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TITLE: A Phase I Study to Evaluate PSCA-targeting Chimeric Antigen Receptor (CAR)-T cells for Patients with PSCA+ Metastatic Castration Resistant Prostate Cancer

CITY OF HOPE PROTOCOL NUMBER: IRB # 17483 **PROTOCOL DATE: 06/27/2023**

COH Initial Submission	Protocol dated 09/26/18	Version: 00

DATE(S) OF AMENDMENT(S)/REVISION(S):

COH Amendment 01	Protocol dated 04/03/2019	Version: 01
COH Amendment 02	Protocol dated 07/17/2019	Version: 02
COH Amendment 03	Protocol dated 12/18/2019	Version: 03
COH Amendment 04	Protocol dated 05/06/2020	Version: 04
COH Amendment 05	Protocol dated 08/06/2020	Packet: 05
COH Amendment 06	Protocol dated 03/03/2021	Packet: 06
COH Amendment 07	Protocol dated 11/04/2021	Packet: 07
COH Amendment 08	Protocol dated 02/21/2022	Packet: 08
COH Amendment 09 at Cont	Protocol dated 02/21/2022	Packet: 09
COH Amendment 10	Protocol dated 08/10/2022	Packet: 10
COH Amendment 11	Protocol dated 08/10/2022; TP	Packet: 11
COH Amendment 12 at Cont	Protocol dated 08/10/2022 (tp)	Packet: 12
COH Amendment 13	Protocol dated 06/27/2023	Packet: 13

IND NUMBER: 18812

SPONSOR/ IND HOLDER: City of Hope

SITE: Prostate

STAGE (If applicable): Metastatic Castration Resistant Prostate Cancer

TYPE: Phase I

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Clinical Trial Protocol

A Phase I Study to Evaluate PSCA-targeting Chimeric Antigen Receptor (CAR)-T cells for Patients with PSCA+ Metastatic Castration Resistant Prostate Cancer

Version Date: 06/27/2023
Protocol Version: 11
City of Hope Protocol #: 17483
Agent: PSCA(dCH2)BBζ-CAR T cells

IND Number: 18812
Sponsor/ IND Holder: City of Hope
Funding Support: Prostate Cancer Foundation; Mustang Bio, Inc.
NCT Number: NCT03873805

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EXPERIMENTAL DESIGN SCHEMA

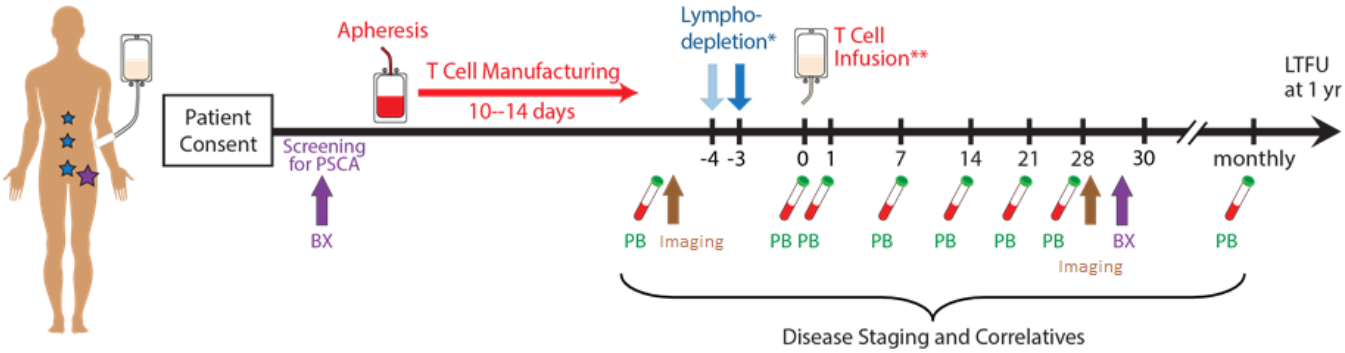


Figure 1: CAR T cell product manufacturing and participant treatment plan. Screening for PSCA can be conducted on archival tissue or fresh biopsy tissue. BX = biopsy, PB = peripheral blood for correlative assays, LTFU = long-term follow-up. *Lymphodepleting regimen is at the discretion of PI. **CAR T cell infusion may be given within a window of 3-14 days after last dose of lymphodepleting regimen. Participants may receive additional infusions that will not exceed the initial dose assigned and will be administered not less than 28 days post the prior infusion.

PROTOCOL SYNOPSIS

Protocol Title

A Phase I Study to Evaluate PSCA-targeting Chimeric Antigen Receptor (CAR)-T cells for Patients with PSCA+ Metastatic Castration Resistant Prostate Cancer

Study Detail

Population/Indication(s):	Adult patients with metastatic castration resistant prostate cancer
Phase:	I
Sample Size:	6-30
Estimated Accrual Duration:	3-4years
Estimated Study Duration	4 years
Participant Duration:	1 year short term follow-up Up to 15 years long-term follow-up
Participating Sites:	City of Hope Duarte, CA
Study Agents:	PSCA(dCH2)BBZ-CAR T cells (a.k.a., PSCA-CAR T cells)
Sponsor:	City of Hope; Prostate Cancer Foundation (financial)
Industry Partner:	Mustang Bio, Inc. (financial)

Rationale for this Study

Over the past decade, multiple therapies have been approved for metastatic castration resistant prostate cancer (mCRPC), including novel endocrine therapies (abiraterone and enzalutamide), novel radioisotopes (radium-223), vaccine therapy (sipuleucel-T) and chemotherapy (cabazitaxel or docetaxel). Despite the availability of these agents, mCRPC remains incurable and highly lethal. In hematologic malignancies, programmed CAR T cells have produced durable remissions in selected settings. Prostate cancer expresses a unique antigen, prostate stem cell antigen (PSCA), which we hypothesize will be an effective target for cellular immunotherapy. We propose to clinically test PSCA-targeting CAR T cell therapy in adult patients with recurrent mCRPC who have been treated with prior abiraterone and enzalutamide for mCRPC. Prior docetaxel is permitted but not required.

Treatment Plan

Subjects will enroll in cohorts of 3 starting with a first cohort where subjects will receive a single CAR-T cell infusion. After this single cohort (3 participants) without pre-conditioning therapy has been deemed safe, subjects will enroll at the same dose cohort to receive a single CAR-T cell infusion following lymphodepletion with the option of additional cycles. Subjects will then enroll at escalating doses according to **Tables 12.1a** and **12.1b** (CAR+ Cell Dose Schedule). Final DLT assessment and first disease assessment are at 28-33 days depending on the dose cohort, and subjects will be actively followed on this study for 1 year, after which they will shift to a long-term follow-up study for gene therapy patients.

Study Design

This is a single-center, open-label, Phase I dose–escalation trial of adoptive T cell therapy of PSCA-CAR T cells for adult patients with PSCA+ mCRPC. This trial seeks to determine a recommended phase 2 dose (RP2D) to test in future phase 2 trials. RP2D will be based on maximum tolerated dose (MTD), toxicity profile and activity data. The toxicity equivalence range (TEQR) design of Blanchard and Longmate[1] will be used to evaluate select doses of PSCA-CAR T cells and determine the MTD. The dose schedule is shown in **Tables 12.1a** and **12.1b**. The starting dose will be dose level 1.

Tables 12.1a and 12.1b: PSCA-CAR T cell dose schedule. The first nine study participants were treated according to **Table 12.1a** (3 participants at Dose 1, and 6 participants at Dose 1b).

As of February 2021, Doses 1 and 1b (shaded in blue) have been completed.

Table 12.1a. CAR+ Cell Dose Schedule

Dose Level	Lymphodepletion (Standard Flu/Cy)	#CAR+ cells
-1	No	50 M
-1a*	Yes	50 M
Starting Dose 1	No	100 M
1b	Yes	100 M
2	Yes	300 M
3	Yes	600 M

^Dose range allows for -20% of listed dose. Listed dose is the upper limit of the dose cohort
*Dose level -1a should be considered a ½ step above dose level -1. We get to -1a if the toxicity at dose level -1 is acceptable and the toxicity level at dose level 1 is too toxic.

Table 12.1b.^ Revised CAR+ Cell Dose Schedule (initiated at protocol V06)

Dose Level	Lymphodepletion (Modified Flu/Cy)	#CAR+ cells[+]
1c*	No (Cy alone)	100 M
Starting Dose 1d	Yes	100 M
2	Yes	300 M

*Dose 1c will be used as a de-escalation dose if 1d is too toxic
^Cystitis mitigation plan in place for subjects enrolling in Table 12.1.b cohorts per V06 of the protocol
[+]Initiated at protocol V07: Participants may receive additional infusions that will not exceed the initial dose assigned and will be administered not less than 28 days post the prior infusion.

Objectives

Primary Objectives

- Define the safety and tolerability of PSCA-CAR T cells in patients with PSCA+ mCRPC
- Define the recommended phase 2 dose (RP2D) of PSCA-CAR T cells in patients with PSCA+ mCRPC

Secondary Objectives

- Assess the expansion and persistence of PSCA-CAR T cells
- Assess disease response
- Assess survival outcomes (including PFS based on RECIST and OS)
- Assess serum cytokine profiles in peripheral blood pre- and post-therapy

Describe the PSCA expression level on tumor cells prior to CAR T cell infusion, and the relationship it may have with disease response and observed toxicities.

Evaluation Criteria and Endpoints

Primary Endpoints

- All toxicities post CAR T cell infusion

- Dose limiting toxicities (DLTs)

Secondary Endpoints

- Persistence of T cells to 28 days post infusion defined as CAR T cells >0.1% of total CD3 cells by flow-cytometry;
- Expansion of CAR T cells (Max log10 copies/µg of genomic DNA).
- Disease Response
  - RECIST
  - Prostate Cancer Working Group (PCWG3) criteria
  - Changes in PSA
- Survival
  - Overall survival (death from all causes from date of CAR T cell infusion)
  - Progression-free survival (PFS) time defined as survival without radiographic evidence of disease progression based on RECIST starting at the date of CAR T cell infusion or lymphodepletion
- PSCA expression on tumor cells by IHC and/or flow cytometry
- Serum cytokine profile before and after CAR T cell infusion to assess potential CRS toxicity and CAR T cell effector function

Exploratory Endpoints

- Phenotypes and frequencies of immune cell subsets in the peripheral blood pre- and post-therapy: analysis will include CD4:CD8 ratios, differentiation status (CD62L, CD27, CD45 RA/RO), and exhaustion markers (PD1, Tim3, LAG3), trafficking (CCR7, α4β7), proliferation markers (ki67) and effector functions (cytotoxicity, Th1/Th2 cytokines, and CD107a degranulation) on endogenous and CAR+ T cells
- Phenotype of tumor-infiltrating lymphocytes
- Gene expression (by RNA-seq) of CTCs
- cfDNA in peripheral blood by whole exome sequencing
  CAR immunogenicity based on the presence of anti-PSCA CAR antibodies or T cell mediated immune responses.

Statistical Considerations

TEQR Trial Design: The TEQR can be viewed as a minimal elaboration of the 3+3 design to include an explicit toxicity target range, and permit intuitive specification of a too toxic level for closing a dose level. In this implementation of the TEQR design, we define the target equivalence range of DLT as 0.20-0.35. Toxicity levels of 0.51 or higher will be considered too toxic and doses that achieve that level will not be revisited. If the rate of participants that experience a DLT is below 0.20 we will escalate, if the rate is above 0.35 we will de-escalate and if it is between 0.20-0.35 we will stay at the current dose. Participants will enter the protocol in cohorts of 3. This dose escalation portion of this study will end when 6 research participants are studied at a single dose level with a toxicity level below 0.51. The MTD will be the dose closest to target of 0.25 below 0.51 based on isotonic regression.

