not confirmed by the central review should continue tumor assessments (if clinically feasible) according to the SOE (or more frequently if clinically indicated) until progression has been confirmed by central review.

For clinical decision making, investigators will assess tumor response according to RECIST v1.1 PET scans performed at Baseline, Week 4, Week 12, and end of Active Follow-up (EOA) will be assessed by investigators for metabolic response. To the extent that it is feasible, local tumor assessments should be performed by the same radiologist. Additionally, scans will be submitted to a central imaging facility for independent read and to give a RECIST criteria response (eg, RECIST v1.1 or mesothelioma-specific RECIST, if applicable). Results from both reads will be summarized and reconciled at the end of the study phase.

For patients who have new lesions, response by RECIST (Nishino et al, 2013) will be assessed by the Investigator for exploratory purposes. For new lesions, information on whether the lesion is measurable or non-measurable will be recorded in the CRF.

Study images will be sent to a TCR^2 Therapeutics designated central vendor for assessment of tumor response according to protocol-defined imaging schedule. Review and interpretation of

tumor response according to RECIST v1.1 (or mesothelioma-specific RECIST criteria, if applicable) will be conducted by an appropriately qualified, trained, and experienced independent radiologist in accordance with an imaging review charter. The imaging review charter will also describe the procedures for CT/MRI and PET data handling after the images have been received by the central vendor from the sites.

9.3.11 Period of Hospitalization for Lymphodepletion and/or TC-510 Infusion

Both the lymphodepleting chemotherapy as well as the TC-510 infusion may either be given as an outpatient treatment or patients may be hospitalized at the discretion of the Investigator. However, for the phase 1 portion of the study, patients should be observed overnight following T cell infusion.

Hospitalization may be required after study treatment to manage certain treatment-associated toxicities. Therefore, patients should remain within 2 hours travel time of the treatment facility for at least 4 weeks following TC-510 infusion. Should a patient require hospitalization for administration of lymphodepleting chemotherapy, information of such hospitalization (e.g., reason, number of days) will be recorded in the CRF.

For the phase 2 portion of the study, it is recommended that patients are observed overnight following TC-510 infusion.

9.4 TC-510 Retreatment (Applicable for Phase 1 RP2D Expansion Cohort and During Phase 2)

In the phase 1 RP2D expansion cohort and in the phase 2 portion of the study, patients will be candidates for TC-510 retreatment if they fall into either of the following groups:

- Patients who have an objective response (i.e., PR or CR as assessed by the Investigator) after a TC-510 infusion and develop signs and symptoms of progression.
- Patients whose best response to a prior TC-510 infusion is SD (as assessed by the Investigator) sustained for at least 8 weeks post-TC-510 infusion.

The decision to undergo subsequent TC-510 regimen will be made by the treating Investigator and after consultation and in alignment with the TCR^2 Therapeutics Medical Monitor. TC-510 retreatment will follow similar procedural requirements as the initial dose, including the post-treatment study requirements, unless otherwise specified below:

- Patients will be required to meet the original treatment eligibility criteria again and should not have received any other therapy for their underlying malignancy.
- Patients will be required to undergo a biopsy, however, they will not be required to undergo MSLN testing and demonstrate MSLN expression. A post infusion, on-study biopsy (e.g.

Week 6) within 3 months prior to the planned retreatment may fulfill the retreatment biopsy requirement (provided there is adequate tissue for correlative testing).

- Retreatment will consist of an abbreviated course of lymphodepleting chemotherapy followed by TC-510 infusion:
  - Lymphodepleting chemotherapy will consist of fludarabine 30 mg/m2/day on days -7 through -5 (i.e., 3 doses) and cyclophosphamide 600 mg/m2/day on days -6 through -5 (i.e., 2 doses). Please note, this regimen differs from that given prior to the initial TC-510 infusion. In the event an alternative lymphodepleting chemotherapy regimen is required due to the fludarabine shortage, please refer to section X and discuss with the Sponsor's Medical Monitor.
  - Please Refer to Section 7.3 for guidelines regarding lymphodepleting chemotherapy administration.

Patients meeting treatment eligibility criteria for a second dose may proceed to TC-510 infusion no sooner than 8 weeks and no later than 1 year (52 weeks) following completion of the first TC-510 dose.

Patients eligible for retreatment should be reconsented following discussion regarding benefits and risks of TC-510 therapy and if applicable, a careful explanation about the need to undergo leukapheresis a second time for the manufacturing of TC-510 prior to performing any study related procedures or treatment. This conversation should be recorded in the patient's source document.

9.5 Correlative Studies and Research Assessments

Correlative studies and research assays will be performed during the trial to monitor the biological parameters that may impact TC-510 treatment outcome. Such studies include assessing the phenotype, function, and persistence of the infused TC-510, cytokine measurements, and immunogenicity studies, among others. Studies will be conducted on tumor biopsies, PBMCs, serum or plasma, and TC-510 drug product and manufacturing retains as specified in the SOE (Appendix A). All samples will be processed and/or frozen following trial specific SOPs and analyzed at TCR^2 Therapeutics designated central laboratory facilities. Documentation for sample receipt, processing and storage, and primary data from the research analyses will be collected and stored at the central laboratory or at TCR^2 Therapeutics, as appropriate.

Research studies conducted on blood samples, manufactured TC-510 drug product and manufacturing retains, ascites and serosal effusions from patient samples may include the following exploratory endpoints, among others:

- Analysis of PBMC's and TC-510 phenotype and persistence by flow cytometry

- Determination of TC-510 persistence using PCR-based assays
- Characterization of PBMCs or TC-510 (pre- and post-infusion) using molecular or functional assays to assess cytotoxicity, cytokine release, proliferative capacity, or gene expression profiles
- Assessment of cytokine release by TC-510 using multiplexed assays to measure proinflammatory cytokines
- Detection of anti-TC-510 antibody levels pre- and post-infusion using enzyme-linked immunosorbent assay (ELISA)
- Quantitation of circulating tumor cells and circulating tumor DNA pre- and post TC-510 infusion

Biopsy research studies may include, among others:

- Tissue expression of the MSLN antigen and PD-L1 by IHC
- Detection and quantification of tumor infiltrating T-cells and TC-510 in longitudinal biopsies using multiplexed immunofluorescence and image analysis
- Gene expression profiling (RNASeq) and whole exome sequencing.

If a patient has an adverse event, an additional biopsy (e.g., skin, ascites, serosal fluid, gastrointestinal tract, bone marrow, tumor) or blood (serum and PBMC) samples may be requested, and studies may be performed on those samples aimed at understanding the underlying pathophysiology of the ongoing adverse event.

9.5.1 Cytokine and Anti-TC-510 Antibody Analysis

Plasma is collected at baseline and at select visits post-infusion, to allow for measurement of cytokines in the blood. Plasma is also collected from patients with suspected CRS with samples being taken approximately every 24-48 hours until symptoms improve or an alternative diagnosis is confirmed. Details regarding plasma collection are provided in the laboratory manual.

Cytokines, growth factors and soluble receptors including but not limited to IL-1 β, IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, and GM-CSF will be measured utilizing a multiplexed assay and will be performed following Good Laboratory Practice procedures. All other measurements may be performed as exploratory.

Plasma samples will also be used to detect the presence of anti-MH1 antibodies prior to infusion and at week 8 post-infusion. For plasma samples that demonstrate positivity of anti-MH1 human antibodies at the week 8 visit, an attempt should be made to obtain and test additional plasma samples at week 12, week 24, and at 12-week intervals thereafter until the antibody levels return to baseline (or become negative) or up to 1 year from date of TC-510 infusion, whichever occurs first.

9.5.2 Tumor Biopsies

The activity of TC-510 will be impacted by other cellular elements within the tumor microenvironment (e.g., regulatory T cells). Evaluating the "immune landscape" within the tumor pre- and post-infusion of TC-510 may provide valuable insight into characteristics of the tumor microenvironment that influence clinical outcomes. For this reason, core needle biopsies will be requested at 4 specific time points:

- **Pre-screening (For Ovarian, Pancreatic, Colorectal, TNBC, NSCLC, and Cholangiocarcinoma only):** Archival tissue biopsy may be used for pathological confirmation of diagnosis and MSLN expression determination if obtained within 5 years of the pre-screening ICF date. If adequate archival tissue is unavailable, a fresh tissue biopsy should be obtained
- **Baseline**: a tissue biopsy within 6 months of the planned first protocol defined therapy will be obtained for MSLN expression and the conduct of translational studies to evaluate the immune status of the tumor prior to TC-510 infusion, as described in section 9.6, including, among others: T cell infiltration, presence of immune activation/exhaustion markers, and tissue expression of immune checkpoints. The pre-screening biopsy may fulfill the Baseline biopsy requirement if collected within 6 months of the start of treatment (provided there is adequate tissue for correlative testing). In the event a fresh biopsy is performed at baseline and the patient experiences unforeseen delays in dosing, the window for the fresh biopsy may be extended with approval from the Sponsor. PD-L1 expression may also be assessed on this tumor biopsy for research purposes.

- **Week 6 (± 7 days):** a fresh tissue biopsy will be obtained at this time point, which is when an active anti-tumor response by the infused TC-510 is expected. Translational studies will be conducted on this biopsy to evaluate TC-510 infiltration and the immune status of the tumor upon TC-510 infusion as described in section 9.6, MSLN and PD-L1 expression will also be assessed on this tumor biopsy for research purposes. If lesion is deemed inaccessible or unsafe to biopsy, biopsy may be omitted.
- **After confirmation of disease progression** (within 3 months or prior to receiving subsequent therapy, whichever is shorter): a fresh tissue biopsy will be obtained at this time point (unless tumor is not safely accessible) to evaluate the immune status of the tumor upon TC-510 infusion, as described in section 9.5, and mechanisms of immune escape (e.g., target antigen downregulation, T cell immune exclusion). Ideally, biopsy material obtained after confirmation of disease progression should be collected from lesions that have progressed and from new lesions to best address mechanisms of resistance and to determine eligibility for retreatment (refer to section 9.5 for details regarding retreatment).
- **Autopsy Tissue Collection**: In the event a patient dies, and an autopsy is performed upon request of the participant's family or next of kin, study investigators may ask permission from the participant's family to collect tissue during the autopsy for research purposes. This may help investigators learn about the effects of treatment with genetically modified cells. Subject to the consent of the participant's family, the Sponsor may use tissue collected

during the autopsy for future testing. The Sponsor will only request tissue if an autopsy is requested by the participant's family, the Sponsor will not require an autopsy to be performed solely for the purpose of the study.