Analysis Plan: RP2D will be based on the MTD as well as the available activity and correlative data. Rates and associated 90% Clopper and Pearson binomial confidence limits will be estimated for *i)* DLTs within 28 days of CAR T cell infusion at the RP2D, and *ii)* disease response. Tables will be created to summarize all toxicities and side

effects by attribution to treatment, dose, organ, and severity. Maximum persistence (in days) and peak expansion (log10 copies/ug of genomic DNA) will be described. Kaplan Meier methods will be used to estimate median PFS and OS and graph the results. Statistical and graphical methods will be used to describe persistence and expansion of the CAR T cells (peripheral blood), cytokine levels (peripheral blood) and PSA levels over the study period. Linear regression will be used to assess the relationship between PSCA expression and disease response, and toxicities experienced. We will provide descriptive statistics for patient demographics and exploratory studies.

Abbreviated Eligibility Criteria

Main Inclusion Criteria

- Metastatic castration resistant prostate cancer (mCRPC) with evaluable disease
- PSCA positive tumor antigen expression
- Progression on either abiraterone or enzalutamide, or both
- Prior chemotherapy allowed but not mandatory
- Prior radiotherapy allowed provided it was not administered to the only evaluable site of disease and was >14 days prior to leukapheresis.

Main Exclusion Criteria

- Active autoimmune disease requiring systemic immunosuppressive therapy.
- Any known contraindications to tocilizumab.
- Clinically significant arrhythmia or not stable on medical management within 2 weeks of signing the consents.
- Patients with a known history or prior diagnosis of optic neuritis or other immunologic or inflammatory disease affecting the central nervous system.
- Known bleeding disorders (e.g., von Willebrand's disease) or hemophilia
- History of stroke or intracranial hemorrhage within 6 months prior to signing the consents.

Investigational Product Dosage and Administration

Autologous PSCA-CAR T cell product will be administered by intravenous (I.V.) infusion in a single dose (100, 300 or 600 × 10^6 CAR+ T cells depending on cohort) on Day 0. Participants may receive additional infusions that will not exceed the initial dose assigned and will be administered not less than 28 days post the prior infusion. Reference is made to **Tables 12.1a** and **12.1b**.

Clinical Observations and Tests to be Performed

- Medical history and physical exam
- Safety assessments (CBCs with differential, comprehensive chemistry panel, and coagulation)
- Bone & CT scans
- Correlative tumor tissue (biopsy pre and post treatment) and blood samples.

TABLE OF CONTENTS

ABBREVIATIONS

Abbreviation	Meaning
ACIT	Adoptive Cellular Immunotherapy
ADCC	Antibody-dependent Cell-mediated Cytotoxicity
AE	Adverse Event
ALT	Alanine Aminotransferase
AlloSCT	Allogeneic Stem Cell Transplantation
ANC	Absolute Neutrophil Count
aPTT	Activated Partial Thromboplastin Time
ARV-7	Androgen-receptor splice variant 7
AST	Aspartate Aminotransferase
BM	Bone Marrow
BMT	Bone Marrow Transplantation
BSA	Body Surface Area
CATD	Center for Applied Technology Development
CAR	Chimeric Antigen Receptor
CBC	Complete Blood Count
CBG	Center for Biomedicine and Genetics
cfDNA	Circulating Cell-Free DNA
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
CMP	Comprehensive Metabolic Panel
CMV	Cytomegalovirus
CofA	Certificate of Analysis
COH	City of Hope
ConMed	Concomitant Medications
CR	Complete Response
CRA	Clinical Research Associate
CRF	Case Report Form
CRS	Cytokine Release Syndrome
CTC	Circulating Tumor Cells
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T Lymphocytes
CXR	Chest X-Ray
CY	Cyclophosphamide

DAC	Donor Apheresis Center
dCH2	Deleted Heavy Chain Constant Region 2
DLCO	Diffusing Capacity of the Lung for Carbon Monoxide
DLI	Donor Lymphocyte Infusion
DLT	Dose Limiting Toxicity
DSMC	Data Safety Monitoring Committee
FcR	Fc Receptors
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume in 1 second
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
GVHD	Graft versus Host Disease
HLA	Human Leukocyte Antigen
HSCT	Hematopoietic Stem Cell Transplant
ICF	Informed Consent Form
IND	Investigational New Drug
INR	International Normalized Ratio
IRB	Institutional Review Board
IV	Intravenous
KPS	Karnofsky Performance Scale
LDH	Lactate Dehydrogenase
LDR	Lymphodepleting Preparative Regimen
LN	Lymph Node
LTR	Long Terminal Repeat
mCPRC	Metastatic Castration Resistant Prostate Cancer
MG	Malignant Glioma
mHAs	Minor Histocompatibility Antigens
MRD	Minimal Residual Disease
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
MUGA	Multi Gated Acquisition Scan
NCI	National Cancer Institute
NE	Neurotoxicity
NHL	Non-Hodgkin Lymphoma
NIH OSP	National Institute of Health, Office of Science Policy
NSG	NOD scid IL-2R gamma knockout

NYHA	New York Heart Association
OIDRA	Office of IND Development and Regulatory Affairs
OQS	Office of Quality Systems
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cell
PCWG3	Prostate Cancer Working Group 3
PD	Progressive Disease
PFS	Progression Free Survival
PFT	Pulmonary Function Tests
PI	Principal Investigator
PMT	Protocol Monitoring Team
PR	Partial Response
PSCA	Prostate Stem Cell Antigen
PT	Prothrombin
QA	Quality Assurance
QS	Quality Systems
QPCR	Quantitative real time Polymerase Chain Reaction
RP2D	Recommended Phase 2 Dose
SAE	Serious Adverse Event
SCPL	Stem Cell Processing Laboratory
SD	Stable Disease
SIN	Self-Inactivating
SOP	Standard Operating Procedure
Tcm	Central Memory T Cells
TCTRL	T Cell Therapeutics Research Laboratory
TILs	Tumor Infiltrating Lymphocytes
Treg	Regulatory T Cells
UP	Unanticipated Problem
WBC	White Blood Count
WPRE	Woodchuck Posttranscriptional Regulatory Element

1 OBJECTIVES

1.1 Primary Objectives:

- Define the safety and tolerability of PSCA-CAR T cells in patients with PSCA+ mCRPC.
- Define the recommended phase 2 dose (RP2D) of PSCA-CAR T cells in patients with PSCA+ mCRPC.

1.2 Secondary Objectives:

- Assess the expansion and persistence of PSCA-CAR T cells.
- Assess disease response
- Assess survival outcomes (including PFS based on RECIST and overall survival [OS]).
- Assess serum cytokine profiles in peripheral blood pre- and post-therapy.
- Describe the PSCA expression level on tumor cells prior to CAR T cell infusion, and the relationship it may have with disease response and observed toxicities.

1.3 Exploratory Objectives

- Characterize the phenotypes and frequencies of immune cell subsets in the peripheral blood pre- and post-therapy.
- Enumerate and characterize tumor-infiltrating lymphocytes (TILs) pre- and post-therapy.
- Enumerate and analyze gene expression of circulating tumor cells (CTC) pre- and post-therapy.
- Analyze circulating cell-free DNA (cfDNA).
- Determine the immunogenicity of PSCA-CAR T cells.

2 BACKGROUND

2.1 Introduction/Rationale for Development

2.1.1 Treatment landscape for mCRPC and immunotherapy in prostate cancer

Treatment for mCRPC has improved considerably in the last decade, with approval of 5 life-extending therapies: abiraterone, cabazitaxel, enzalutamide, radium223 and sipuleucel-T [3-7]. Nevertheless, the disease remains highly lethal, causing more than 25,000 deaths each year in the United States. New treatments which can yield durable remissions are needed.

Immunotherapy has met with variable success in mCRPC; sipuleucel-T was found to prolong overall survival but rarely induces objective responses [8]. Ipilimumab, the CTLA-4 antibody approved for melanoma, failed to prolong overall survival compared to placebo in 2 phase III mCRPC trials, although objective responses were seen in 8/32 patients in an early phase dose finding study, with 1 CR [9]. More recently pembrolizumab, the PD-1 antibody, has shown promise with 30% response rate in a small series of men whose disease is progressing on enzalutamide [10].

Prostate cancer presents many novel antigens which can be targeted using immunotherapy constructs. Sipuleucel-T exposes patient's autologous cells to prostate acid phosphatase conjugated to GM-CSF; other vaccine strategies have focused on targeting PSA (PSA-Tricom) or multiple antigens from deactivated

cancer cell lines (GVAX). While these have made it to phase III testing, there continues to be a need for targeted immunotherapies that can produce durable remissions.

PSCA is a cell-surface protein uniquely and highly expressed on prostate cancer tumors and their metastases [11-13], making it a promising target for immunotherapy. One series assessed 126 prostatectomy specimens (including 9 which were hormone therapy pre-treated) with mRNA in situ hybridization and identified 88% expression overall (81% strong staining, 7% moderate); normal glands had markedly lower expression. Importantly, normal human tissues were evaluated for expression of PSCA and there were only small amounts detected in the kidney and small intestine after prolonged exposure, at a level 1% of that seen in prostate tissue [13]. In another experience (using immunohistochemistry with antibody 1G8 to amino acids 46-85), 100% of 9 bone metastases from prostate cancer strongly expressed PSCA and 94% of primary prostate tumors expressed PSCA; stronger expression was associated with higher Gleason score and progression to castration resistance [12]; the only extra-prostatic staining identified was weak staining in renal collecting ducts, urothelium, and neuroendocrine cells of stomach and colon.

2.1.2 Adoptive cellular immunotherapy background

Adoptive cellular immunotherapy (ACIT) using chimeric antigen receptor (CAR)-modified T cells is a treatment approach that is being evaluated in a variety of cancers in recent years. Lymphodepletion chemotherapy followed by CD19-targeted CAR T cell infusion has produced high response rates in patients with refractory B-cell acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL). In the ZUMA-1 trial (NCT02348216), patients with refractory diffuse large B cell lymphoma including transformed follicular lymphoma and primary mediastinal large cell lymphoma were treated with autologous anti-CD19 CAR T cells (KTE-C19) after fludarabine and cyclophosphamide lymphodepletion. The overall response rate was 82% and CR rate was 54% [14]. Rates of grade ≥3 cytokine release syndrome (CRS) and neurotoxicity (NE) were 13% and 28%, respectively. There were 2 deaths related to KTE-C19: 1 hemophagocytic lymphohistiocytosis, and 1 case of cardiac arrest [14]. In the TRANSCEND-NHL-001 trial (NCT02631044) using autologous CD19-targeted CAR T Cells (JCAR017), a best overall response rate of 80% and CR of 60% were recently reported in patients with relapsed or refractory aggressive B-NHL including 2 MCL patients [15]. No severe CRS was observed; 10 of 28 patients had grade 1-2 CRS. Five patients developed neurotoxicity, including 4 grade 3-4; all events resolved. Four patients died due to disease progression and not related to JCAR017.

City of Hope's T Cell Therapeutics Research Laboratory (TCTRL), under Dr. Stephen Forman's leadership, has tested CD19-targeted CAR T cells generated and manufactured by our group in patients with B-ALL (NCT02146924) and B-NHL undergoing autologous stem cell transplant (NCT02051257). The treatment is safe with manageable side effects at the 200 million CAR T dose level with no treatment related deaths. The MTD has not been reached; and we have tested doses up to 600 million CAR+ T cells. All AEs related to CD19-targeted CAR T infusion have been reversible.

City of Hope's TCTRL also has a solid tumor CAR T cell program, the flagship of which is IL13Rα2-targeted CAR T cells for malignant glioma (MG). We have completed two clinical trials evaluating autologous (NCT00730613, 3 patients) and allogeneic (NCT01082926, 6 patients) first-generation IL13Rα2-CAR CD8+ T cells for resectable and non-resectable recurrent GBM, respectively [16-18], and reported the feasibility and safety of repetitive intracranial delivery of CAR T cells ($\leq 1\times10^8$ cells) through a reservoir/catheter system. This first-generation CAR T cell had limited persistence, and all tumors recurred.

More recently, we have optimized a second-generation IL13Rα2-targeted 41BB-costimulatory CAR and a manufacturing platform that utilizes central memory T cells (Tcm) for lentivirus engineering (IL13BBζ-Tcm). The ongoing first-in-human clinical trial (NCT02208362) is evaluating the safety, feasibility and bioactivity of these autologous second-generation IL13BBζ-Tcm in patients with recurrent IL13Rα2+ MG, via locoregional delivery. Interim findings for the first 34 research participants (February 2018) demonstrate that locoregional delivery of IL13BBζ-Tcm post-surgical resection has been well-tolerated with no observed DLTs, or therapy-related SAEs.

Our study team is also highly encouraged by preliminary patient outcomes for this heavily pre-treated recurrent MG patient population. In a case study recently published, a patient with recurrent multifocal glioblastoma treated with IL13Rα2-targeted CAR T cells experienced complete remission of all brain and spinal lesions [19]. While this patient has shown the most dramatic response to COH's IL13Rα2-targeted CAR therapy, interim data suggests a survival benefit in other patients, with median survival time for all individuals that received at least 3 doses of CAR T cells being 7.9 months [95% CI; 4.8 mo.–undefined] as compared to an expected survival of 3–5 months for this heavily pretreated patient population (2nd or later recurrence; 38% prior Avastin treatment)[20]. These finding suggest that CAR therapy can mediate profound antitumor effects against MG and holds promise for our other solid tumor CAR programs.

2.2 Development of PSCA-CAR T Cell Therapy

There is a longstanding interest in developing PSCA-targeting CAR T cells as PSCA is a well validated therapeutic target for various cancers, including that of the prostate, pancreas, and bladder (reviewed in [21]). To date, there are four phase I clinical trials at other institutions evaluating PSCA-specific CAR T cells that are actively recruiting research subjects (NCT03198052, NCT02744287, NCT03356808, NCT03267173). One of these has recently reported results (NCT02744287), where the PSCA-targeted CAR T cell strategy to treat metastatic pancreatic cancer has been well-tolerated with no CRS or neurotoxicity in the initial cell-dose escalation, and there is initial evidence for stable disease and biologic activity in some patients.[22].

2.2.1 Development of a 41BB-costimulatory PSCA-CAR

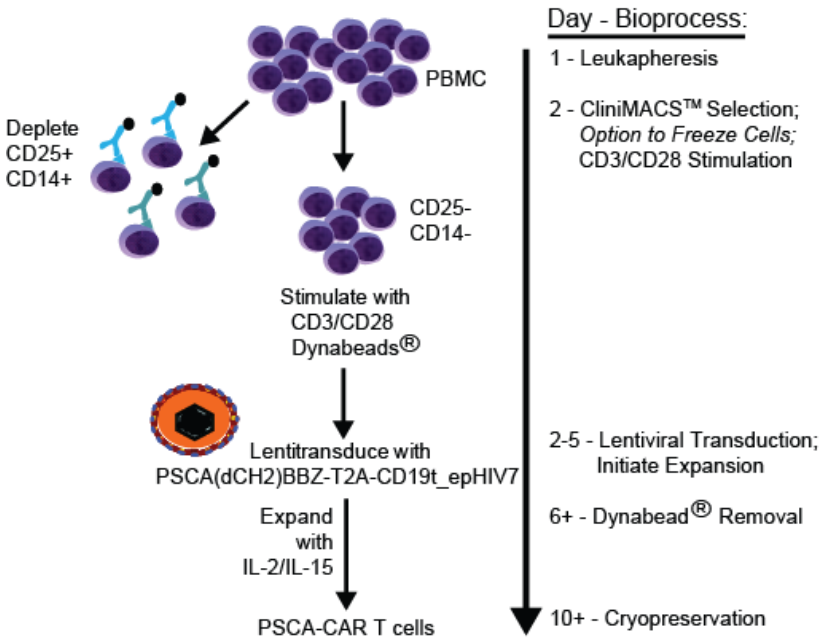
The preclinical manuscript reporting on the optimization and selection of a lead candidate for translation has recently been published [23]. This work revealed that a 4-1BB intracellular co-stimulatory signaling domain within a PSCA-targeting CAR confers improved selectivity for higher tumor antigen density, reduced T cell exhaustion phenotype, and equivalent tumor killing ability compared to a PSCA-targeting CAR containing the CD28 co-stimulatory signaling domain [23]. Furthermore, the PSCA-CARs exhibited robust *in vivo* anti-tumor activity in patient-derived bone-metastatic prostate cancer xenograft models, with the 4-1BB-containing CARs exhibiting superior *in vivo* T cell persistence and control of disease compared to that of the CD28-containing CARs [23]. Based on these studies, we are proposing the use of the 41BB-containing CAR, here termed PSCA(dCH2)BBZ, for the PSCA-CAR T cells in this clinical protocol.

2.2.2 T Cell population for CAR T cell modification

This study will use autologous PBMC that have been immunomagnetically depleted of CD14+ myeloid cells and CD25+ regulatory T cells (Treg) for CAR engineering. Depletion of these cells is based on studies suggesting that the manufacturing and efficaciousness of bulk PBMC-derived CAR T cells may be hampered by such populations, including myeloid cells that limit T cell expansion *ex vivo* [24, 25]. Additionally, this enrichment strategy will capture both effector as well as naïve/stem-like T cell subsets. Thus, we hypothesize that autologous PSCA-CAR T cells manufactured with this initial depletion step will

maximally retain their effector function and capacity to persist after their genetic modification and *ex vivo* propagation, and, upon adoptive transfer to prostate cancer patients, will produce robust anti-tumor responses against PSCA+ mCRPC. **Figure 2.1** depicts the manufacturing platform for this trial.

Figure 2.1. PSCA-CAR T Cell Product Manufacturing Process



2.3 Manufacturing Qualification Run Data

We have performed three qualification runs on cells procured from normal donors using the manufacturing platform proposed for this protocol. Importantly, these preclinical studies suggest that the desired PSCA-CAR T cells can be expanded ≥27-fold within 14 days, and the expanded T cells expressed the PSCA(dCH2)BBζ/CD19t transgenes (**Table 2.1** and **Figure 2.2**). These PSCA-CAR T cells also exhibited PSCA-specific effector function *in vitro* (**Figure 2.3**). Since PSCA is expressed at low levels in some normal tissue, which could cause on-target off-tumor toxicity, it is important to note that the PSCA-CAR T cells were evaluated using prostate cancer cell lines with varying levels of PSCA expression (**Figure 2.3a**). Specifically, the human prostate cancer cell line PC-3 was engineered to express the human PSCA gene under the control of either the EF1α promoter to derive a high-antigen-density cell line (PC3-PSCA) or a mutant PGK promoter [26] to derive a low-antigen-density cell line (PC3-PGK100p). HPAC is a pancreatic cancer line with high endogenous PSCA expression (data not shown). Overall, the activation of PSCA-CAR T cells was significantly less with the low-antigen-density PC3-PGK100p targets (**Figure 2.3b-d**), suggestive of an acceptable safety profile.

Table 2.1. Summary of CAR expression and fold-expansion					
Cell Product	**CD3**	**CD19t**	**CD4***	**CD8***	**Fold-Expansion**
HD007.10	98.8%	45.4%	26.7%	64.6%	27-fold; 14 days
HD013.4	99.5%	82.3%	67.5%	29.0%	59-fold 14 days
HD406	99.5%	69.4%	34.6%	57.3%	60-fold; 14 days

*Percentages of CD4 and CD8 are donor specific and within the normal expected range

Figure 2.2. Surface phenotype of qualification run products. PBMC were depleted of CD25+ and CD14+ cells, transduced with PSCA(dCH2)BBZ-T2A-CD19t_epHIV7 and expanded *in vitro* as proposed for clinical use. The resulting cells were stained with fluorochrome-conjugated antibodies to detect either the T cell markers CD3, CD4, or CD8, or the CD19t transgene marker. Percentages of viable cells (DAPI negative) with immunoreactivity above control staining are indicated in each histogram.

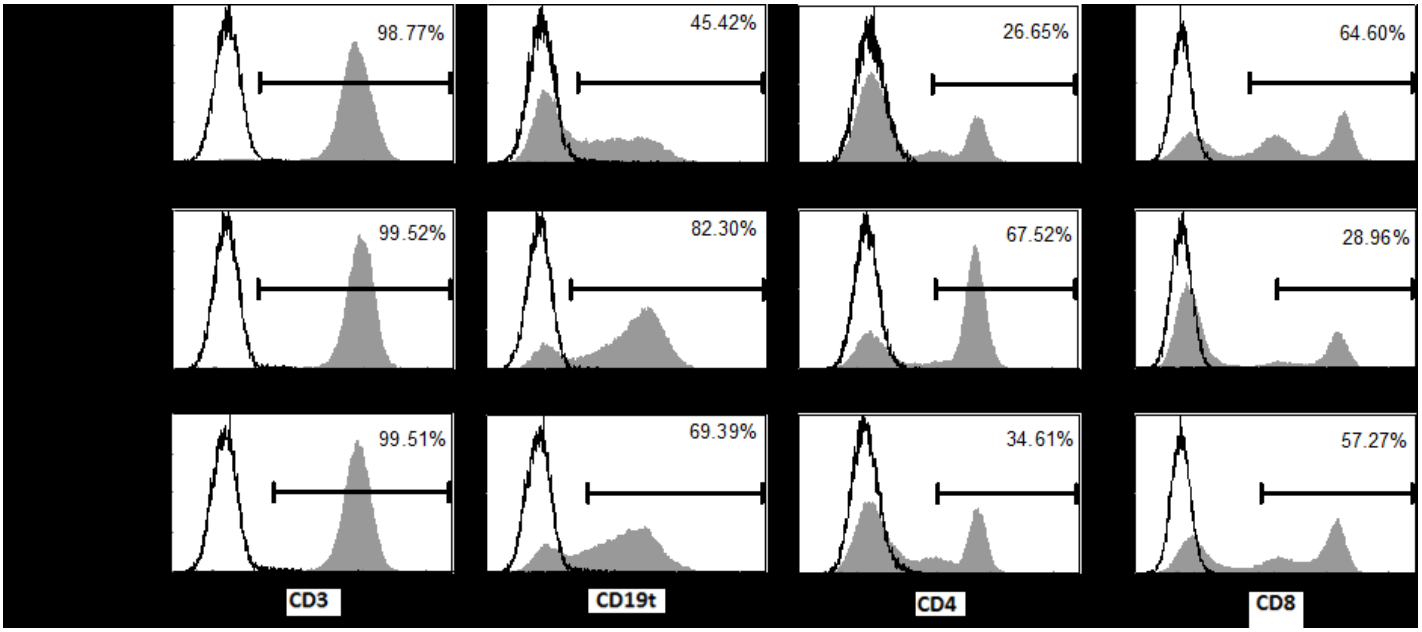
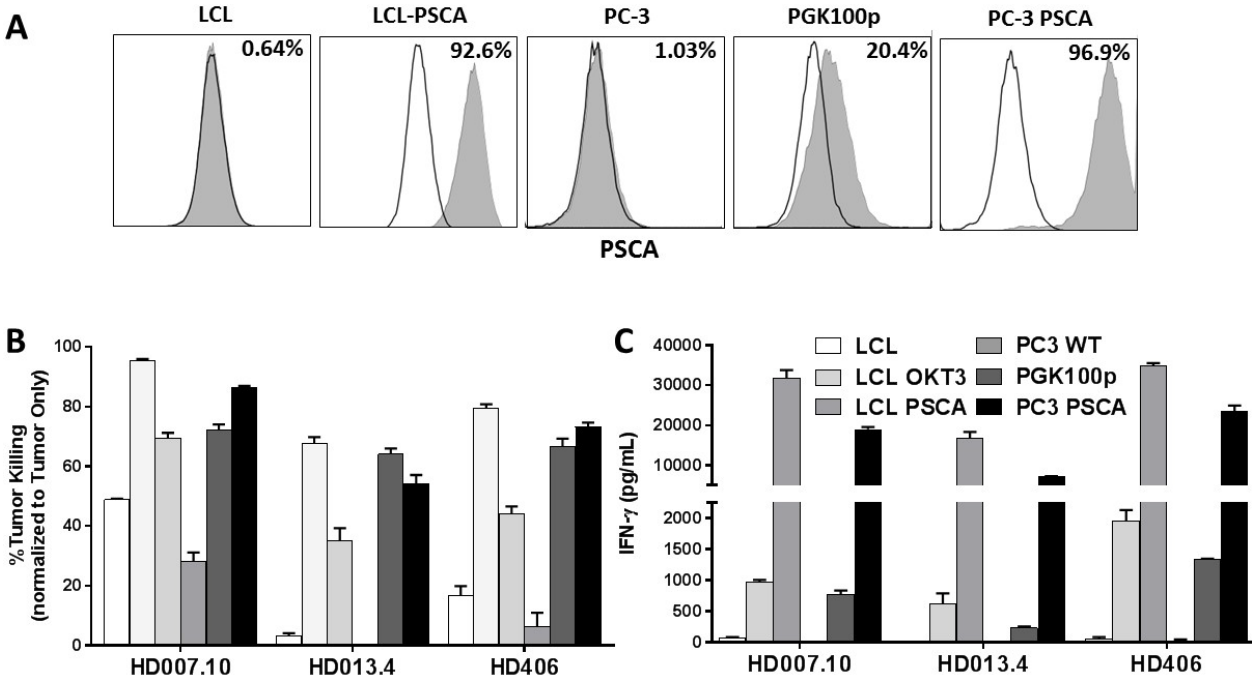