- **Retreatment Baseline**: Patients will be required to undergo a biopsy, if clinically feasible, if they are proceeding with retreatment > 180 days (6 months) post their most recent TC-510 infusion. However, they will not be required to undergo MSLN testing and demonstrate MSLN expression. In the event a patient will be undergoing retreatment within 180 days (6 months) of the prior TC-510 infusion, they will not be required to undergo a biopsy prior to retreatment. The same translational studies as for the initial Baseline biopsy will be conducted to evaluate the immune status of the tumor prior to TC-510 retreatment. MSLN expression analysis and PD-L1 expression may also be assessed on this tumor biopsy for research purposes. Refer to section 9.5 for details regarding MSLN expression requirements for TC-510 retreatment.

When possible, biopsies should consist of multiple cores taken from more than one lesion. In cases where tumor lesions may not be amenable to core needle biopsy, fine needle aspirates may be obtained based on interventional radiology recommendations.

Tumor tissue should either be taken from non-target lesions or from target lesions > 2 cm. Every attempt should be made to obtain biopsies at all time points from the same lesion(s). The radiological (or clinical) status of the lesion(s) biopsied should be noted at the time (e.g., decreased, stable, increased size or activity).

In patients who have ascites and/or serosal effusion, should there be a clinical requirement for removal of the effusion fluids at any time during study, samples are requested to be collected for the conduct of research studies. When available, ascites, serosal effusion fluids, and lumbar fluid, should be collected in addition to, and not instead of the requested tumor biopsies, with the exception of MPM cases where tumor biopsy is not accessible and/or available.

Ascites, serosal effusion, and lumbar fluid specimens will be used to interrogate the tumor microenvironment prior to and after TC-510 infusion to address mechanisms of sensitivity or resistance to therapy as well as kinetics of tumor clearance.

9.5.3 TC-510 Persistence

The presence, expansion, and persistence of TC-510 will be monitored in peripheral blood by both quantitative polymerase chain reaction (qPCR) and flow cytometry.

9.5.4 TC-510 T Cells Phenotype and Activity

A range of assays will be performed to elucidate the phenotype and activity of the infused TC-510 including (but not limited to):

- Immunophenotyping of TC-510 in manufactured cell product prior to infusion and in the blood (and tumor, when available) post-infusion by flow cytometry to assess differentiation and activation status.
- Detection of antibody responses against the anti-MSLN MH1 binder of TC-510.
- Ex-vivo activity of PBMCs and/or TC-510 pre- and post-infusion to assess T cell functionality by cytotoxicity, cytokine release, proliferative capacity, or molecular characteristics.

9.5.5 Liquid Biopsies

The presence of circulating tumor DNA (ctDNA) will be assessed in plasma samples as an early response biomarker at Baseline and up to 4 time points post TC-510 infusion.

9.5.6 Tissue and Sample Retention Policy

Tissue samples and biological specimens may be stored for up to 15 years to conduct exploratory research aimed at addressing scientific questions related either to the study treatment or to the specific tumor type. Each patient will have the right to have the specimen material destroyed at any time by contacting the Investigator who in turn can contact the laboratory. The Investigator should provide TCR^2 Therapeutics the study and patient number so that the sample can be identified and destroyed.

For patients who withdraw consent, any samples that were not requested to be returned or destroyed will remain with the Sponsor and any data that may be generated will be entered in the study database.

9.5.7 Blood Sampling Volumes

The amount of blood drawn from patients for the purposes of this study shall not exceed 600 mL in an 8-week period.

In the event blood draws are limited due to this restriction, blood samples for research will be performed in order of priority as defined by the Sponsor.

9.5.8 Patient-Reported Outcome Measures

The following quality of life and patient-reported outcome (PRO) instruments will be used to collect patient experience data during phase 2 of the study.

9.5.9 EuroQOL Group EQ-5D 3 Level Version (EQ-5D-3L)

EQ-5D is a standardized measure of health status developed by the EuroQol Group in order to provide a simple, generic measure of health for clinical and economic appraisal (EuroQOL, 1990). The EQ-5D is applicable to a wide range of health conditions and therapies

and provides a simple descriptive profile and a single index value for health status that can be used in the clinical and economic evaluation of health care. The EQ-5D-3L descriptive system comprises the following 5 dimensions: mobility, self-care, usual activities pain/discomfort, and anxiety/depression. Each dimension has 3 levels: no problems, some problems, extreme problems. The patient is asked to indicate his/her health state by selecting the most appropriate statement in each of the 5 dimensions. The EQ visual analog scale records the respondent's self-rated health on a vertical, visual analogue scale where the endpoints are labelled "Best imaginable health state" and "Worst imaginable health state." This information can be used as a quantitative measure of health outcome as judged by the individual respondents.

The EQ-5D-3L will be administered at Baseline, prior to TC-510 infusion on day 0, then at Day 10, Day 21, Week 4, Week 8, Week 12, and then at Week 24, 36, 48, 60, 72, 84, and 96 or until study completion, disease progression, or withdrawal, whichever comes first. It is recommended that patients complete the Quality of Life questionnaires at the beginning of each required study visit prior to conducting any procedures or clinical evaluation.

9.5.10 Patient-Reported Outcome Version of the Common Term Criteria for Adverse Events

The PROs Version of the CTCAE is a patient-reported outcome measure developed to evaluate symptomatic toxicity in patients on cancer clinical trials (Basch, 2014). The PRO-CTCAE was designed to be used as a companion to the CTCAE, the standard lexicon for adverse event reporting in cancer trials. The PRO-CTCAE includes an item library of 124 items representing 80 symptomatic toxicities drawn from the CTCAE. Patient-reported outcomes-CTCAE provides a systematic yet flexible tool for descriptive reporting of symptomatic treatment side effects in cancer clinical trials. In the present study, a subset of 20 items selected from the PRO-CTCAE version 1.0 item library will be administered.

10 SAFETY REPORTING

10.1 Safety Summary

This will be the first-in-human trial of TC-510 and therefore, no clinical safety information with this therapeutic is currently available. However, TC-510 represents the third TRuC T cell product to be studied in subjects with cancer, the first 2 being gavo-cel and TC-110. TC-510 results from engineering gavo-cel T cells to have them express a PD-1:CD28 switch. A series of common toxicities have been identified across a growing number of trials involving autologous T cells genetically engineered to express either chimeric antigen receptors or T cell receptors targeting tumor antigens. The most important of such toxicities are CRS, neurotoxicity, GvHD, and myelosuppression. CRS events have also been reported in patients receiving gavo-cel therapy but they have been generally manageable upon institution of standard CRS management measures (e.g., corticosteroids, tocilizumab). As of January 10, 2023, no GVHD events and only two low grade self-limited neurotoxic events have been reported among patients treated with

gavo-cel to date. A detailed guidance on appropriate monitoring and management of these toxicities is included in Section 9 of this protocol.

Most adoptive T cell therapy trials involve the use of lymphodepleting chemotherapy prior to T cell infusion. Most frequently, lymphodepletion chemotherapy involves the combination of cyclophosphamide and fludarabine, which are expected to be associated with toxicities such as cytopenias.

To manage the risk of CRS and other potential TC-510 mediated toxicities, TCR^2 will implement specific AE pages in the eCRF to carefully document the events and to enable evaluation and identification of potential risk factors. To help Investigators manage TC-510 mediated toxicities, TCR^2 has developed treatment guidelines based on published literature which are included in the protocol. TCR^2 has also developed standard protocol guidance on the management of myelosuppression, including a recommendation to consult with physicians with expertise in bone marrow transplant, infectious diseases, and the management of aplastic anemia.

The theoretical risks of RCL and insertional oncogenesis will be monitored in accordance with FDA and EMEA guidance in the long-term follow-up study protocol.

10.2 Adverse Events

In accordance with the ICH, an AE is any untoward medical occurrence in a patient or clinical investigation patient who receives a pharmaceutical product, whether or not considered related to product. The event does not necessarily have a causal relationship with study treatment to be an AE. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product. Pre-existing conditions which worsen during the study are to be recorded as AEs. Interventions for pre-treatment conditions (such as elective cosmetic surgery) or medical procedures that were planned before study participation are not considered AEs. Hospitalization for study treatment infusions or precautionary measures per institutional policy is not considered an AE.

AEs or abnormal laboratory findings should be recorded in the CRF using a diagnosis or possible diagnosis, and rated for intensity, causality, and seriousness. In the absence of a diagnosis, individual symptoms or findings may be recorded and the CRF updated to reflect a final diagnosis once additional information becomes available. If photographs are requested by the Sponsor, for example, of a rash AE, for example, the patient will sign a medical photograph release prior to any photographs being taken.

The term “disease progression” as assessed by measurement of malignant lesions on radiographs or other methods should not be reported as an AE term. If a patient dies due to disease progression and the disease progression was documented prior to the patient’s death, it should not be reported as an SAE or an AESI. If disease progression was not documented prior to the patient’s death due to disease progression, then the direct cause of death (for example, respiratory failure, sepsis, etc.), if known, should be reported as an SAE with fatal outcome. If

the direct cause of death is not known, then the primary tumor type (eg, NSCLC) should be reported as an SAE with fatal outcome. For situations when an adverse event or SAE is due to the disease under investigation report the signs and symptoms. Worsening of signs and symptoms of the malignancy under study should also be recorded as AEs in the CRF. The event reporting period ends at the time of disease progression.

10.2.1 Adverse Events of Special Interest

An adverse event of special interest (AESI) (serious or nonserious) is an adverse event of greater scientific and medical concerns specific to TC-510. Current defined AESIs for TC-510 include the following and, other than events leading to death, must be considered possibly related to TC-510 in order to be considered reportable:

- Events leading to death, except for Disease Progression documented before the patient's death.
- Serious or opportunistic infections, defined as bacterial, viral, fungal, or parasitic infections that fulfill 1 of the following criteria: require anti-infective treatment or, lead to significant disability or hospitalization or, need surgical or other intervention.
- New incidence or exacerbation of a pre-existing neurologic disorder
- New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder
- Positive RCL test result
- Vector insertion site sequencing result with a mono- or oligoclonality pattern or in a location near a known human oncogene
- New malignancy (T cell and non-T cell), other than the primary malignancy
- CRS
- Tumor lysis syndrome
- Neutropenic fever
- ≥ Grade 3 Cytopenia > 28 days
- Drop in cardiac ejection fraction following treatment with TC-510
- Neurotoxicity

All AESIs must be recorded on the adverse event CRF. All deaths must also be reported to TCR^2 Clinical Safety or designee within 24 hours of learning of its occurrence. All other AESIs (serious and non-serious) must be reported to TCR^2 Clinical Safety or designee within 24 hours of learning of its occurrence only if causality is assessed as possibly, probably, or definitely related to TC-510 by the Investigator.

AESIs that occur within 8 weeks of the TC-510 infusion will be summarized by group term and preferred term.