Figure 2.3. Effector activity of PSCA-CAR T cells. A, PSCA staining profiles of target cells used in effector activity assays. Human lymphoblastic cell line (LCL) and human prostate cancer cell line PC-3 were used as PSCA negative control targets. LCL were also lentivirally engineered to express membrane bound CD3 agonist OKT3 (LCL-OKT3) as a positive stimulator control (same staining profile as LCL), or the PSCA target antigen (LCL-PSCA). PC-3 cells were also lentivirally transduced and FACS sorted to uniformly express high levels of PSCA (PC3-PSCA) or low levels of PSCA (PC3-PGK100p). Percent immunoreactivity above isotype control staining is indicated in each histogram. **B**, PSCA-CAR T cell qualification runs were used as effectors in a flow cytometry based 48-hour long term killing assay at an E:T ratio of 1:2 total cells. % Tumor killing was calculated by comparing the viable numbers of the indicated target line cocultured with CAR+ T cells to that of tumor only; i.e., using the following equation: 100 – (100 x (counts in coculture/counts in tumor only)). **C**, PSCA-CAR T cell qualification runs were used as effectors in a cytokine production assay in which supernatants were collected after overnight coculture with the indicated targets at an E:T ratio of 1:1 total cells. IFN-γ levels in the supernatants were then measured by ELISA.



2.4 Overview and Rationale of Study Design

Research participants with mCRPC progression on at least abiraterone and/or enzalutamide are selected due to increased immune responsiveness seen in more refractory populations with immune checkpoint inhibitors [10]. Participants will be required to exhibit tumor expression of PSCA, the target of the investigational product, PSCA-CAR T cells. Tissue testing will be performed within the department of Clinical Pathology using a standardized research assay.

Studies of CAR-T cell therapy in hematologic malignancies have utilized lymphocyte depletion to make space in the lymphoid tissue for CAR T cell engraftment. While we believe this is important for efficacy, this may also exacerbate cytokine release syndrome. Therefore, in this first-in-human study, we have chosen to initiate the study with the first cohort of 3 participants at Dose level 1 with 100 million CAR+ T cells alone (i.e., no prior lymphodepletion) to evaluate the safety of PSCA-CAR T cells alone. If this first dose is deemed safe, we will add a new cohort of 3 participants at Dose level 1 with 100 million CAR+ T cells AND lymphodepletion, with the goal of increasing CAR T cell engraftment and tumor targeting success. If Dose level 1 is again deemed safe, the study will dose escalate with prior lymphodepletion as described in **Section 12**.

The feasibility, efficacy and safety of the proposed clinical trial and study model is supported by our significant experience (over 15 years) with clinical ACIT using genetically-modified T cells [16-19, 27-29]. Our studies to date have demonstrated the tolerability of cell doses and lack of lymphoproliferative sequelae based on *in vivo* proliferation of transferred cells. Our clinical trials (**BB-INDs 14645**, **15490, 15918**, **16226,** and **16457**) demonstrate the feasibility of manufacturing gene-modified CAR+ T cells.

Overall, with the protocol proposed in this application, we will continue to build on our past clinical trial experience, now targeting PSCA for the treatment of mCRPC, utilizing i.v. delivery methods with a dosing schedule that is comparable to our current CD19-targeting CAR T cell for B-cell malignancy trials (**BB-IND 14645**, **15490, and 15918**).

3 ELIGIBILITY CRITERIA

Patient MRN:	Patient Initials (F, M, L):

Participants must meet the following criteria to be eligible to participate in the study:

3.1 Inclusion Criteria

Informed Consent and Willingness to Participate

__1. All participants must have the ability to understand and the willingness to sign a written informed consent.

Note: For research participants who do not speak English, a short form consent may be used with a COH certified interpreter/translator to proceed with screening/leukapheresis, while the request for translated consents is processed. However, the research participant is allowed to proceed with lymphodepletion and CAR T cell infusion only after the translated treatment consent form is signed.

Age Criteria

__2. Age ≥ 18 years.

Performance Status

__3. ECOG Performance status 0 – 2 or KPS ≥70%.

Nature of Illness and Treatment-Related Criteria

__4. Documented castration resistant prostate cancer (mCRPC) (Note: castration will be defined by a testosterone <50 ng/dL achieved by orchiectomy or LHRH agonist/antagonist therapy).

a. Documented PSCA+ tumor expression as evaluated by COH Pathology Core.

b. Progression of disease manifest by **one** of the following means during treatment with at least one advanced androgen targeted therapy (e.g., abiraterone or enzalutamide):

i. rising PSA documented on 2 occasions at least 7 days apart, with absolute increase >2 ng/dL despite testosterone <50 OR

ii. radiographic evidence of new metastatic foci on CT or bone scan, or soft tissue progression by RECIST (See **Section 11** for RECIST criteria).

__5. Prior chemotherapy with cabazitaxel and/or docetaxel is allowed but not required. If there has been prior chemotherapy, at least 2 weeks must have elapsed prior to leukapheresis.

__6. Prior radiotherapy is allowed provided it was not administered to the only evaluable site of disease and was >14 days prior to leukapheresis.

__7. No known contraindications to leukapheresis, steroids or tocilizumab.

Clinical Laboratory Criteria (To be performed within 42 days of signing the main study consent)

__8. Total serum bilirubin ≤ 2.0 mg/dL __9. Patients with Gilbert syndrome may be included if their total bilirubin is ≤ 3.0 x ULN and direct bilirubin ≤ 1.5 x ULN.	ULN: Bil:	Date:
__10. AST ≤ 5 x ULN	ULN: AST:	Date:
__11. ALT ≤ 5 x ULN	ULN: ALT:	Date:
__12. Creatinine clearance of ≥ 50 mL/min per the Cockcroft-Gault formula $\text{CrCl (mL/min)} = \frac{(140\text{-age}) \times \text{actual body weight (kg)}}{72 \times \text{serum creatinine (mg/dL)}}$ (x 0.85 for females) **Or** $\text{CrCl (mL/min)} = \frac{(140\text{-age}) \times \text{actual body weight (kg)}}{0.8136 \times \text{serum creatinine (umol/L)}}$ (x 0.85 for females)	Serum Cr: Cr Clearance:	Date:
__13. Cardiac function (12 lead-ECG) without acute abnormalities requiring investigation or intervention	Result:	Date:
__14. Left ventricular ejection fraction >40%	Result:	Date:

Contraception

__15. Participants of reproductive potential must agree to use acceptable birth control methods throughout study therapy and for 3 months after final dose of study treatment.

3.2 Exclusion Criteria

Other illnesses or conditions

__1. Participants with clinically significant arrhythmia or arrhythmias not stable on medical management within two weeks of signing the consents.

__2. Participants with a known history or prior diagnosis of optic neuritis or other immunologic or inflammatory disease affecting the central nervous system, including seizure disorder.

__3. History of allergic reactions attributed to compounds of similar chemical or biologic composition or other agents used in this study.

__4. Known bleeding disorders (e.g., von Willebrand's disease) or hemophilia.

__5. History of stroke or intracranial hemorrhage within 6 months prior to signing the consents.

__6. History of other malignancies, except for malignancy surgically resected (or treated with other modalities) with curative intent, basal cell carcinoma of the skin or localized squamous cell carcinoma of the skin; non-muscle invasive bladder cancer; malignancy treated with curative intent with no known active disease present for ≥ 3 years.

__7. Uncontrolled active infection.

__8. Active hepatitis B or hepatitis C infection.

__9. HIV infection.

__10. Any other condition that would, in the Investigator's judgment, contraindicate the subject's participation in the clinical study due to safety concerns with clinical study procedures.

Noncompliance

__11. Prospective participants who, in the opinion of the Investigator, may not be able to comply with all study procedures (including compliance issues related to feasibility/logistics).

Eligibility Confirmed* by (Choose as applicable):	**Print Name**	**Signature**	**Date**
☐ Site PI			
☐ Authorized study MD			
☐ Study Nurse			
☐ Study CRA/ CRC			
☐ Other:_____ ___			
*Eligibility should be confirmed per institutional policies.			

4 PARTICIPANT SCREENING AND REGISTRATION

4.1 Screening Procedures

A screening consent will be utilized to consent participants to allow for screening of their existing tumor tissue for PSCA tumor expression and to proceed with screening assessments and leukapheresis. Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done only after obtaining written informed consent. Studies or procedures that were for clinical indications (not exclusively to determine study eligibility) may be used for baseline values, even if the studies were done before informed consent was obtained. Reference is made to **Section 10.0 – Study Calendar**.

- PSCA Screening

A CLIA approved clinical assay for the detection of surface PSCA is **not** currently available. In order to screen subjects for PSCA positive tumor expression, the COH Pathology Core team has developed an immunohistochemistry protocol to be used specifically for patient tumor screening. Thus far, the majority of prostate tumor samples screened have tested positive for membrane/cytoplasmic expression of the PSCA marker (n>11) in tumor cells. Therefore, in this phase I study, it is the study team's intent to enroll all subjects that test positive for PSCA expression using this test on soft-tissue tumor specimens as developed by the COH Pathology Core. This phase I study will then also describe, as a secondary aim, if percentages of prostate cancer cell PSCA surface expression are different between research participants and/or change within a research participant over the course of treatment.

4.2 Informed Consent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the subject and a signed informed consent will be obtained. Documentation of informed consent will be maintained in the subject's research chart and medical record.

4.3 Registration Requirements/Process

Research participants will be identified through the clinical practices of the PI, co-Is and participating clinicians and through direct referrals from outside hospitals and physicians. No direct-to-patient advertising will be performed without IRB approval.