10.2.2 Laboratory Test Abnormalities

Laboratory abnormalities that constitute an adverse event (i.e., are considered clinically significant, induce clinical signs or symptoms, require concomitant therapy, or require changes in study treatment), should be recorded on the adverse event CRF. Whenever possible, a diagnosis, rather than a symptom should be provided (e.g., anemia instead of low hemoglobin) or per CTCAE 5.0 category (i.e., decreased platelet count, decreased neutrophil count). Laboratory abnormalities that meet the criteria for adverse events should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory or test result corresponds to a sign/symptom of an already reported adverse event, it is not necessary to separately record the lab/test result as an additional event.

Laboratory abnormalities that do not meet the definition of an adverse event should not be reported as AEs. A grade 3 or 4 event (severe) as per CTCAE does not automatically indicate a SAE unless it meets the definition of serious as defined below and/or as per investigator's discretion. A medication for the lab abnormality may be required by the protocol in which case the lab abnormality would still, by definition, be an adverse event and must be reported as such.

10.3 Reporting of Adverse Events

The Principal Investigator is responsible for ensuring that all adverse events observed by the study team and/or reported by the patient that occur after signing of the study consent are recorded in the patient's source documents. Safety monitoring will begin once the patient has signed the pre-screening ICF.

From the signing of the pre-screening ICF until the start of the first protocol defined therapy (i.e., lymphodepleting chemotherapy) adverse events will only be reported when considered related to a protocol-specified procedure (e.g., protocol-required blood draw or tumor biopsy, leukapheresis, etc.) starting at time of procedure until 24 hours afterwards for blood sampling and leukapheresis, or until 2 weeks post-biopsy.

Once the patient receives the first protocol defined therapy (e.g. lymphodepleting chemotherapy), all new or worsening adverse events including abnormalities deemed clinically significant by the Investigator, regardless of causality will be monitored and recorded in the adverse event CRF through 3 months after the last infusion of TC-510, unless the patient's disease progresses.

AE monitoring should be continued through the Year 5 (end of study [EOS]) visit..

For patients receiving TC-510, following the Week 12 post-infusion visit, and through the End of Active Follow-up (EOA) visit, only targeted AEs that meet 1 of the following criteria should be recorded in the AE CRF:

- Events resulting in death, except death due disease progression when disease progression was previously documented.
- Events related to a study procedure
- Infections:
  - Serious or opportunistic infections, defined as bacterial, viral, fungal, or parasitic infections that fulfill one of the following criteria: require anti-infective treatment or, lead to significant disability or hospitalization or, need surgical or other intervention
- New incident or exacerbation of a pre-existing neurologic disorder
- New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder
- All SAEs the Investigator believes may have a reasonable relationship to TC-510 therapy
- Positive RCL test result
- Vector insertion site sequencing result with a mono or oligoclonality pattern or in a location near a known human oncogene
- New malignancy (T cell and non-T cell), other than the primary malignancy
- Any severe adverse event or condition the investigator believes may have a reasonable relationship to TC-510 T cells

For patients who do not receive TC-510, the reporting period ends 30 days after the last dose of lymphodepleting chemotherapy.

If a patient is retreated with TC-510, all adverse events will be collected from the date the patient signs the retreatment ICF, and the safety reporting schema will be the same as for the initial TC-510 dose.

Some patients receiving TC-510 may require inpatient and/or intensive-care unit (ICU) care within 28 days post TC-510 infusion. The events leading to hospitalization are most often due to CRS, neurotoxicity, or tumor lysis syndrome, although there may be some contribution from the lymphodepletion chemotherapy. These events are expected on-target effects of TC-510 expansion. A single inpatient or ICU day can generate hundreds of data points and many therapeutic dose changes throughout a given day. While expected in some patients, these inpatient events and days are not scheduled protocol defined visits. Revised inpatient data capture will be utilized for this study to systematically collect selected subsets of patient data to describe the management of safety events associated with TC-510 therapy for the purpose of:

- Adequately informing physicians and patients of the expected risks of TC-510 and the recommended interventions to manage these risks
- Health authority submission

This is done through a targeted collection of concomitant medications and laboratory data and CRS CRFs specifically designed to capture TC-510-related toxicity, severity, interventions, and response/resolution following intervention.

The Investigator must address the below items regarding AEs:

- AE diagnosis or syndrome (if not known, signs or symptoms)
- Dates of onset and resolution
- Severity
- Assessment of causality to investigational product, conditioning chemotherapy or study procedures
- Action taken

The Investigator will grade all adverse events (besides CRS and ICANS) according to the NCI CTCAE v 5.0 on a 5-point scale (grade 1-5) and report in detail on the CRF. CRS will be graded per the ASTCT consensus grading scale (Table 4). Any new onset of neurotoxicity shall include a neurological examination based on ICE score, and any event of ICANS will be graded according to the ASTCT ICANS Consensus Grading for Adults (Table 6).

Adverse events not specifically listed on the CTCAE should be graded according to Table 9.

Table 9. Grading of Adverse Events Not Specified in Common Terminology Criteria for Adverse Events Version 5.0

CTCAE Grade	Equivalent to	Definition
Grade 1	Mild	Discomfort noticed but no disruption of normal daily activity
Grade 2	Moderate	Discomfort sufficient to reduce or affect daily activity; minimal medical intervention is indicated
Grade 3	Severe	Incapacitating with inability to work or perform normal daily activity; treatment or medical intervention is indicated in order to improve the overall well-being or symptoms; delaying the onset of treatment is not putting the survival of the patient at direct risk
Grade 4	Life threatening/disabling	An immediate threat to life that requires urgent medical intervention
Grade 5	Death	AE resulting in death

AE = adverse event; CTCAE = Common Terminology Criteria for Adverse Events.

The Investigator is responsible for reviewing laboratory test results and determining whether an abnormal value in an individual study patient represents a clinically significant change from the patient's baseline values. Abnormal laboratory findings without clinical significance (based on investigator's assessment) are not to be recorded as adverse events. Where applicable, clinical sequelae (not the laboratory abnormality) are to be recorded as the adverse event.
The Investigator is expected to follow reported adverse events until stabilization or resolution.

The Investigator will also assess the causal relationship between the adverse event and investigational product (i.e., TC-510) according to his/her best clinical judgement.
An assessment of possibly/probably/definitely related is meant to convey there is evidence of a causal relationship, not that a relationship cannot be ruled out. The Investigator should consider alternative causes such as natural history of the underlying disease, lymphodepleting chemotherapy, concomitant medications, and other risk factors when making an assessment.
The following scale will be used as guidance:

- **Not related** – The patient did not receive the investigational product; the temporal sequence of the AE onset relative to administration of the investigational product is not reasonable; or there is another obvious cause of the AE.
- **Possibly related** – There is evidence of exposure to the investigational product; the temporal sequence of the AE onset relative to T cell infusion is plausible; or the AE could have been due to another equally likely cause.

- **Probably related** – There is evidence of exposure to the investigational product; the temporal sequence of the AE onset relative to T cell infusion is plausible; the AE shows a pattern consistent with previous knowledge of the investigational product; or the AE is more likely explained by the investigational product than any other cause.**Definitely related** – There is evidence of exposure to the investigational product; the temporal sequence of the AE onset relative to T cell infusion is plausible; the AE shows a pattern consistent with previous knowledge of the investigational product, or the AE is most likely explained by the investigational product and any other cause is improbable.

The Investigator may change his/her opinion of causality if additional information is received and amend the SAE CRF accordingly. The Investigator causality assessment is one of the criteria TCR^2 Therapeutics will use to determine regulatory reporting requirements for an SAE.

10.4 Definition of Serious Adverse Events

The Investigator is responsible for reporting all the observed SAEs and SAEs reported by the patient that occur after signing of the consent through the timelines specified below. For patients receiving TC-510, all SAEs must be reported through 12 weeks post TC-510 treatment or until disease progression. After Week 12, **only targeted SAEs** will be reported until Year 5. If disease progression is documented prior to Week 12, targeted SAEs will be reported according to the LTFU SAE reporting requirements (see Section 10.5.5). For patients who do not receive TC-510, the reporting period ends 30 days after the last dose of lymphodepleting chemotherapy.

An SAE is defined as an AE that meets at least one of the following serious criteria:

- Fatal
- Life threatening (places the patient at immediate risk of death)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Congenital anomaly/birth defect
- Other medically important serious event

An AE would meet the criterion of "requires hospitalization" if the event necessitated an admission to a health care facility (e.g., overnight stay).

Events that require an escalation of care when the patient is already hospitalized should be recorded as an SAE. Examples of such events include movement from routine care in the hospital to the ICU or if that event resulted in a prolongation of the existing planned hospitalization. The event should be documented in the CRF with the highest degree of severity that resulted in the hospitalization extension.

If an Investigator considers an event to be clinically important, but it does not meet any of the serious criteria, the event could be classified as a SAE with the criterion of "other medically important serious event."

Progression of the malignancy during the study and death due to progressive disease is not considered a reportable SAE. The SAE terms should instead be recorded as described in section 10.2, as applicable.

10.5 Reporting of Serious Adverse Events

10.5.1 Screening/Pretreatment

Safety monitoring will begin once the patient has signed the pre-screening ICF. From the signing of the pre-screening ICF until the patient receives the first protocol defined therapy (i.e. lymphodepleting chemotherapy), SAEs must be recorded on the adverse events CRF and reported to TCR^2 Clinical Safety or designee only when considered related to a protocol-specified procedure (e.g., protocol-required blood draw or tumor biopsy, leukapheresis, etc.), starting at time of procedure until 24 hours afterwards for blood sampling and leukapheresis, or until 2 weeks post-biopsy.

10.5.2 Study-Related Treatment to Week 12

To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient has begun study-related treatment (i.e., lymphodepleting chemotherapy) through the week 12 visit or until disease progression, whichever comes first, must be recorded on the adverse events CRF and reported to TCR^2 Therapeutics Clinical Safety or designee within 24 hours of learning of its occurrence. The same process applies to patients that are retreated with TC-510.

10.5.3 Week 12 to EOA

Any SAEs experienced after the week 12 visit, and through the EOA visit must be recorded on the AEs CRF and reported to TCR^2 Therapeutics Clinical Safety or designee within 24 hours only if it meets one of the following criteria:

- Events leading to death except for death due to disease progression when disease progression was documented prior to the patient's death
- Events related to a study procedure
- Serious or opportunistic infections, defined as bacterial, viral, fungal, or parasitic infections that fulfill 1 of the following criteria: require anti-infective treatment or, lead to significant disability or hospitalization or, need surgical or other intervention
- New incidence or exacerbation of a pre-existing neurologic disorder
- New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder

- New incidence of other hematologic disorder
- Any SAE or condition the Investigator believes may have a reasonable relationship to TC-510 therapy
- Positive RCL test result
- Vector insertion site sequencing result with a mono- or oligoclonality pattern or in a location near a known human oncogene
- New malignancy (T cell and non-T cell), other than the primary malignancy
- Progressive multifocal leucoencephalopathy (PML)
- Hepatitis B reactivation

10.5.4 Other SAE Reporting

In addition, the following SAEs will be reported in an expedited manner to health authorities (e.g. FDA) by TCR^2 Therapeutics or designee::

- Any SAE related to a study procedure
- All occurrences of CRS grade ≥ 4
- All occurrences of neurotoxicity grade ≥ 4
- All fatal SAEs regardless of attribution following lymphodepleting chemotherapy and/or TC-510 infusion

10.5.5 Long-Term Follow-Up SAE Reporting

Patients that complete the EOA visit before the Week 108 visit for any reason (e.g. radiographically confirmed or clinically assessed disease progression, adverse events, investigator decision, patient decision) and patients who have completed the Active Follow-up phase by completing the Week 108 visit enter into the Long Term Follow-Up phase until they reach approximately 5 years post TC-510 infusion. Ongoing SAEs at the time of disease progression/active follow-up completion should be followed by the Investigator until resolution. New SAEs do not need to be recorded on the adverse events CRF or reported to TCR^2 Clinical Safety or designee from the time of documented disease progression or the time of active follow-up completion, unless the following criteria apply:

- Events leading to death, except for death due disease progression when disease progression was documented prior to the patient's death
- Events related to a study procedure

- Opportunistic and/or serious infections defined as bacterial, viral, fungal or parasitic infections that fulfill one of the following criteria: require anti-infective treatment, lead to significant disability or hospitalization, or need surgical or other intervention
- New malignancies (and anti-cancer therapies when available)
- New incidence or exacerbation of a pre-existing neurological disorder
- New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder
- New incidence of other hematologic disorder
- Any severe adverse event or condition the Investigator believes may have a reasonable relationship to TC-510 therapy
- Positive RCL test result
- Vector insertion site sequencing result with a mono- or oligoclonality pattern or in a location near a known human oncogene
- New malignancy (T cell and non-T cell), other than the primary malignancy
- Progressive multifocal leukoencephalopathy (PML)
- Hepatitis B reactivation

10.6 Pregnancy and Lactation

The safety of TC-510 during pregnancy and lactation has not been established in humans. Therefore, TC-510 should not be administered to pregnant women, women intending to become pregnant, or women who are breastfeeding.

The effects of TC-510 therapy on breast milk are unknown. Therefore, breastfeeding should be discontinued starting at the first dose of chemotherapy and for at least 12 months after receiving the investigational product and for 4 months from the time no evidence of persistence/gene modified cells is documented in the patient's blood.

Pregnancy (or pregnancy of a male patient's partner) is not considered an adverse event/SAE unless there is reason to believe that the pregnancy may be the result of failure of the contraceptive being used due to interaction with the study drug. However, the Investigator shall report all pregnancies within 24 hours of learning of its occurrence to TCR^2 Clinical Safety or designee. A woman who becomes and remains pregnant during the study will be discontinued from the study and be placed into the appropriate Sponsor LTFU study. The outcome of the pregnancy must also be reported to the Sponsor. The contraception guidelines in the inclusion criteria of this protocol should continue to be followed during the LTFU.

11 SAFETY MONITORING

11.1 Safety Review Team

TCR^2 Therapeutics will perform ongoing comprehensive evaluation of the safety profile for the TC-510 product. This includes routine periodic reviews of available safety data by TCR^2 Therapeutics' SRT. The SRT will consist of at least 2 study investigators and the Sponsor's chief medical officer. The SRT will be specifically chartered to review safety and efficacy data during phase 1 of the study and make a recommendation to progress the study from phase 1 to phase 2 based on the incidence of TC-510 DLTs and review of SAEs.

For the phase 2 portion of the trial, the SRT will convene to review the safety and efficacy data of the patients treated at the RP2D, as described in the SRT charter.

11.2 Dose-Limiting Toxicity (Applies to Phase 1 Only)

DLT is defined as the following TC-510-related events with onset within the ensuing 28 days following day 0 TC-510 infusion:

- Any CTCAE grade 5 organ toxicity.
- Any CTCAE grade 4 organ toxicity (cardiac, dermatologic, gastrointestinal, hepatic, pulmonary, renal/genitourinary, or neurologic) with an attribution of definitely or probably related to the TC-510 infusion that does not improve to grade ≤ 2 within 4 weeks.
- Any grade 3 or greater autoimmune toxicity related to TC-510.
- Grade 4 CRS that does not improve to ≤ 3 within 72 hours. If grade 4 CRS resolves to grade 3 within 72 hours, then the grade 3 CRS must further resolve to grade ≤ 2 within the following 11 days so that the time between grade 4 CRS and its resolution to grade ≤ 2 does not exceed 14 days.
- Grade 4 neurotoxicity that does not improve to ≤ 3 within 72 hours. If grade 4 neurotoxicity resolves to grade 3 within 72 hours, then the grade 3 neurotoxicity must further resolve to grade ≤ 2 within the following 11 days so that the time between grade 4 neurotoxicity and its resolution to grade ≤ 2 does not exceed 14 days.

The study will be suspended in the event of any death within 28 days of receiving TC-510 or occurrence of 2 or more grade 4 toxicities (with the exception of toxicities expected with lymphodepleting chemotherapy), pending comprehensive safety review before enrolling additional subjects.

The following adverse events will NOT be considered DLTs:

- Grade 1 to 4 toxicities expected with lymphodepleting chemotherapy (e.g., cytopenias).
- Fever grade ≤ 3.

- Myelosuppression (includes bleeding in the setting of platelet count less than 50×10^9/L and documented bacterial infections in the setting of neutropenia), defined as lymphopenia, decreased hemoglobin, neutropenia and/or thrombocytopenia.
- Immediate hypersensitivity reactions occurring with 2 hours of TC-510 infusion (related to cell infusion) that are not reversible to a grade 2 or less within 24 hours of TC-510 administration with standard therapy.

11.3 Monitoring and Management of Replication Competent Lentivirus

While no cases of RCL have been reported either in vitro or in vivo, RCL remains a theoretical risk associated with the use of lentiviral vectors. The risk is derived from the detection of replication competent retrovirus (RCR) during the use of early γ retroviral vector packaging systems which were inadequately designed to avoid recombination events between the vector and packaging components (Miller, 1990).

Replication competent retrovirus/lentivirus should be rigorously evaluated in vector and cell lots, and in patients post infusion with any product involving a lentivirus or a retrovirus for a total of 15 years (FDA Guidance "Long Term Follow-Up After Administration of Human Gene Therapy Products Guidance for Industry. U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research," January 2020). Post-infusion monitoring for RCL will be performed by detecting vesicular stomatitis virus, glycoprotein (VSV-g) by qPCR.

An RCL may be generated during the production phase or subsequently after introduction of vector transduced cells into the patient. Replication competent lentivirus may be generated by homologous or non-homologous recombination between the transfer vector and packaging elements, or endogenous retroviral elements. An RCL resulting from the production phase of the lentivirus used in this trial is highly unlikely since elements are incorporated in the design of the vector system that minimize vector recombination and generation of RCL. Nevertheless, generation of an RCL following infusion of the vector product remains a theoretical possibility. The development of RCL could pose a risk to both the patient and their close contact(s), and therefore, monitoring for RCL will be conducted during the course of the trial. If a positive RCL assay result is obtained from a patient blood specimen, (as detected by VSV-g qPCR), the Investigator will be informed, and the patient will be rescheduled for a retest of the DNA test. Regulatory agencies and the gene therapy community have previously discussed measures to be taken should an RCL be confirmed in a patient. However, because the probability and characteristics of an RCL are unknown, no guidelines have been put in place.

Approaches that have been discussed for managing the patient are the following:

- Provide targeted antiretroviral therapies based on genotyping of the RCL
- Intensive follow up of patient in consultation with gene therapy experts, study investigators, HIV physicians, FDA, and NIH.

11.4 Testing for Replication Competent Lentivirus in Clinical Studies

Replication competent lentivirus will be monitored using a PCR-based assay that detects and measures copies of the gene coding for the vector's envelope protein, namely VSV-g that is necessary for the assembly of pseudotyped infectious lentiviral particles but absent from the vector's backbone. Replication competent lentivirus testing and monitoring will take place on:

- The cell product tested by or under the direction of the manufacturing facility responsible for the manufacturing and release of the vector.
- Patient PBMCs which will be collected for RCL testing at Baseline and then at week 12, 24, and 48 post day 0 infusion and then yearly for up to 15 years. If these tests are negative at all time points during the first year, collection of the yearly follow-up RCL samples may be discontinued for that patient. PBMC samples will be archived for up to 15 years post infusion. If VSV-g DNA copies are detected at any time point in the first year post-infusion, at a TCR^2 Therapeutics' designated centralized biorepository. Patient samples will continue to be tested for VSV-g DNA copies until VSV-g DNA copies are not detected for 3 consecutive annual assessments, then patient samples will be collected annually until year 15 and archived at a TCR^2 Therapeutics' designated centralized biorepository.

11.4.1 Safety Monitoring-Results

If a positive VSV-g DNA signal is obtained, the study Investigator will be informed, and the patient scheduled as soon as possible for a retest within one month after the initial positive result is reported to the Sponsor. A review by the SRT at TCR^2 Therapeutics will take place.

If the second test is positive, infusions for all patients receiving cells modified with the same vector lot will be postponed. The patient with the confirmed positive VSV-g signal will be scheduled for leukapheresis and a biological RCL test will be performed on the leukapheresis product. The biological RCL test assesses whether there is active production of infectious viral particles from the leukapheresis product.

If the biological RCL test is positive, TC-510 infusions will be halted in all patients. An action plan will be discussed with regulatory authorities and other experts as appropriate. Additional patients will not be treated with TC-510 until such a plan is completed, reviewed, and agreed upon with Regulatory Authorities.

11.4.2 Clonality and Insertional Oncogenesis

The occurrence of adverse events caused by insertional mutagenesis in 3 patients in a gene therapy trial for X-linked severe combined immunodeficiency following stem cell therapy emphasizes the potential for problems in translating this approach to the clinic. To date, clinically evident insertional mutagenesis has not been reported following the infusion of engineered adoptive T cells. Lentiviral vectors may have a lower risk than oncoretroviral vectors based on several considerations. Monitoring for T cell clonal outgrowth will be performed by

qPCR quantitation of the TC-510 transgene, and by complete blood count. If monoclonality is found, further studies including insertion site analysis will be considered.