Evaluation of research participant's eligibility after signing the main informed consent will include the following. However, procedures completed within 6 weeks of the informed consent signing, unless otherwise specified, will not be duplicated and will, therefore, be used to document eligibility:

- Documentation of recurrence/progression/residual disease following prior therapy.
- Review of available radiological images to assess for extent of disease, active infection or second malignancy.
- Documented PSCA+ tumor expression by immunohistochemistry as assessed by COH Pathology Core
    - *Fresh or archival biopsy samples, for staining with PSCA (prior soft-tissue tumor specimen, including prostate primary), may be tested for PSCA expression during screening for eligibility purposes. However, a study-mandated fresh biopsy must occur within 6 weeks prior to lymphodepletion [or prior to CAR T infusion ONLY for cohorts 1 and -1]. The results from soft tissue biopsies will be used to confirm eligibility, but bone biopsy staining results will not impact eligibility since IHC staining for PSCA has not*

been optimized in bone specimens. Subjects who undergo bone biopsy on study will be qualified based on the original pre-screening tissue result.

- Review of available Echo.
- History and Physical Exam, Vital Signs, ECOG.
- Men with female partners of reproductive potential must use a physician-approved contraceptive method for at least two weeks prior to, during, and two months after CAR T cell infusion.
- Up to 35 cc peripheral blood draw for Correlative Studies
- Initiation of the following tests:
    - Hepatitis
    - HIV Ag/Ab Combo Assay
    - CBC, Differential, ANC
    - Chemistry Panel (includes CMP, LDH, uric acid, phos, Mg)

Once research participants sign the main informed consent, they undergo all screening assessments. Once they have met all eligibility criteria, they will proceed with the leukapheresis procedure to manufacture a CAR T cell product for infusion. They are considered 'accrued' and registered to the study at the time of initiating study therapy (e.g., cohorts 1 and -1 at the time of CAR T cell infusion on Day 0, and for all other cohorts at the start of lymphodepletion).

4.4 Dose Level Assignment

Participants will be accrued to dose levels in cohorts of 3. After toxicities in a cohort are assessed by the protocol management team (PMT), the decision will be made on whether to escalate or not. Dose escalation rules follow the TEQR design [1] and are further described in **Section 12.**

5 TREATMENT PROGRAM

5.1 Study Design Overview

Tables 12.1a and 12.1b: PSCA-CAR T cell dose schedule. The first nine study participants were treated according to **Table 12.1a** (3 participants at Dose 1, and 6 participants at Dose 1b).

As of February 2021, Doses 1 and 1b (shaded in blue) have been completed.

Table 12.1a. CAR+ Cell Dose Schedule

Dose Level	Lymphodepletion (Standard Flu/Cy)	#CAR+ cells
-1	No	50 M
-1a*	Yes	50 M
Starting Dose 1	No	100 M
1b	Yes	100 M
2	Yes	300 M
3	Yes	600 M

^Dose range allows for -20% of listed dose. Listed dose is the upper limit of the dose cohort
*Dose level -1a should be considered a ½ step above dose level -1. We get to -1a if the toxicity at dose level -1 is acceptable and the toxicity level at dose level 1 is too toxic.

Table 12.1b.^ Revised CAR+ Cell Dose Schedule (initiated at protocol V06)

Dose Level	Lymphodepletion (Modified Flu/Cy)	#CAR+ cells[+]
1c*	No (Cy alone)	100 M
Starting Dose 1d	Yes	100 M
2	Yes	300 M

*Dose 1c will be used as a de-escalation dose if 1d is too toxic
^ Cystitis mitigation plan in place for subjects enrolling in Table 12.1.b cohorts per V06 of the protocol
[+]Initiated at protocol V07: Participants may receive additional infusions that will not exceed the initial dose assigned and will be administered not less than 28 days post the prior infusion.

Participants will receive treatment on this Phase I trial in cohorts of 3 with dose level assignment based on TEQR design rules [1] for dose escalation/de-escalation as described in **Section 12**. Possible doses being explored are listed in **Tables 12.1a** and **12.1b**, with the first cohort enrolled at Starting Dose 1 (100×10^6 CAR+ T cells). Twelve patients will be accrued at the MTD. The RP2D will be based on the MTD but may be lower than MTD taking into account toxicities occurring after the DLT period and disease response. Research participants whose modified cellular product cannot be generated in sufficient numbers to achieve the assigned cell dose level can be assigned to a lower dose that has already been deemed safe.

5.2 Treatment Cycle Definition

A cycle is defined as study therapy plus 28-days of post CAR T cell assessment. For example, cohort 1, where only CAR T cells are infused, has a 28-day cycle starting with CAR T cell infusion (day 0). For cohorts that include prior lymphodepletion, a cycle will be ~33 days starting with the first dose of lymphodepletion (~day -5) followed by CAR T cell infusion (day 0) and 28-days of post CAR T cell assessments.

Optional infusions: In patients who: i) have evidence of disease at 28 days or later following initial infusion of CAR T cells, and ii) who did not have a DLT, will be allowed to receive a second infusion. The second infusion of CAR T cells will not exceed the initial dose assigned and will be administered not less than 28 days post the initial infusion once the patient meets all CAR T cell infusion eligibility criteria. Additional CAR T cell infusions after a second infusion will be allowed not less than 28 days apart following the same rules as the second infusion. Additional lymphodepletion prior to the second and any subsequent doses will be optional and will be given at a dose not greater than the initial lymphodepletion. Toxicity evaluation and disease response evaluations will follow as described for the initial CAR T cell infusion. This data will not be used in determining dose escalation other than to define a dose as too toxic for further study based on achieving a DLT rate of 0.51 or above in three or more participants. This data will be included in the determination of the RP2D.

5.3 Treatment Plan and Enrollment Criteria

The treatment schedule is depicted graphically in the **Figure 1** Schema, and detailed in the **Section 10** Study Calendar. In addition to the initial eligibility criteria for study screening, there are criteria that must be met prior to specific pre-treatment activities as well as study enrollment as described below.

5.3.1 PBMC collection for CAR T cell manufacturing

5.3.1.1 Criteria to proceed with Leukapheresis

__1. Research participant must have an ECOG 0-2 or KPS ≥70%, ANC >1000 and Platelets >75,000.

__2. Research participant must not require concurrent use of systemic corticosteroids or chronic use of immunosuppressant medications above physiologic replacement doses (prednisone ≤ 10 mg/day, or hydrocortisone ≤ 20mg/day is allowed). Recent or current use of inhaled steroids is not exclusionary.

__3. Research participant must have appropriate venous access or be willing to undergo temporary line placement.

__4. At least 7 days must have elapsed since the research participant received his last dose of prior enzalutamide/other AR targeted therapy (excluding LHRH therapy which will continue).

__5. At least 2 days must have elapsed since the research participant received his last dose of prior abiraterone.

__6. At least 2 weeks must have elapsed since the research participant received his last dose of prior chemotherapy.

__*7.* At least 4 weeks must have elapsed since the research participant received his last dose of radium223.

5.3.1.2 Pre-treatment Leukapheresis Activities

The research participant will need to have a vein assessment completed at the COH Donor Apheresis Center (DAC) prior to scheduling the apheresis procedure to determine if the participant will require a temporary line to support the procedure. Temporary line placement needs to be coordinated for the morning of apheresis to be completed prior to 9am OR the manufacturing team needs to be notified of the late apheresis procedure.

The leukapheresis product will be collected at the COH DAC according to the DAC operating procedures. The research participant must be evaluated by a physician prior to leukapheresis and not have a standard contraindication for this procedure per COH standard practices which this process may take approximately 1 hour. The procedure will be a single apheresis run of approximately 2–4 hours. Apheresis duration may be modified by the DAC physician as required by DAC policies without prior notification to the PI and will not result in a deviation. Should a technical issue arise during the procedure or in the immediate processing of the product such that it cannot be used for CAR T cell production, a second procedure may be ordered.

Upon completion of the leukapheresis procedure, the DAC will notify Manufacturing staff to collect the product. The product will then be checked into a COH GMP facility and will be under the control and monitoring of COH Quality Systems.

CAR T Cell product manufacturing, expansion, and cryopreservation is anticipated to be approximately 3 to 4 weeks, during which time participants' medical care will be managed by their treating oncologist. During this time, research participants may receive chemotherapy, hormone therapy such as abiraterone or enzalutamide; however, the treatment will be subject to the same washout requirements as specified prior to leukapheresis. No radium223 should be administered during this gap.

5.3.2 Criteria to Proceed with Lymphodepletion (*not applicable to cohorts 1 and -1*)

A study-mandated biopsy must occur following completion of bridging chemotherapy as long as it is within 6 weeks prior to lymphodepletion [or prior to CAR T infusion ONLY for cohorts 1 and -1].

Research participants (except for cohorts 1 and -1) will be administered the appropriate lymphodepleting regimen per PI and protocol team's discretion. The time between the last therapy and start of lymphodepletion must be at least 3 weeks for chemotherapy and at least 7 days for oral agents. Lymphodepletion may be given as an outpatient. Lymphodepletion may be given 3 to 14 days prior to CAR T cell infusion based on PI discretion and patient eligibility (please refer to **Section 8.2.1**).

__1. Before participants can be assigned to a cohort, tumor tissue testing for PSCA from study mandated biopsy must be completed and documented as positive.

Additionally, if applicable, the following washouts must be met prior to start of lymphodepletion:

__2. At least 7 days must have elapsed since the research participant received his last dose of prior enzalutamide/other AR targeted therapy (excluding LHRH therapy which will continue).

__3. At least 2 days must have elapsed since the research participant received his last dose of prior abiraterone.

__4. At least 2 weeks must have elapsed since the research participant received his last dose of prior chemotherapy.

The following evaluations will be performed no more than 7 days prior to start of lymphodepletion, unless otherwise specified, to show that subjects meet eligibility criteria to proceed:

__1. Research participant has a released cryopreserved CAR T cell product.

CofA date: __ /__ /__

__2. Progress notes including vital signs (pulse rate, temperature, respiratory rate, blood pressure)

__3. ECOG ≤ 2 or KPS ≥70%

__4. Complete blood count, differential, ANC

__5. Chemistry panel (CMP, LDH, uric acid, phos, Mg)

__6. Pulmonary: Not requiring supplemental oxygen or mechanical ventilation, oxygen saturation 90% or higher on room air

__7. Cardiovascular: Not requiring pressor support, no symptomatic cardiac arrhythmias, no acute coronary syndrome, or uncontrolled hypertension.

__8. Renal Function: Preservation of renal function, serum creatinine did NOT increase by more than 2-fold above the normal reference range.

__9. Liver Function: Adequate liver function defined as: Total bilirubin ≤ 2.0 mg/dL, ALT and AST ≤5 times the institutional upper limits of normal.

__10. Neurological: Research participant without clinically significant encephalopathy/new focal deficits.

__11. Infectious diseases: No clinical evidence of uncontrolled active infectious process.

__12. Metastasis soft-tissue biopsy and testing for PSCA expression for participants who underwent salvage therapy following screening and those who have NOT yet had a biopsy on trial. This may be performed following final salvage therapy, and up to 6 weeks prior to start of lymphodepletion.

- *Please note, bone biopsy specimens may be screened for PSCA expression, and archived for further correlative analysis, but will not dictate study enrollment as this tissue staining has yet to be optimized.*

__13. Brain MRI/CT as well as a neurological consultation, for participants who have experienced focal neurological signs or mental status changes in the last 3 months.

__14. IF applicable, research participant has recovered from bridging therapy-related toxicities to ≤ Grade 2 (except for alopecia or neuropathy) prior to initiation of lymphodepletion

__16. Serum creatinine ≤2.5 x ULN or estimated creatinine clearance of >30 mL/min per the Cockcroft-Gault formula, and the participant is not on hemodialysis	ULN: Serum Cr: Cr Clearance:	Date:

$\text{CrCl (mL/min)} = \frac{(140\text{-age}) \times \text{actual body weight (kg)}}{72 \times \text{serum creatinine (mg/dL)}}$ (x 0.85 for females) **Or** $\text{CrCl (mL/min)} = \frac{(140\text{-age}) \times \text{actual body weight (kg)}}{0.8136 \times \text{serum creatinine (umol/L)}}$ (x 0.85 for females)		
__17. Absolute neutrophil count ≥1000/µL, hemoglobin (Hb) ≥8 g/dl and platelet count ≥50,000/µL. Transfusions and growth factors may be used to meet these requirements prior to lymphodepletion.	ANC: PT:	Date:

5.3.3 Criteria to proceed with CAR T cell infusion(s)

Research participants will be evaluated for eligibility to proceed with CAR T cell infusions no more than 1 day prior to CAR T cell infusion. For cohorts 1 and -1 where subjects ONLY receive CAR T cell infusion, eligibility to proceed with CAR T cell infusion will constitute study accrual. If a research participant is not eligible to receive CAR T cell infusion following completion of lymphodepletion regimen, infusion may be delayed up to 14 days after the last dose of lymphodepletion (reference **Section 7**).

The morning of infusion should include a physical exam including vital signs (pulse rate, temperature, respiratory rate, blood pressure), complete blood count, differential, ANC, chemistry panel (CMP, LDH, uric acid, phos, Mg), and a 35 cc peripheral blood will be collected for Correlative Studies (drawn prior to CAR T cell infusion). Additionally, baseline CTCAE evaluation using version 5, and modified CRS grading as applicable (reference is made to **Appendix A**) should be collected.

The following criteria must be met prior to CAR T Cell infusion(s):

__1. Research participant has a released cryopreserved CAR T cell product.

CofA date: __ /__ /__

__2. ECOG ≤ 2 or KPS ≥70%

__3. Body temperature < 38^{o}C

__4. Pulmonary: Not requiring supplemental oxygen or mechanical ventilation, oxygen saturation 90% or higher on room air

__5. Cardiovascular: Not requiring pressor support, no symptomatic cardiac arrhythmias, no acute coronary syndrome, or uncontrolled hypertension.

__6. Renal Function: Preservation of renal function, serum creatinine did NOT increase by more than 2-fold above the normal reference range.

__7. Liver Function: Adequate liver function defined as: Total bilirubin ≤ 2.0 mg/dL, ALT and AST ≤5 times the institutional upper limits of normal.

__8. Neurological: Research participant without clinically significant encephalopathy/new focal deficits.

__9. Infectious diseases: No clinical evidence of uncontrolled active infectious process.

__10. Required ONLY if clinically indicated and/or at the discretion of the PI: Chest X-Ray within normal limits

ONLY for cohorts 1 and -1:

__1. Before participants can be assigned to a cohort, soft-tissue tumor testing for PSCA expression from study mandated biopsy must be completed and documented as positive within 6 weeks prior to CAR T infusion. For patients whose on-study biopsy is a bone biopsy, PSCA staining will not be used to determine eligibility to proceed with infusion because IHC staining has not been optimized for bone specimens.

Additionally, if applicable, the following washouts must be met prior to start of CAR T cell infusion:

__2. At least 7 days must have elapsed since the research participant received his last dose of prior enzalutamide/other AR targeted therapy (excluding LHRH therapy which will continue).

__3. At least 2 days must have elapsed since the research participant received his last dose of prior abiraterone.

__4. At least 2 weeks must have elapsed since the research participant received his last dose of prior chemotherapy.

5.4 Agent Administration

5.4.1 Lymphodepletion

Research participants will be administered a lymphodepleting regimen as deemed appropriate by the protocol PI in consultation with the treating physician. **Section 8.2.1** has a list of recommended regimens. Lymphodepletion may be given as an outpatient. Lymphodepletion may be given 3 to 14 days prior to CAR T cell infusion based on PI discretion and subject eligibility. For participants receiving optional CAR T cell infusions, additional lymphodepletion may be given prior to optional CAR T cycles at a dose not greater than the initial lymphodepletion.

5.4.2 PSCA-CAR T cells

After a research participant is deemed eligible to proceed with CAR T cell infusion, the PI or designee will submit orders to the Department of Transfusion Medicine, Stem Cell Processing Laboratory (SCPL) for the transfer of the cryopreserved cell product to patient bedside. CAR T cell infusion may occur in the inpatient or outpatient setting per PI discretion and patients may be hospitalized for observation afterwards.

Research participants will be pre-medicated approximately 30 minutes (+/- 15 minutes) prior to CAR T cell infusion with 15 mg/kg of acetaminophen P.O. (max. 650 mg), and diphenhydramine 25-50mg I.V. or PO (max dose 50 mg). Clinically acceptable alternatives may be used if research participant is intolerant.

The CAR T cell infusion will be administered through an I.V. over approximately a 10-15 minute period at an infusion rate of 250 ml/hour. The infusion rate can also be adjusted if subjects experience mild infusion related adverse events (grade 2 or lower). Immediately following CAR T cell infusion, research participants will be monitored for no less than 5 hours per COH HCT standards (e.g., vital status, O_2, BP and temp).

If research participants do not meet the eligibility criteria to proceed with CAR T cell infusion on Day 0 then CAR T cell infusion may be delayed up to 14 days after the last dose of lymphodepletion. Eligibility will be reassessed after the medical issue(s) is/are appropriately addressed, and once all eligibility criteria for this event are achieved, otherwise CAR T cell infusion may be cancelled (reference **Section 5.7.1** for cancellation criteria).

Samples of the cryopreserved CAR T cell product will be sent for sterility analysis. Final results may be obtained after the product has been infused. If there is a positive microbiology culture, the PI or designee must be notified immediately. Standard practice of care will be followed to treat participants having a bacterial, fungal or viral infection in addition to standard prophylaxis, with the agent chosen to cover the specific organism identified while taking into account any patient specific antibiotic allergies. Treatment will be at the PI or designee's discretion.

5.5 Assessments and Special Monitoring

Required study assessments are shown in the study calendar, **Section 10.0**. Special monitoring is required for CRS and neurotoxicity. Research participants who exhibit and/or experience cytokine release syndrome symptoms or focal neurological signs or mental status changes following CAR T cell infusion will be admitted to the hospital for observation, management of clinical symptoms (as applicable), and additional testing which may include a neurological consultation, MRI/CT and a lumbar puncture. Subsequent correlative studies will include analysis of CSF for CAR+ cells and/or serum cytokine profiles (reference **Section 10.0**).

Repeat metastasis biopsy and marrow aspirate will occur at day 28 (+/- 7 days) unless there is contraindication.

5.6 EPIC Definition of Active Treatment

Participants will receive only one infusion of CAR T cells, so the active treatment portion of the trial as recorded in EPIC will include for (i) cohort one: the start of CAR T cell infusion through the end of the DLT period and first disease-response evaluation (~28-days), and for (ii) all other cohorts: the start of lymphodepletion through the end of the DLT period and first disease-response evaluation (~33 days, depending on the regimen).

5.7 Criteria for Removal from Protocol Therapy

5.7.1 Premature Discontinuation (*without* receiving CAR T cells)

Research participants who DO NOT receive any CAR T cell infusion will be considered to have prematurely discontinued the study. The reasons may include:

- The judgment of the PI, or his designee, is that the research participant is too ill to receive CAR T cell infusion,
- Significant and rapid progression of disease prior to CAR T cell infusion requiring alternative medical intervention that, in the opinion of the PI or his designee, is not compatible with CAR T cell therapy. If a research participant had successful generation of a therapeutic CAR T cell product but screen failed s/he may be eligible to re-enroll on the trial at a future date if they meet the eligibility criteria and a study slot is available.
- Inability to manufacture and/or release a CAR T cell product.

- Research participant's failure to meet criteria in **Section 5.3.3**, Criteria to proceed with CAR T cell infusion within 14 days after last dose of lymphodepletion.
- Research participant/family noncompliance with study therapy and/or clinic appointments,
- Voluntary withdrawal: a research participant may remove himself/herself from the study at any time without prejudice.

5.7.2 Discontinuation of Protocol Therapy (*after* receiving CAR T cells)

Research participants who discontinue study therapy AFTER receiving any CAR T cell infusion will still be required to adhere to long term follow up guidelines as mandated for research participants who receive gene modified cells (follow-up for 15 years from the time a CAR T cell product is infused).

All research participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Potential reasons for discontinued study therapy include:

- The judgment of the PI, or his designee, the research participant is too ill to continue, such as significant progression of disease or complication related to progressive disease requiring alternative medical interventions that in the opinion of the PI or his/her, designee, are not compatible with CAR T cell therapy.
- Research participant requires or elects to start disallowed treatment/care,
- Research participant/family noncompliance with study therapy and/or clinic appointments,
- Pregnancy,
- Voluntary withdrawal: a research participant may withdraw consent himself/herself from further study therapy at any time without prejudice,
- Death.

Once a participant meets criteria for removal from protocol therapy, the participant should then proceed to End of Protocol Therapy assessments, and then to follow-up (reference **Section 10.2**).

Documentation of the reason for discontinuing protocol therapy and the off-therapy date should be recorded in the Electronic Health Record/medical record and appropriate eCRF. The COH CRC and the Study PI should be promptly notified of the change in participant status.

5.8 Follow-Up

5.8.1 First Year Follow-Up Post CAR T cell Infusion

- Immediate follow-up: Research participants will have follow-up evaluations (reference **Section 10.1 study calendar** for details) that start on day 1 post CAR T cell infusion and then evaluations will be done at least every two days for toxicity for up to a minimum of 14 days post CAR T cell infusion; subsequently weekly evaluations for the first month post CAR T cell infusion for toxicities, serum analysis and correlative studies will continue.
- Short-term follow-up: Research participants will continue with monthly evaluations for the first year post CAR T cell infusion for toxicities, and correlative studies (as applicable, reference **Section 10.1**).

5.8.2 Long-term follow-up:

Research participants will continue with annual evaluations for a minimum of 15 years in accordance with the FDA guidance "Long Term Follow-up After Administration of Human Gene Therapy Products".

Long term follow-up may include the following:

- Physical examination which will include assessment for reproductive risk and risk to the fetus; autoimmune toxicities (such as new evidence or exacerbation of a prior rheumatologic or other autoimmune disorder);
- Disease status until first progression (or when subject is deemed censored for PFS, if applicable) following study treatment, while still enrolled in this protocol.
- Continued annual testing with an HIV Test by qPCR or equivalent as determined by the PI for the diagnosis of HIV positivity which could result from an RCL mediated event from the investigational agent (initial testing at the pre, 3, 6, and 12 month time points followed by annual testing thereafter, reference **Section 10.0**);
    - In the event of a confirmed positive HIV result, COH will require further testing to interrogate for evidence of a recombination event due to the investigational agent to distinguish from HIV infection. This will include qPCR testing for VSV-G and transgene.
    - Reports of all confirmed positive HIV results must be submitted to the FDA. Contact the Office of IND Development and Regulatory Affairs for such reporting requirements.
- Evaluation of CAR T cell persistence which will include testing for long-term vector persistence and insertional mutagenesis until long-term follow-up has completed.
    - In the event of suspected continued CAR T cell persistence and/or CAR T cell related late effects, the participant will be asked to return to COH, or a kit may be shipped to their local oncologist or designee, so that additional peripheral blood samples (up to 35cc may be collected, reference **Section 10.2**) may be evaluated for clonal T cell population(s), including the gene expression profile (i.e., insertional site mutagenesis) and the cytokine independent growth potential of any such population.

All long-term toxicities will be reported to the Agency in accordance with CFR 312.32.

5.8.3 AE and ConMed data collection:

AE and ConMed collection will begin at the start of study therapy (be it CAR T cell infusion for cohorts 1 and -1 or lymphodepletion for all other cohorts) and will continue based on the following provided below. Only serious adverse events attributed to protocol-related procedures (such as leukapheresis) will be collected and reported prior to the start of conditioning and/or start of CAR T cell infusion.