11.4.2.1 Historical Experience with Retrovirally/Lentivirally Modified Gene Therapies

To date, malignant cell transformation after vector-mediated insertional mutagenesis has been observed in three clinical entities (X-linked Severe Combined Immunodeficiency [SCID-X1], Chronic Granulomatous Disease, and Wiskott-Aldrich Syndrome), which have involved the use of first-generation gamma-retroviral vectors harboring long terminal repeats (LTRs) with strong enhancer/promoter sequences (Hacein-Bey-Abina et al, 2003; Howe et al, 2008; Boztug et al, 2010; Stein et al, 2010; Persons and Baum, 2011). In contrast, data from lentiviral vector trials have demonstrated more polyclonal patterns of vector insertion (Cartier et al, 2009, Biffi et al, 2011), and despite the very high transduction efficiency achieved using lentiviral vectors, molecular clonality studies in patients enrolled in clinical trials have not indicated any reasons for concern to date (Schambach et al, 2013).

The integration pattern of lentiviral vectors tends to be inside active transcription units as opposed to upstream in the locus control region where the insertion would have a greater chance of up-regulating gene expression. In addition, lentiviral vectors have no enhancer activity in their LTR regions and have lower levels of poly-A read-through; all factors which may improve gene transfer safety (Zaiss et al, 2002). Available data suggest that lentiviral vectors are a safer alternative to oncoretroviral vectors for gene transfer, as shown in animal models (Montini et al, 2006).

Both retroviral and lentiviral safety have been demonstrated in modified T cell gene therapy trials. A long-term retrospective study of > 500 patient-years of collective patient samples tested for at least 11 years after infusion from three clinical trials using γ-retroviral modified T cells to express CD4-ζ CAR did not show evidence of transgene silencing, atypical gamma-retroviral integration patterns, or clonal expansion (Scholler et al, 2012). A favorable safety profile was also determined for a conditionally replicating HIV-derived lentivirus that delivered HIV envelope antisense to patient T cells. In 2 separate treatment cohort analyses, no evidence for insertional mutagenesis or enrichment of vector copies near proto-oncogenes was observed (Levine et al, 2006, and Wang et al, 2009). These data represent follow-up after 21 to 36 months (Levine et al, 2006) and 28 to 32 weeks (Wang et al, 2009). Another group reported no apparent risk of vector related adverse events following 263 infusions of autologous, lentiviral transduced T cells with a long RNA antisense to HIV-1 envelope (McGarrity et al, 2013). More recently ex vivo lentiviral transduced hematopoietic stem cells were used to correct an inherited storage disease in 3 children and in an inherited Wiskott-Aldrich Syndrome in 3 children with follow up for up to 24 months and 20 to 32 months, respectively. Finally, no evidence of in vivo vector-induced genotoxicity has been reported to date in any of the multiple CAR T cell trials, either completed or ongoing, for patients with cancer.

12 DOSE ESCALATION

The objective of the dose-escalation phase of the study (phase 1) will be the evaluation of DLTs and the determination of the RP2D. If the MTD (defined as the dose administered at 1 dose level below the dose in which DLTs were observed in > 33% of patients) is determined during the dose escalation phase, then the MTD will be the recommended RP2D. See table below for the TC-510 dose levels.

Each patient will receive a single dose of TC-510. At each dose, TC-510 will be given to at least 3 patients following lymphodepleting chemotherapy (fludarabine 30 mg/m^2/d on days -7 through -4 and cyclophosphamide 600 mg/m^2/d on days -6 through -4). Patients will be enrolled at the following dose levels (DL) to determine the RP2D:

Dose Level	**Transduced T cells on day 0**
DL -1	25×10^6
DL 0 (initial dose level)	**50×10^6**
DL 1	100×10^6
DL 2	130×10^6
DL 3	160×10^6
DL 4	200×10^6

- A variation on the target dose of 15% (i.e., ± 15%) will be allowed at each dose level.
- A planned stagger of 14 days will be instituted between the first patient infused at each dose level and the 2nd patient.
- Patients 2 and 3 in any given cohort may be infused simultaneously.
- In the event of excessive toxicity at the initial dose level (DL0), the study will resume at DL-1 (i.e., 25×10^6 transduced T cells on day 0).
- In the event a patient requires urgent treatment, they may be treated at a dose level previously cleared by the SRT (sponsor approval required)
- Dose escalation will be directed by the SRT upon review of the safety data.
- Once identified, up to 10 patients may be subsequently treated at the RP2D in an expansion cohort to further delineate the toxicity profile of TC-510 prior to advancing to the Phase 2 portion of the study. TC-510 retreatment may be possible for patients in the expansion cohort at the RP2D.
- For the purpose of safety onboarding, a 14-day stagger will be implemented for the first 3 patients treated at each new site (i.e., with no prior experience administering TC-510).
- In the event the RP2D is determined for one or more specific indications, dose escalation may continue further to determine the indication-specific RP2D for other indications where TC-510 may be tolerated at higher doses. Once the RP2D for a specific indication is

determined, enrollment of the Phase 2 specific arm for such indication may commence. Protocol stagger, safety observation and dose escalation rules will continue to apply to patients within the indication-specific dose escalation cohort(s) until the indication-specific RP2D is determined.

- The SRT may declare the RP2D at any time based on available safety data independent of whether the MTD has been reached or not.

13 SAFETY REVIEW TEAM

An SRT will be implemented for this study and will consist of at least 2 study investigators and the sponsor's chief medical officer, who will review the safety data during dose escalation stage of phase 1 of the study. An SRT charter, defining roles, responsibilities, and the process for safety review, will be available prior to the start of the study. The SRT will review safety and efficacy data and be chartered to make dose escalation recommendations based on safety data.

14 STATISTICAL AND DATA ANALYSIS

The objectives and endpoints for this study are described in section 4.1. Section 13 focuses on key aspects for the analysis and reporting of the primary and secondary efficacy and safety endpoints. Details for all endpoint analyses will be provided in the final statistical analysis plan, which will be finalized prior to the last patient last visit. All efficacy and safety data will be summarized by cohort and across cohorts. Any notable differences between cohorts will be described. The primary analysis will occur at the time of clinical cut-off. There are no planned formal interim analyses; however, at various time points throughout the clinical trial there may be informal database cuts to review and present data externally. There will be no impact on statistical significance

14.1 Study Populations

- **ITT Population**: all patients who sign the informed consent form and undergo leukapheresis.
- **mITT Population**: all patients enrolled and treated with the planned dose of study drug. This analysis set will be used for all analyses of objective response and endpoints based on objective response (objective response, DoR, PFS) for both, the phase 1 and phase 2 portions of the study.
- The mITT population is the primary analysis population for efficacy and safety evaluations. The ITT population is the secondary analysis population for efficacy and safety evaluations. If the ITT and mITT populations are identical, only analyses associated to the ITT population will be reported.
- **DLT Evaluable Set:** (phase 1 only) will include patients treated in the phase 1 portion of the study who received study drug and were followed for at least 28 days post-infusion or who have a DLT within the 28 days of the first study drug.
- **Safety Set**: the safety set is defined as all patients treated with any dose of study drug.

14.2 Sample Size Calculation

The co-primary clinical endpoints for efficacy are ORR defined as the proportion of patients with a CR or PR via independently reviewed RECIST v 1.1 relative to the total number of patients in the analysis population, and DCR, defined as the ORR plus the proportion of patients with SD for at least 12 weeks via independently reviewed RECIST v 1.1 relative to the total number of patients in the analysis population. TC-510 will be considered for further development if there is at least 1 response in a disease cohort. For each planned ph2 cohort, 20 patients will have an 98% probability of having at least 1 response if the true response rate is $p_A = 0.2$.

14.3 Statistical Analysis

The data will be analyzed by the Sponsor and/or designated clinical research organization (CRO). Any data analysis carried out independently by the Investigator should be submitted to the Sponsor before publication or presentation.

14.3.1 General Considerations

Tabulations of summary statistics, graphical presentations, and statistical analyses will be performed with SAS® software, Version 9.2 or higher. No specific statistical hypotheses are being evaluated with respect to primary, secondary, or exploratory objectives and endpoints. All analyses will be descriptive and exploratory.

Descriptive statistics for continuous variables in summary tables will include the number of patients in the analysis (n), mean, standard deviation, median, and range (minimum, maximum). Descriptive statistics for categorical variables in summary tables will include counts and percentages. Graphical summaries of the data may be presented. Assessments will be displayed by cohort and time as well as across cohorts for specific parameters. Time to event endpoints will be summarized and displayed graphically using Kaplan-Meier (KM) methodology to estimate the median, and the 25th and 75th percentiles. Two-sided 80% confidence intervals will be produced. Overall survival may be assessed at fixed time points such as 6 months and 1 year using KM methods.

The most recent non-missing measurement collected prior to the first administration of study drug in each phase (i.e., lymphodepleting chemotherapy) will be used as the baseline value in the corresponding phase.

All data obtained on the CRF and entered into the database will be provided in separate data listings showing individual patient values by cohort and time, if applicable. The planning and reporting of statistical analysis will be carried out as described in CRO's SOPs governing clinical studies.

14.3.2 Patient Demographics and Other Baseline Characteristics

For phase 1, the number and percentage of patients in the following disposition categories will be summarized by dosing group and overall:

- Patients screened
- Screen failures and reasons for screen failure
- Included in the ITT
- Included in the DLT evaluable set (phase 1 only)
- Included in the safety set
- Included in the mITT
- Completed the follow-up visit (safety follow-up at 28 days post TC-510 infusion)
- Discontinued from the study with reasons for discontinuation

For phase 2, the number and percentage of patients in the following disposition categories will be summarized by cancer cohort (MPM, serous ovarian adenocarcinoma, pancreatic adenocarcinoma, colorectal cancer, and triple negative breast cancer) and overall:

- Patients screened
- Screen failures and reasons for screen failure
- Included in the ITT
- Included in the safety set
- Included in the mITT
- Completed the follow-up visit (Week 24 disease response assessment)
- Discontinued from the study with reasons for discontinuation

Demographic information: age, sex, race, ethnicity, height, weight, body mass index, and other baseline measures such as ECOG performance status score, cancer type, and number of prior chemotherapy regimens, will be summarized by dosing group and overall, using descriptive statistics.

Medical history will be summarized using descriptive statistics.

14.3.3 Study Treatments and Concomitant Therapy

Study drug information, including the dose of TC-510, duration of the infusion, saline flush volume, and the exposure to lymphodepleting chemotherapy, will be summarized by dosing group.

Any prior anti-cancer drug therapies, surgeries, and radiotherapies will be summarized by dosing group using variables as reported in CRF.

Other prior and concurrent therapy or medication for each phase, given to a patient from the date of enrollment into the study (when the patient completes the leukapheresis eligibility testing required during screening) up to the follow-up visit in each of the phase, will be tabulated by counts and percentages using the latest version of World Health Organization Drug Dictionary Enhanced in the study.

14.3.4 Phase 1 Analysis

14.3.4.1 Primary Analysis

The primary analysis of phase 1 study is to evaluate the safety profile of TC-510 and to establish the RP2D.

The number and percent of patients in the DLT evaluable set who experienced DLTs from the first administration of study drug up to 28 days post study drug treatment will be summarized by dosing group, separately for patients with and without the lymphodepleting chemotherapy regimen. The RP2D will be declared by the SRT upon evaluation of the safety data. If during phase 1 of the study the MTD (i.e., the highest dose of study drug that does not cause a DLT in > 33% of patients) is determined, then the latter will be declared to be the RP2D.

The overall safety profile of the study drug will be evaluated in the safety set by:

- AEs
- Vital signs
- Clinical laboratory measurements

All safety data will be presented in patient data listings.

Adverse events will be coded according to the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) during the study. Adverse events will be classified as pre-treatment or treatment-emergent as follows:

- Pre-treatment AEs are defined as adverse events that were reported or worsened after signing the Treatment ICF up to the first administration of protocol defined therapy (i.e. lymphodepleting chemotherapy).
- Treatment-emergent adverse events (TEAEs) are defined as adverse events that were reported or worsened on or after the first administration of protocol defined therapy (i.e. lymphodepleting chemotherapy) through the follow-up visit.

The number and percent of patients with TEAEs, drug-related TEAEs, TEAEs of NCI-CTCAE grade 3 or higher, SAEs, drug-related SAEs, and premature discontinuation from the study drug

and from the study participation due to adverse events will be presented by the dosing group, MedDRA preferred term, and system organ class, separately for patients with and without the lymphodepleting chemotherapy regimen.

Mortality rates will be summarized during entire phase 1, and up to day 15 and day 25 by dosing group. The 80% confidence intervals will be provided in mortality rates for these time intervals. In addition, KM curves will be provided for time to death.

Descriptive statistics for actual values and change from baseline values for vital signs and clinical laboratory measurements will be presented by visit and dosing group. In addition, for each safety parameter and each visit, the occurrence of post-baseline values outside the normal limits will be summarized by dosing group (not applicable to qualitative parameters). Shift tables based on the normal range will also be presented.

For laboratory evaluations, the worst grades at baseline, each post-baseline visit and on-treatment for applicable hematological, biochemistry, and coagulation parameters will be derived according to NCI-CTCAE. The number and percentage of patients by grade at each visit and change in grade from baseline will be summarized.

14.3.4.2 Secondary Analysis

The secondary analysis will be performed to assess the preliminary efficacy profile of the study drug, using outcome measures of ORR, DCR, DoR, TTR, PFS, and OS. The primary analysis population for efficacy analysis is the mITT population. The tumor response will be based on the reconciled results assessed by investigators/radiologist and by the independent read of image scans performed in a central imaging facility.

The ORR will be estimated as the proportion of responders, defined as a patient whose best overall response is CR or PR during the treatment period, considering patients without response assessment as non-responders. The DCR is defined as the ORR plus the proportion of patients with SD lasting at least 12 weeks. The estimates and 80% exact binomial confidence intervals for ORR and DCR will be presented by dosing group, separately for patients with and without the lymphodepleting chemotherapy. If the total number of patients in the dosing group is < 3, the confidence interval (CI) will not be calculated.

The DoR is the interval from date of initial documented response (CR or PR) to the first documented date of disease progression or death. Patients who do not relapse or die are censored at the day of their last radiographic tumor assessment.

The TTR is defined as the time from the first administration of study drug to the first objective tumor response (CR or PR). Patients who do not have any observed tumor response are censored at the day of disease progression, death, or last radiographic tumor assessment, whichever comes first.

The PFS is defined as the time from the date of TC-510 infusion to the date of progressive disease or death from any cause. Patients who have neither progressed nor died will be censored at the day of their last radiographic tumor assessment if available, or date of enrollment if no post initial tumor assessment is available. If deaths or progressive disease occurs after 2 or more missing radiographic visits, censoring will occur at the date of last radiographic visit.

The OS is defined as the time from the date of TC-510 infusion to the death from any cause. Patients who do not die on study will be censored at their last date known to be alive.

For time-to-event endpoints (DoR, TTR, PFS, and OS), the KM curves and estimates will be provided for each dosing group if estimable, separately for patients with and without the lymphodepleting chemotherapy. The median, 25th percentile, and 75th percentile of PFS and associated 80% CIs will be provided based on the KM estimates. The KM estimates of overall survival may be assessed at fixed time points such as 6 months and 1 year.

14.3.5 Phase 2 Analysis

14.3.5.1 Primary Analysis

The co-primary endpoints ORR and DCR will be estimated by the same method as described in section 14.3.4.2. The estimate and 80% exact binomial confidence interval will be presented by cancer cohort (MPM, serous ovarian adenocarcinoma, pancreatic adenocarcinoma, colorectal cancer, and triple negative breast cancer). The primary analysis will be performed in the mITT population. All analysis will be repeated in the ITT population if the ITT and mITT populations are not identical.

14.3.5.2 Secondary Analyses

The primary analysis population for the secondary analyses is the mITT population. All analysis will be repeated in the ITT population if the ITT and mITT populations are not identical.

The definitions of TTR, DoR, PFS, and OS are the same as described in section 14.3.4.2. The KM curves and estimates of these time-to-event variables will be provided for each cancer cohort. The median, 25th percentile, and 75th percentile of PFS and associated 80% CIs will be provided based on the KM estimates. The KM estimates of overall survival may be assessed at fixed time points such as 6 months and 1 year.

For patients who experience progressive disease following TC-510 therapy and receive a second TC-510 infusion, the response rate for the second infusion will be estimated as the proportion of responders whose best overall response is CR or PR post the second infusion, considering patients without response assessment as non-responders. The estimate and 80% exact binomial confidence interval will be presented by cancer cohort.

14.3.5.3 Exploratory Analyses

The TC-510 kinetics and other correlative analyses to be performed upon TC-510 infusion, such as T cell phenotype, persistence, infiltration, genomic sequencing data, tissue expression data, and tumor mutation profile will be included in separate analysis plans.

The patient-reported outcomes will be summarized descriptively in the mITT population. For EQ-5D-3L, the health status index scores derived using the individual responses to the EQ-5D-3L descriptive system and country specific value sets will be summarized as categorical variables for each domain (mobility, self-care, usual activities, pain, and anxiety) and as a continue variable for the overall by visit and cancer cohort. The change from baseline in the overall index score will also be summarized by visit and cancer cohort. The EuroQoL Visual Analog Scale and change from baseline will also be summarized by visit and cancer cohort.

14.3.5.4 Safety Analyses

The overall safety profile of the study drug in phase 2 will be evaluated in the safety set by

- AEs
- Vital signs
- Clinical laboratory measurements

The analyses will follow what has been described in section 14.3.4.1. The phase 2 safety analysis will be descriptively and presented by cancer cohort and the overall safety set.

For PRO-CTCAE, the scores will be summarized as categorical variables for each AE item and each attribute (frequency, severity, interference, and presence/absence). The PRO-CTCAE maximum score post-baseline will also be summarized for each item and attribute.

15 CLINICAL SUPPLIES

15.1 Packaging and Labelling

Selected, qualified manufacturing sites will manufacture, package, and label cell product for each individual patient in accordance with applicable regulatory requirements.

Labels will include batch number, protocol number, number of transduced cells, and the patient's unique study identification number.

15.2 Standard Policies and Product Return

TC-510 investigational product must be received by a designated person at the site, handled, and stored safely and properly, and kept in a secure location to which only the Investigator and designated personnel have access. TC-510 will be dispensed only in accordance with the

protocol. The Investigator is responsible for keeping accurate records of the investigational product received from the sponsor, the amount dispensed to and any unused investigational product remaining at the conclusion of the study. Contact the sponsor or designee regarding any questions concerning the investigational product. Sites should contact the sponsor or designee for specific instructions for investigational product returns or destruction and appropriate documentation for drug accountability.

15.3 Storage and Handling

The TC-510 product that is received at the site from the manufacturer will be stored at ≤ -150°C until being ordered by the Investigator (or designee) to be infused. The cells will be thawed and infused as specified in section 7.

15.4 Product Accountability

The TC-510 product provided for this study is for investigational use only as directed in the protocol. It is the Investigator/institution's responsibility to establish a system for handling study treatments, including the investigational product TC-510, so as to ensure that:

- Delivery of TC-510 product is correctly received and recorded by a responsible person
- TC-510 product is handled and stored safely and properly as stated on the label
- TC-510 product is only administered to study patients in accordance with the protocol
- Any unused TC-510 product is accounted for in the site records before returning to the Sponsor (or designee)

At the end of the study, it must be possible to reconcile delivery records with records of usage and destroyed/returned stock. Records of usage should include the identification of the person to whom the investigational product was dispensed and the quantity and date of dispensing. This record is in addition to any drug accountability information recorded on the CRF. Any discrepancies must be accounted for on the appropriate forms.

16 DATA HANDLING, RECORD KEEPING, AND STUDY MONITORING

16.1 Data Management

An electronic data capture (EDC) system will be used to collect data pertaining to this trial. Trial data will be captured through a CRF. Within the EDC system the CRF data will be entered by the site staff and all source document verification and data cleaning will be performed by the sponsor or designee (e.g., CRO).

The specifications for the EDC system will be documented and approved before the EDC system is released for live use. The validation of the CRF data will be defined in a data management

plan. As data are entered into the CRF, the validation checks will be performed and where necessary, queries will be raised. All queries raised will be held in the EDC database.

The EDC system is a validated software program that has been designed to comply with 21 CFR Part 11 requirements. All users will access the system via unique user name and password. A full audit history of all actions performed within the system will be maintained. User accounts ensure that each user can only perform the tasks applicable to their role and only have access to the data applicable to their role.

Standard coding dictionaries, World Health Organization Drug and MedDRA will be used to code medical history, concomitant medications, and AEs.

When all data have been entered and all data cleaning is complete the data will be locked and made available for analysis and reporting. Upon study completion all CRF data, including all associated queries and audit history, will be made available in PDF format to both the study Sponsor and the sites.

16.2 Case Report Forms

For each patient enrolled, the completed CRF must be reviewed and signed by the principal Investigator or authorized delegate. If a patient withdraws from the study, the reason must be noted on the CRF. The Investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports.

16.3 Site Documentation and Source Data

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents are classified into 2 different categories: (1) investigator site file (ISF), and (2) patient specific source documents.

The Investigator is responsible for maintaining a complete and accurate ISF containing essential documents as required by ICH GCP.

Source documents contain the results of all the activities of a clinical investigation. Source documents include but are not limited to patient medical records/progress notes, appointment book, original laboratory reports, ECG printouts, CT/MRI scans, pathology, and special assessment reports, and signed ICFs. The CRF will never be considered source data.
The Investigator must ensure the availability of source documents from which the information on the CRF was derived.

The Investigator must permit authorized representatives of the sponsor, the respective national, local, or foreign regulatory authorities, the IRB/Independent Ethics Committee (IEC) and auditors to inspect facilities and to have direct access to the ISF and all source documents relevant to this study regardless of the type of media.