- Research participants will be considered "on active therapy" from the start of lymphodepletion (or start of CAR T cell infusion for cohorts 1 and -1) through ~28 days post CAR T cell infusion.
- DLT period is defined as start of CAR T cell infusion through ~28 days post infusion.
- During the DLT period, all AE grade changes and ConMeds (as new, ongoing or discontinued) will be captured regardless of disease status or additional therapy (reference Section 10.1).
- Once the patient has completed the DLT period, they will be deemed in follow up.

- Short-term follow up is defined as patients who have completed study therapy (i.e., no longer receiving CAR T cell infusions), and have not yet progressed or received disallowed therapy.
- During short-term follow-up, AEs (including worst grade and attribution) and ConMeds (as new, ongoing or discontinued) will be captured at the set evaluation periods for the first year (reference **Sections 5.8.1** and **10.1**).
- "Long-Term Follow-up or Modified Follow-Up" (**Sections 5.8.2** and **10.2**) is defined as patients who are no longer receiving CAR T cells, AND have either progressed or received disallowed therapy.
- During "Long-Term Follow-up or Modified Follow-Up" (Sections 5.8.2 and 10.2), participants may then continue to be followed per Section 10.2 where only select AEs attributable to gene therapy are collected while ConMed collection is no longer required. At the end of the first year post last CAR T cell infusion, the research participant will continue to be followed per FDA guidance on gene therapy clinical trials.
- All SAEs and deaths that occur within 30 days of the last active treatment will be reported to the COH regulatory committees per protocol **Section 14.3.2**.

5.9 Duration of Study Participation

Participants will undergo study screening procedures on this study for ~1-2 months prior to CAR T cell treatment, and then will continue to be actively followed for 1 year following the last CAR T cell infusion, after which they will switch to a 15-year long-term follow up.

5.10 Supportive Care

5.10.1 Supportive care for regimen-related toxicities

All standard supportive care measures will be at the discretion of the research participant's COH physician. Decisions regarding antibiotics choices, when hyperalimentation is used and when to use blood products will be determined by the treating clinician.

5.10.2 Infectious disease prophylaxis and monitoring

Active infections occurring after study enrollment will be treated according to the standard of care as defined by the COH Standard Operating Policies, Procedures, and Protocols.

5.10.3 Management of acute adverse event(s) attributable to infused CAR T cells:

Following CAR T cell infusions, the following general rules will be applied for adverse events that are attributed to CAR T cell infusion:

- Research participants experiencing a CRS and/or Neurotoxicity with an attribution of ≥3 (possible, probable or definite) to the infused CAR T cells rather than expected toxicities attributable to lymphodepletion, that in the opinion of the Principal Investigator puts the research participant in significant risk of an untoward outcome if measures are not taken to ameliorate the toxicity, should commence with corticosteroids, and/or IL-6 antagonist tocilizumab treatment (reference COH institutional SOP 'Management of Cytokine Release Syndrome and Neurologic Toxicities in patients receiving CAR T cell Therapy').
- Additional measures may also be taken to resolve the toxicity should the protocol specified CAR T cell toxicity treatment plan fails to ablate side effects associated with CAR T cell infusion such

as, but not limited to, immunosuppressive medications, or chemotherapy agents with immunosuppressive properties.

- If applicable, research participants will be hospitalized for at least the first 72 hours of receiving corticosteroids.

5.10.4 Management of potential exposure to microbial contaminants

Autologous CAR T cell products must meet release specification prior to clinical use, however, final sterility testing may not be resulted prior to clinical use, and therefore, in the event a CAR T cell product sterility test report microbial contaminants the Clinical Microbiology Laboratory at COH will immediately notify the study PI.

The organism identification and sensitivities will be determined, and the participant will be assessed for clinical status. An Infectious Disease consultation will be obtained for recommendations regarding appropriate treatment of the isolate that grows out. Based on the assessment, a decision regarding further management and monitoring of the participant will be made and documented in the medical record. When the organism is identified, an investigation to determine the source of the contamination will be initiated by the COH Quality Systems team and corrective actions will be implemented based on the investigations.

Additionally, the incident will be reported to COH DSMC and IRB and the FDA within 15 calendar days from receipt of notification, in accordance with COH Institutional Policy and 21CFR312.32.

5.10.5 Management of chronic toxicities

For severe chronic toxicities associated with transferred T cell (insertional mutagenesis, tumorigenesis of infused T cells, treatment-related persistent profound neutropenia [ANC <100/µL] lasting longer than 1 month) requiring the ablation of T cells, high dose steroids can be given. Chemotherapy treatment of the treatment physician's choice is also allowed to eradicate aberrant T cells.

Management of Constitutional Symptoms Associated with CAR T cell Infusion

Fever, and Chills: Fever, chills and temperature elevations >101˚F will be managed with additional Tylenol (or equivalent) as clinically indicated. Demerol I.V. (max dose 50 mg) may be given for chills. Additional methods such as cooling blankets may be employed for fevers resistant to these measures. Research participants that develop fever or chills will have a blood culture drawn. Appropriate selection of empiric antibiotics for treatment of neutropenic fever will be administered to research participants who, in the opinion of the physician in attendance, appear septic; alternate antibiotic choices will be used as clinically indicated.

Headache: Headaches may be managed with Tylenol (or equivalent). If unresponsive to Tylenol, manage with good clinical judgment.

Nausea and Vomiting: Nausea and vomiting will be managed with ondansetron IV or PO as well as additional standard anti-emetic treatments per patient preference.

Hypotension: Transient hypotension will initially be managed by IV fluid administration.

If significant hypotension occurs during the infusion, the infusion should be immediately suspended. Significant hypotension is defined as symptomatic and/or systolic blood pressure < 80 mm/hg, or a 15% drop from baseline, whichever value is lower. Treatment for significant hypotension will begin with normal saline 10 mL/kg over 30 minutes, repeat additional boluses, up to a total of 60 mL/kg. Alternately, treatment may be to use PRBC in place of normal saline if research participant Hct is < 25%. If hydration

is ineffective, the ICU should be notified, and the research participant transferred if it is anticipated that there will be a need for vasopressors.

Hypoxemia: Hypoxemia will be managed with supplemental oxygen. An etiology for hypoxemia will be worked up per standard clinical practice.

Potential Exposure to Microbial Contaminants: Autologous CAR T cell products must meet release specification prior to clinical use, however, final sterility testing may not be resulted prior to clinical use, and therefore, in the event a CAR T cell product sterility test report microbial contaminants the Clinical Microbiology Laboratory at COH will immediately notify the study PI. The organism identification and sensitivities will be determined, and the patient will be assessed for clinical status. An Infectious Disease consultation will be obtained for recommendations regarding appropriate treatment of the isolate that grows out. Based on the assessment, a decision regarding further management and monitoring of the patient will be made and documented in the medical record. When the organism is identified, an investigation to determine the source of the contamination will be initiated by the COH Quality Systems team and corrective actions will be implemented based on the investigations.

Additionally, the incident will be reported to COH DSMC and IRB and the FDA within 15 calendar days from receipt of notification, in accordance with COH Institutional Policy and 21CFR312.32.

Schedule of Systemic Corticosteroids to Ablate Side Effects of Genetically Modified T cells:

Steroids may be used per COH institutional SOPs such as:

- **Management of Cytokine Release Syndrome and Neurotoxicities in Patients Receiving (CAR) T or Immune Effector (IEC) Cell Therapy**: (https://cityofhope.my.salesforce.com/069d0000001W24A) to ablate unexpected side effects attributed to T cell infusion, expansion. Steroid treatment strategies have been clinically shown to reverse the side effects of adoptively transferred cytolytic immune cells such as T cells [30-32]. Please note that the above link is an example of the various steroid ablation options available, and the PI/study team may use alternative ablation methods that are deemed for the research participants.
- **Department of Genitourinary (GU) procedures for management of toxicities associated with the GU system**: In the event subjects have adverse toxicities, such as cystitis, related to study therapy (lymphodepletion and/or CAR T cells), the PI may give dexamethasone by intravesical or IV delivery. Particularly those adverse events that are related to GU can be managed following GU standard practice and policies.

  Specifically, urinary symptoms and urinalysis will be monitored regularly for early identification of cystitis (for instance, if WBC and/or RBC are noted to increase by 1 level (ex: 1+ → 2+ or 0 → 1+), implement cystitis mitigation strategies which may include consultation with urology for intravesical treatment, bladder spasm medications by mouth or by suppository, and/or systemic treatment with dexamethasone.

5.10.6 Management of potential off tumor PSCA-targeting toxicities

An unknown but potential toxicity risk in this study is the ability of these first-in-human PSCA-targeting CAR T cells to recognize target antigen on normal tissue, otherwise called off tumor/on-target toxicities. As described in **Section 2.1.1** normal tissue such as kidney, renal collecting ducts, urothelium, small intestine, and neuroendocrine cells of stomach and colon may express low levels of PSCA and therefore may potentially be targeted and killed by the PSCA-targeting CAR T cells. As a consequence, research participants could possibly experience toxicities such as dysphagia/odynophagia, dyspepsia, abdominal pain or diarrhea. Based on results from our preclinical studies using the PSCA-targeting CAR T cells (using normal cell lines, although not specific to these tissues) and reports from similar antibody clinical trials, we do not anticipate off tumor targeting of these tissues. However, the actual risk is unknown. The management plan for such toxicities, should they be observed, will be to request a GI consultation early and urgently for evaluation (to include endoscopy). All related findings, management plan and outcomes will be reported to the FDA in a timely manner. Reference is also made to **Section 5.10.5**, subsection "GU Procedures for management of toxicities associated with the GU system" for treatment options.

5.11 Concomitant Medications

5.11.1 Allowed concurrent medications

Subjects will continue on LHRH agonist/antagonist therapy (unless they have undergone bilateral orchiectomy) and bone supportive therapy (e.g., denosumab, zoledronic acid) but will discontinue abiraterone, enzalutamide or any additional oral or intravenous anti-prostate cancer therapy 2 weeks prior to leukapheresis. These may be resumed or initiated after leukapheresis has been performed but will be discontinued at least 7 days prior to CAR T infusion.

5.11.2 Contra-indicated medications with CAR T cells

Unless the Principal Investigator or designee provides an exception the following agents, other than specified in the protocol, are not allowed once CAR T cell infusion commences through day 100:

- systemic corticosteroids
- chemotherapy
- immunosuppressive agents
- immunotherapy
- other investigational agents

6 ANTICIPATED TOXICITIES

It is anticipated that research participants will experience a number of "expected" adverse events associated with the infusion of genetically modified T cells (usually occurring within the first 48 hours), and the *in vivo* expansion as well as the CAR-directed therapy (usually occurring after the first 48 hours but within the first 21 days after PSCA-CAR T cell infusion). The following is a list of highest **allowable*** "expected" adverse events (including grade and duration):

- Clinical Laboratory Abnormalities
  - Grade 3 or 4 asymptomatic, non-hematological clinical laboratory abnormalities, with the exception of grade 4 laboratory patterns (such as ferritin, D-dimer, lactic acid dehydrogenase, and fibrinogen levels) consistent with MAS/HLH, that return to ≤ Grade 2 within 7 days or electrolyte abnormalities that resolve with replacement.
  - Grade 4 neutropenia lasting up to 14 days.
- Dyspnea
  - Grade 3 Dyspnea lasting up to 72 hours with intervention
- Hypoxia
  - Grade 3 Hypoxia improving to grade 2 within 24 hours
- Fever, Chills, Cough
  - Grade 3 Fever lasting up to 14 days with intervention
  - Grade 4 Fever lasting up to 72 hours with intervention
  - Grade 2 Chills lasting up to 72 hours with intervention
  - Grade 3 Cough lasting for less than 24 hours after CAR T cell infusion
- Headache
  - Grade 3 Headache lasting up to 72 hours with intervention
- Hypotension
  - Grade 3 Hypotension not responding to intervention lasting up to 72 hours
- Hypertension: Grade 3 Hypertension that is either related to G3 headache/pain or does not require intervention
- Nausea
  - Grade 3 Nausea with intervention
- Rash
  - Grade 3 Rash lasting up to 72 hours with intervention
- Tachycardia
  - Grade 3 Tachycardia not responding to intervention and lasting up to 72 hours
- Transaminases
  - Grade 3 Transaminases lasting for less than 7 days after CAR T cell infusion
- Transient neurological complications (confusion, aphasia, seizure-like activity) manageable with intervention.
- Cytokine Release Syndrome
  - Grade 3 CRS with hypotension alone requiring a single vasopressor for support (not requiring intubation) that resolves to < Grade 2 in ≤72 hours
  - Grade 3 hypotension (without other CRS symptoms) requiring a single vasopressor for support that resolves to < Grade 2 in ≤72 hours
  - Grade 3 encephalopathy for ≤72 hours and resolves to baseline in ≤28 days

As cytokine release syndrome consists of a combination of toxicities and can only be diagnosed through exclusion it is believed that a grade 3 event lasting up to 3 days is toxicity that can be

managed and diagnosed within the given timeframes. In the event the grade continues beyond the allowable timeframe with intervention, the event will be considered a DLT (Reference **Appendix A**).