The Investigator must keep all essential study documents including source data on file for at least 25 years after completion or discontinuation of the study. After that period of time the documents may be destroyed, patient to local regulations. The Investigator will not dispose of any records relevant to this study without written permission from the sponsor.

16.4 Study Monitoring

Study monitoring will be conducted by the sponsor or designated CRO. The key sponsor contact, monitors, auditors, or regulatory inspectors are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and verifying source documents and records assuring that patient confidentially is respected.

The monitor is responsible for source document verification of CRF data at regular intervals during the study. Protocol adherence, accuracy, and consistency of study conduct and data collection with respect to local regulations will be confirmed. Monitors will have access to patient records. By signing the Investigator agreement, the Investigator agrees to cooperate with the monitor to address and resolve issues identified during monitoring visits.

In accordance with ICH GCP and the audit plan, a site may be chosen for a site audit. A site audit would include, but is not limited to, an inspection of the facility(ies), review of patient and study related records, and compliance with protocol requirements as well as ICH GCP and applicable regulatory policies. Regulatory agencies may also inspect investigative sites during or after the study. The Investigator (or designee) should contact TCR^2 Therapeutics immediately if this occurs and it will provide copies of correspondence relating to requests for an inspection of the site facilities.

All data will be collected in a CRF system. All entries must be completed in English and concomitant medications should be identified by tradenames. For further details surrounding the completion of CRFs, please refer to the CRF completion guidelines.

17 REGULATORY AND ETHICAL CONSIDERATIONS

17.1 Independent Ethics Committees

The final study protocol and patient Informed Consent documentation will be approved by the IRB/IEC and any other site level committee deemed appropriate by the institution. Approval from each applicable committee will be received in writing before initiation of the study.

Protocol amendments must also be approved by the IRB/IEC (and other committees as applicable) before implementation, except in the case of changes made to protect patients from immediate hazard, which may be implemented immediately.

17.2 Local Regulations/Declaration of Helsinki

The Investigator will ensure that this study is conducted in full compliance with the principals of the "Declaration of Helsinki" or with the laws and regulations of the country in which the research is conducted, whichever, affords the greater protection to the patient. The study must fully adhere to the principles outlined in "Guideline for Good Clinical practice" ICH Tripartite Guideline (January 1997) or with local law if it affords greater protection to the patient.

17.3 Informed Consent

Eligible patients may only be included in the study after providing written (witnessed, where required by law or regulation), IRB/IEC/Research Ethics Board (REB)-approved informed consent.

Informed consent must be obtained before conducting any study-specific procedures (i.e., all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the patient source documents. The date when a patient's informed consent was actually obtained will be captured in his/her CRF.

TCR^2 Therapeutics will provide to investigators, in a separate document, proposed ICFs (Pre-Screening ICF and Treatment ICF) that is considered appropriate for this study and complies with the ICH GCP guideline and regulatory requirements. Any changes to this ICF suggested by the Investigator must be agreed to by TCR^2 Therapeutics before submission to the IRB/IEC/REB, and a copy of the approved version must be provided to the TCR^2 Therapeutics monitor after IRB/IEC/REB approval.

Female patients of child-bearing potential should be informed that taking the study medication may involve unknown risks to the fetus if pregnancy were to occur during the study and agree that in order to participate in the study they must adhere to the contraception requirement for the duration of the study. If there is any question that the patient will not reliably comply, they should not be entered in the study.

17.4 Confidentiality

Patient confidentiality must be contained at all material submitted to the key sponsor contact. The following rules are to be applied:

- Patients will be identified by a unique identification number
- Date of birth will be reported according with local laws and regulations
- Age at the time of enrollment

For reporting of SAEs, patients will be identified by their respective patient identification number, initials, and date of birth (as per their local reporting requirements for both initials and date of birth).

Per federal regulations and ICH/GCP guidelines, investigators and institutions are required to permit authorization to the sponsor, CRO, IRB/IEC, and regulatory agencies to patient's original source documents for verification of study data. The Investigator is responsible for informing potential patients that such individuals will have access to their medical records which includes personal information.

17.5 Protocol Adherence

Investigators ascertain they will apply due diligence to avoid protocol deviations. Under no circumstances should the Investigator contact TCR^2 Therapeutics or its agents, if any, monitoring the study to request approval of a protocol deviation, as no authorized deviations are permitted. If the Investigator feels a protocol deviation would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by TCR^2 Therapeutics and approved by the IRB/IEC/REB it cannot be implemented. All significant protocol deviations will be recorded and reported in the clinical study report.

17.6 Study Suspension, Study Termination, and Study Completion

The study will be suspended in the event of any death within 28 days of receiving TC-510 or occurrence of 2 or more grade 4 toxicities (with the exception of toxicities expected with lymphodepleting chemotherapy), pending comprehensive safety review before enrolling additional subjects.

The sponsor may suspend or terminate the study at any time for any reason. If the study is suspended or terminated the sponsor will ensure that applicable sites, regulatory agencies and IRBs/IECs are notified as appropriate.

If the Investigator stops/terminates the study at his/her site, the sponsor must be notified. The sponsor will ensure that regulatory agencies and IRBs/IECs are notified as appropriate.

The sponsor will ensure that end-of-study declarations are made to the relevant regulatory agencies/IECs in accordance with local regulations.

17.7 Public Posting of Study Information

The sponsor is responsible for posting appropriate study information on applicable clinical study registry websites. Information included in clinical study registries may include participating Investigator's names and contact information.

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Appendix A:PART 1 -Schedule of Events for Study TCR2-21-01

D= Day, W= Week, M= Month, Q3W= Every 3 weeks, Q3M= Every 3 months, EOA= End of Active Follow-Up, PD= Progressive disease.

	Pre-Screening				Interventional Phase																							
		Leuka-pheresis Eligibil-ity[4]	Leuka phere-sis	Base-line[4]	Lymphodepleting Chemotherapy				TC-510 Infusion	Post-Infusion Assessments[19]																Follow Up Every 12 Weeks	End of Active Follow-up (EOA)[20]
Timepoint	-	-	Enroll-ment	D-21 to D-8[4]	D-7	D-6	D-5	D-4	D0	D1	D2	D3	D4	D7	D10	D21	W4	W6	W8	W9	W 12	W 15	W 18	W 21	W 24	Q12W	Week 108/ EOS
Visit Window	-	-14 Days of Visit 3	-	-	-	-	-	-	-	± 1 Day					± 2 Days						± 7 Days					± 14 Days	
Informed Consent[1]	X			X																							
IRT[2]	X	X	X						X																		
Demographics	X																										
Leukapheresis Inclusion/Exclusion		X																									
Treatment Inclusion/Exclusion				X																							
Disease History	X	X																									
MSLN expression	X[21]			X[3,21]													X										
Medical History		X																									
Physical Exam		X		X					X					X	X		X	X	X		X	X			X	Weeks 36, 48, 60, 72, 84, and 96	X
Prior Anti-Cancer Therapies		X		X																							
Concomitant Medications		X - Concomitant medications should be assessed at every visit																									
ECOG Performance Status		X		X										X	X	X	X	X	X		X	X			X	Weeks 36, 48, 60, 72, 84, and 96	X
Vital Signs / Weight		X		X				X	X[5]	X	X	X	X	X	X		X	X	X		X	X			X	X	X
ECG				X[6]																							
Echo / MUGA[7]				X[7]											X[7]												
Troponin				X[7]											X[7]												
CT Scan / MRI		X[7]		X[7]													X		X		X				X	Weeks 36, 48, 60, 72, 84, and 96	X
PET Scan				X[7]													X				X						X
Hematology		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical Chemistry		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X	X	X	X
Coagulation[11]		X		X					X			X		X	X		X										
Pregnancy Test				X																							

	Pre-Screening				Interventional Phase																							
		Leuka-pheresis Eligibil-ity[4]	Leuka phere-sis	Base-line[4]	Lymphodepleting Chemotherapy				TC-510 Infusion	Post-Infusion Assessments[19]																Follow Up Every 12 Weeks	End of Active Follow-up (EOA)[20]
Timepoint	-	-	Enroll-ment	D-21 to D-8[4]	D-7	D-6	D-5	D-4	D0	D1	D2	D3	D4	D7	D10	D21	W4	W6	W8	W9	W 12	W 15	W 18	W 21	W 24	Q12W	Week 108/ EOS
Visit Window	-	-14 Days of Visit 3	-	-	-	-	-	-	-	± 1 Day					± 2 Days					± 7 Days						± 14 Days	
Urinalysis				X																							
Infectious Disease Screening[8]		X																									
Rapid Influenza Test and/or Respiratory Viral Panel[9]				X																							
CMV PCR				X					X[10]						X[10]		X[10]	X[10]	X[10]								
Thyroid Function Tests				X																							
C-reactive Protein and Ferritin[11]				X					X			X		X	X		X										
CA-125				X					X			X		X	X	X	X	X	X		X	X	X	X	X	Week 36, and Week 48	X
Renal Function Tests				X																							
Adverse Events	X – Adverse events should be assessed at every visit																										
Leukapheresis			X																								
Fludarabine					X	X	X	X																			
Cyclophosphamide						X	X	X																			
Mesna[12]						X	X	X																			
G-CSF[13]								X																			
TC-510 Infusion									X																		
EQ-5D-3L				X					X						X	X	X		X		X		X		X	Weeks 36, 48, 60, 72, 84, and 96	X
PRO-CTCAE				X					X						X	X	X		X		X		X		X	Weeks 36, 48, 60, 72, 84, and 96	X
Tumor Biopsy	X			X[3]														X									X
Leukapheresis, Excess Manufacturing Material, and T-Cell Product Samples for Correlative Studies[14]			X																								
MSLN and Megakaryocyte Potentiating Factor Level (Serum)				X					X					X	X	X	X	X	X		X	X	X	X	X	Weeks 36 and 48	X

	Pre-Screening				Interventional Phase																						
		Leuka-pheresis Eligibil-ity[4]	Leuka phere-sis	Base-line[4]	Lymphodepleting Chemotherapy				TC-510 Infusion	Post-Infusion Assessments[19]															Follow Up Every 12 Weeks	End of Active Follow-up (EOA)[20]
Timepoint	-	-	Enroll-ment	D-21 to D-8[4]	D-7	D-6	D-5	D-4	D0	D1	D2	D3	D4	D7	D10	D21	W4	W6	W8	W9	W 12	W 15	W 18	W 21	W 24	Q12W	Week 108/ EOS
Visit Window	-	-14 Days of Visit 3	-	-	-	-	-	-	-	± 1 Day					± 2 Days						± 7 Days					± 14 Days	
PBMC (Functional Assays)[15]			X	X					X					X			X				X				X	Weeks 48, 108 and/or EOS	
Anti-TC-510 Antibodies									X[16]										X		X[17]				X[17]	X[17]	
Cytokine Analyses[11]				X					X[11, 16]	X		X		X	X	X	X	X	X	X	X		X		X		X
Collection of Serosal Effusions					If effusion present																						
TC-510 Cellular Kinetics (Flow Cytometry) and Peripheral Blood T Cell Phenotype[15]			X	X					X[16]	X		X		X	X	X	X	X	X	X	X	X	X	X	X	Weeks 48, 72, 108, and/or EOS	
Peripheral Blood TC-510 Cellular Kinetics (qPCR)[15]				X					X[16]	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	
RCL by VSV-g qPCR[18]				X																	X				X	Weeks 48, 108 and/or EOS	
ctDNA				X												X		X		X							X

1. Patients will sign a Pre-Screening informed consent which, for applicable patients, will allow new tumor tissue sample to be obtained or provide permission to use banked tumor samples. This ICF will also allow for leukapheresis eligibility procedures as well as the leukapheresis to occur, for patients meeting eligibility. Before Baseline eligibility can be assessed, patients must provide written Treatment Informed Consent, which will allow for treatment eligibility to be assessed and for the patient to proceed with the first protocol-defined therapy and the rest of the trial.
2. IRT will be utilized for patient registration and cell tracking milestones.
3. At Baseline, MSLN testing must be performed on a biopsy sample obtained within 6 months of the planned first protocol defined therapy. The pre-screening biopsy may fulfill the Baseline biopsy requirement if collected within 6 months of the start of treatment (provided there is adequate tissue for correlative testing). If biopsy is deemed unsafe and/or inaccessible by the PI, please consult the Sponsor's medical monitor.
4. The Leukapheresis Eligibility visit should be ≤ 14 days of the Leukapheresis visit, with the exception of infectious disease testing, CT/MRI scan, PET scan, and echocardiogram/MUGA. See assessment specific footnotes for allowed windows. The Baseline visit should be ≤ 14 days of first protocol-defined treatment (lymphodepletion) with the exception of CT/MRI scan, PET scan, echocardiogram/MUGA, and fresh baseline biopsy. See assessment specific footnotes for allowed windows.
5. Timed vital signs (temperature, pulse, respirations, SPO2, and blood pressure) on day of TC-510 infusion should be taken pre-infusion 10 minutes (± 3 minutes), and at 5 minutes (± 3 minutes), 15 minutes (± 3 minutes), 30 minutes (± 5 minutes), 1 hour (± 5 minutes), 1.5 hours (± 5 minutes), 2 hours (± 5 minutes), and 4 hours (± 5 minutes) after the infusion has started.

6. ECG will be taken at Baseline Visit to determine eligibility.
7. Information regarding CT scan (MRI when CT scan not feasible), PET scan, and echocardiogram/MUGA scans, performed as standard of care assessments within 4 weeks prior to Leukapheresis and/or Treatment Eligibility will be acceptable. Repeat Echocardiogram to be performed between days 10 and 14 post-infusion or at any time point if clinically indicated. Blood troponin levels should be tested on the days when Echocardiogram/MUGA scans are performed.
8. Testing for infectious disease markers is required only at Leukapheresis Eligibility and does not need to be repeated at baseline to satisfy the inclusion / exclusion criteria. Testing performed within 3 weeks of leukapheresis is acceptable. If performed outside the treating institution the results will be accepted as long as the outside lab's corresponding CAP, CLIA, and infectious disease reference ranges are collected. Patients who are hepatitis B surface antigen negative but are hepatitis B core antibody positive must have undetectable hepatitis B DNA. Patients who are HCV antibody positive will be screened for HCV RNA by any RT PCR or bDNA assay.
9. All patients must have undergone a rapid influenza diagnostic test and/or respiratory viral panel (as per institutional guidelines) within 14 days prior to the first protocol defined therapy. Refer to the eligibility criteria for further information regarding testing.
10. Test required only if seropositive at baseline.
11. If cytokine release syndrome is suspected, local and central cytokine (and local if available), C-reactive protein, and ferritin levels as well as coagulation parameters (e.g., PT, aPTT, INR, fibrinogen, D-Dimer) should be measured approximately every 24-48 hours until symptoms are improving or an alternative diagnosis is confirmed.
12. Mesna will be given to cover the duration of cyclophosphamide chemotherapy according to Institutional guidelines.
13. G-CSF may be given from 24 hours after the last dose of cyclophosphamide until resolution of neutropenia following Institutional practice or physician's preference. Long-acting (pegylated) G-CSF may be substituted according to Institutional practice or physician's preference as described in section 8.4 Dispensation/Delivery of GM-CSF is not permitted during the study.
14. Material from leukapheresis collection as well as excess manufacturing material (material collected during the manufacturing process) may be used for correlative testing.
15. These research assessments are collected via PMBC collection tubes, centrifuged at the investigative site, and sent to a central lab before being sent separately to third party laboratories for analysis. Sites should complete sample collection as outlined in the Laboratory Visit Schedule located in the Central Laboratory Services Manual.
16. Samples to be collected day 0 pre-infusion.
17. For plasma samples that demonstrate positive anti-TC-510 human antibodies at the week 8 visit an attempt should be made to obtain and test additional plasma samples at week 12, 24, and then at 3-month intervals until the antibody levels return to baseline (or become negative) or up to 1 year from date of TC-510 infusion, whichever occurs first.
18. RCL safety samples are collected on Day 0 (pre-infusion), and post infusion at Week 12, Week 24, Week 48, Week 108 and/or EOA, and up to year 15 as applicable. If negative at all timepoints, during the first year, collection of the yearly RCL follow-up samples may be discontinued for that patient.
19. If a patient completes the study (i.e., due to withdrawal of consent) after receiving TC-510 infusion, all procedures and assessments listed in the discontinuation/completion visit must be performed, unless performed within the previous 30 days. A tumor biopsy is still requested at progression or post progression confirmation (e.g., from an excisional surgery), irrespective of the previous biopsy occurring within the prior 30 days.
20. Patients that come off Active Follow-Up for any reason should perform an EOA visit and then be followed in Long Term Follow-Up as per Schedule of Events Part 2
21. Patients with Epithelioid MPM are not required to have tissue tested for MSLN expression at pre-screening and baseline

APPENDIX A: PART 2 – LONG-TERM FOLLOW-UP SCHEDULE OF EVENTS

Note: Long-term Follow-up (LTFU) includes all patients who have come off Active Follow-up for any reason (eg, radiographically confirmed or clinically assessed disease progression, adverse events, investigator decision, patient decision, as well as patients who have completed Active Follow-Up by completing Week 108). Patients in LTFU will be followed for up to approximately 5 years post the most recent TC-510 infusion and assessments will be performed according to the schedule below.

	Long Term Follow-Up														
Timepoint (Weeks)	**W12**	**W24**	**W36**	**W48**	**W60**	**W72**	**W84**	**W96**	**W108**	**W132**	**W156**	**W180**	**W204**	**W228**	**W252/ EOS**
Timepoint (Years)				**Y1**					**Y2**		**Y3**		**Y4**		**Y5**
Visit Window	**+/- 7 Days**	**+/- 14 Days**													
Survival Follow-Up[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam	X	X		X		X			X		X		X		X
Hematology	X	X		X					X		X		X		X
Adverse Events	X (protocol defined adverse events including new malignancies and other significant findings should be reported upon investigator knowledge which may not align with scheduled visits and can be obtained via phone contact)														
Concomitant Medications	X (protocol defined concomitant medications including anti-cancer therapies or surgeries should be reported at each visit)														
Progression Information (only for patients who enter LTFU with controlled disease)	X (For all patients who have controlled disease (with stable disease or better), progression status should be assessed (if applicable))														
TC-510 cellular kinetics (flow cytometry) and peripheral blood T cell phenotype[b,c]	X	X		X		X[c]			X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]
Peripheral blood TC-510 cellular kinetics (qPCR)[b,d]	X	X		X		X[d]			X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[d]
RCL by VSV-g qPCR[b,e]	X	X		X					X[e]		X[e]		X[e]		X[e]
Tissue Collection in the event a new or secondary malignancy	X (If a subject has a new malignancy or secondary malignancy, attempts should be made to obtain tissue for transgene analysis)														

a) Survival Follow-Up will occur every 12 weeks until W108/Y2, then every 6 months until Y5/EOS. Clinic visit, telephone contact, and/or email contact are acceptable means of survival confirmation; contact should be documented in the patient's medical record and CRF.
b) These research assessments are collected via PMBC collection tubes, centrifuged at the investigative site, and sent to a central lab before being sent separately to third party laboratories for analysis. Sites should complete sample collection as outlined in the Laboratory Manual.

c) TC-510 cellular kinetics via flow cytometry will be performed at Weeks 12, 24, 48, and every 6 months thereafter. TC-510 detection analysis by flow can be discontinued if undetectable in 2 consecutive analyses performed at least 6 months apart.
d) Peripheral blood TC-510 cellular kinetics via qPCR will be performed at Weeks 12, 24, 48, and then every 6 months until Y5/EOS, or until the time when TRuC product transgene becomes undetectable by 2 consecutive analyses.
e) If RCL safety samples are negative at all timepoints during the first year, collection of the yearly RCL follow-up samples may be discontinued for that patient.

Appendix B: Clinical Laboratory Parameters

Table 10. Clinical Laboratory Parameters		
Cardiac Assessment		
Troponin		
Hematology		
RBC Count	Hemoglobin	Hematocrit
Platelet Count	WBC Total Count	Neutrophils Absolute Count
Lymphocytes Absolute Count	Monocytes Absolute Count	Eosinophils Absolute Count
Basophils Absolute Count	Neutrophils %	Lymphocyte %
Monocytes %	Eosinophils %	Basophils %
Chemistry		
Total Bilirubin	Bilirubin Direct	Alkaline Phosphatase
ALT (SGPT)	AST (SGOT)	Albumin
Total Protein	Creatinine	Urea (BUN)
Glucose (fasting)	Chloride	Sodium
Potassium	Calcium	Bicarbonate
Lipase	Uric Acid	Glomerular Filtration Rate
Magnesium		
Coagulation		
Prothrombin Time	Activated Partial Thromboplastin Time	
Fibrinogen	D-Dimer	
Infectious Disease Screening		
HBsAg	HBsAb	HBcAb
HBV-DNA	Hepatitis C RNA	Human T-lymphotropic Virus Type 1
HCV	HIV	Human T-lymphotropic Virus Type 2
Cytomegalovirus		
Kidney Function		
Urinalysis with Microscopy		
Routine Assessments		
CA-125	Ferritin	
ALT = alanine aminotransferase; AST = aspartate aminotransferase; HCV = hepatitis C virus; HIV = human immunodeficiency virus; RBC = red blood cell; WBC = white blood cell.		