- Tumor Lysis Syndrome
  Grade 3 or 4 tumor lysis syndrome (TLS) for ≤2 weeks

*Allowable 'expected' AEs will not result in administration of T cell ablation methods and will not be considered a DLT.

Adverse events from the above list occurring within the specified time will:

- Not result in an expedited adverse event reporting to the FDA
- Not result in ablation of T cells with corticosteroids

Additionally, a hospitalization/prolongation of a hospitalization for monitoring participants with non-serious adverse events or for management of concomitant medical care issues will not be considered an AE for expedited reporting nor be a factor in MTD determination.

7 DOSE-LIMITING TOXICITIES/DOSE DELAYS/ DOSE MODIFICATIONS

7.1 DLT Definition

Dose limiting toxicity is defined as follows:

- any emergent Grade 3 or greater organ toxicity (cardiac, pulmonary, gastrointestinal, hepatic, or renal) with an attribution of possible, probable or definite to CAR T cells that are not pre-existing or due (i.e., at least possibly related) to underlying malignancy, and lasting more than 72 hours with intervention;
- any grade 3 or greater autoimmune toxicity, and occurring within 28 days of CAR T-cell infusion (not including additional cycles 2+);
- any grade 4 or higher hematological toxicity, with the exception of those listed in **Section 6**; and designated as definitely, probably or possibly related (level of attribution) to the infusion of the CAR T cells; and occurring within 28 days of CAR T cell infusion (not including additional cycles 2+);
- grade 5 toxicity with an attribution of possibly, probably, or definitely related to the infusion of the CAR T cells.

7.2 CAR T cell dose delays

The CAR T cell dose may be delayed for up to 14 days following the last dose of lymphodepleting therapy.

7.3 CAR T cell dose modifications

CAR T cells are given only in 1 dose, so there is no intra-patient dose modification.

8 AGENT INFORMATION

8.1 PSCA(dCH2)BBZ-CAR T cells (IND # 18812) Description

The investigational agent in this protocol is modified PSCA(dCH2)BBζ-CAR T Cells (a.k.a., PSCA-CAR T cells). Autologous T cells will be derived from patient leukapheresis product after ficoll separation. Peripheral blood mononuclear cell (PBMC) preparations will then undergo immunomagnetic selection to deplete CD14+ myeloid cells and CD25+ Treg populations. The resulting cell preparation will then be activated

with anti-CD3/CD28 beads. Activated T cells will undergo lentiviral transduction to express the PSCA(dCH2)BBζ CAR (description of the lentiviral vector is provided below), expanded *in vitro* to achieve cell numbers sufficient for the research participant's planned clinical cell dose and all related product release testing, and then harvested, washed and formulated for cryopreservation until time of infusion.

In the event a research participant does not have sufficient starting material (e.g., less than $20x10^6$ CD3+ T cells from starting apheresis product), excess cryopreserved PBMC from the original leukapheresis may be used as the starting material for another manufacturing campaign as described above.

Lentiviral Vector: Self-inactivating (SIN) lentiviral vectors encoding both a PSCA-targeting CAR and a truncated CD19 (CD19t) surface antigen were produced and released by COH Center for Biomedicine and Genetics (reference is made to COH BB-MF 9778). All raw materials used in the production of the lentiviral vector were released by the Office of Quality Systems (OQS) prior to use. Certificates of Analysis (CofAs) for all materials referenced in any lentiviral production batch record, as well as completion of batch records, labeling and tracking of the product are maintained by OQS. All processes were carried out according to SOPs and include QA oversight for cGMP compliance.

The cloned DNA that will be used for the genetic modification of T cells consists of a codon optimized CAR sequence containing a PSCA-targeting, humanized and affinity matured A11 scFv [33], a human IgG4 Fc spacer lacking the CH2 domain to prevent Fc receptor-mediated recognition [34], a human CD4 transmembrane domain, a costimulatory human 4-1BB cytoplasmic signaling domain, a three-glycine linker and a human CD3ζ cytoplasmic signaling domain. A T2A ribosome skip sequence[35] then separates this PSCA(dCH2)BBζ CAR sequence from a truncated human CD19 (CD19t) sequence, which lacks the cytoplasmic signaling tail (truncated at amino acid 323). Co-expression of CD19t provides an inert, non-immunogenic surface marker that allows for accurate measurement of gene modified cells, as well as efficient cell tracking of the therapeutic T cells *in vivo* following adoptive transfer. The T2A linkage results in the coordinate expression of both PSCA(dCH2)BBζ and CD19t from a single transcript [35]. Transcription is driven by the EF1 promoter.

The PSCA(dCH2)BBZ-T2A-CD19t_epHIV7 construct does not contain an intact 3' long terminal repeat (LTR) promoter, so the resulting expressed and reverse transcribed DNA proviral genome in targeted cells will have inactive LTRs. As a result of this design, no HIV-I derived sequences will be transcribed from the provirus and only the therapeutic PSCA(dCH2)BBζ CAR and CD19t marker will be expressed. The removal of the LTR promoter activity in this self-inactivating vector is also expected to significantly reduce the possibility of unintentional activation of host genes [36].

Furthermore, this study will employ replication-incompetent lentivirus produced by the 4-plasmid co-transfection of producer cells (i.e., 293T renal carcinoma cells). Briefly, the crippled self-inactivating lentivirus was harvested from cultures of 293T cells that had been transiently transfected with the following four plasmids encoding the required components: 1) pCgp containing the HIV-1 gag and pol genes required for viral vector assembly; 2) pCMV-G containing the VSV-G gene required for viral vector infectivity; 3) pCMV-rev containing the rev gene which assists in the transportation of the viral genome for efficient packaging; and 4) the above described PSCA(dCH2)BBζ-T2A-CD19t_epHIV7 transfer vector.

8.1.1 Toxicology

Since PSCA-CAR T cell products are autologous, no animal toxicology is required by the FDA. These products are expected to target PSCA expressing cells, primarily prostate cancer cells. Directed targeting of PSCA expressing cells are associated with a number of expected adverse reactions listed in **Section 6**.

8.1.2 Pharmacology – handling, storage, dispensing and disposal

The PSCA-CAR T cell products will be prepared under **BB IND 18812** by the T Cell Therapeutics Research Laboratory staff under the direction of Dr. Stephen Forman. Cell products are prepared using approved SOPs and manufactured under cGMP conditions on the COH campus. All raw materials used in the production of the cell product are released by OQS prior to use. CofAs for all materials referenced in any cell product batch record and all records for T cell manipulations, including cell selection and transduction, as well as completion of batch records, labeling and tracking of the cell product will be maintained by OQS. All processes are carried out according to SOPs and include QA oversight for cGMP compliance. Cryopreserved cell products are Quality Control tested as required under IND and released for use by the Director of OQS, or designee.

Cryopreserved bag(s) will be frozen using a controlled rate freezer and stored in vapor phase in a controlled access LN_2 freezer within the cGMP facility until transfer to a controlled access LN_2 freezer within the Department of Transfusion Medicine, Stem Cell Processing Laboratory (SCPL). On the day of PSCA-CAR T cell infusion the PI will submit orders to SCPL for transport of the cryopreserved product to participant bedside. The required number of cryopreserved product bags will be thawed and infused via a pump over approximately 10-15 minutes per bag.

Research participants will be pre-medicated approximately 30 minutes (+/- 15 minutes) prior to PSCA-CAR T cell infusion with 15 mg/kg of acetaminophen P.O. (max. 650 mg), and diphenhydramine 25-50mg I.V. or PO (max dose 50 mg). Clinically acceptable alternatives may be used if research participant is intolerant.

Any product released to the PI that is not infused to the patient will either be released back to the SCPL for storage or discarded by clinical staff per institutional policy and documented on the infusion record.

8.2 Recommended Lymphodepleting Regimens

8.2.1 Description

The study PI and the protocol team will choose a chemotherapy regimen, for lymphodepletion prior to the PSCA-CAR T cell infusion (with the exception of cohorts 1 and -1 which will not receive lymphodepletion), based on the research participant's disease type and prior therapies. Therefore, one lymphodepleting regimen may not be adequate for all research participants as some amount of disease control may be attained along with lymphodepletion to allow the CAR-T cells space and time to work *in vivo*. In addition, the best regimen that also limits potential toxicities is not known yet. Lymphodepletion may be given outpatient, at the discretion of the protocol team. For participants receiving optional CAR T cell infusions, additional lymphodepletion may be given prior to optional CAR T cycles at a dose not greater than the initial lymphodepletion.

DRUG(S)	REGIMEN
Standard CAR T Regimen:* Fludarabine/Cyclophosphamide regimen	Fludarabine 30mg/m^2/day IV (3 days) overlapping with Cyclophosphamide 500mg/m^2/day IV (3 days) on days -5, -4, and -3
Modified Fludarabine/Cyclophosphamide regimen	Fludarabine 30mg/m^2/day IV (3 days) overlapping with Cyclophosphamide 300mg/m^2/day IV (3 days) on days -5, -4, and -3

DRUG(S)	REGIMEN
Cyclophosphamide alone	Cyclophosphamide 300mg/m^2/day IV (3 days) on days -5, -4, and -3

***** the above listed dosing regimens may be modified by the PI/designee depending on the research participant's disease and overall health condition at the time of lymphodepletion.

Mesna will be used for cyclophosphamide if cumulative doses greater than 1.5 gm/m^2 are given and adequate IV hydration will be administered with all chemotherapy.
Additionally, dosing will be based on the following research participant body weight guidelines:

- Actual body weight to be used if the research participant's actual body weight is up to 120% of the ideal body weight.
- Adjusted body weight to be used if the research participant's actual body weight is more than 120% of the ideal body weight.

8.2.2 Toxicology

Toxicology and expected adverse events associated with the various lymphodepleting regimens will be dependent on the regimen chosen by the study PI and therefore are not provided in this document. Physicians should consult the current COH HEM/HCT Standard Operating Policies, Procedures and Protocols as well as the drugs' package inserts.

8.2.3 Pharmacology – handling, storage, dispensing and disposal

The lymphodepleting regimens listed above are outlined in the current COH HEM/HCT Standard Operating Policies, Procedures and Protocols and will be used as a guideline to order the disease-specific chemotherapy regimen when chosen for lymphodepletion. Handling, storage, dispensation, and disposal of chemotherapeutic drugs will be done per package insert and within institutional policies.

8.3 ACTEMRA® (Tocilizumab)

8.3.1 Description:

ACTEMRA® (tocilizumab) is indicated for the treatment of adult patients with moderately to severely active rheumatoid arthritis who have had an inadequate response to one or more Disease-Modifying Anti-Rheumatic Drugs (DMARDs), active polyarticular juvenile idiopathic arthritis in patients 2 years of age and older, and active systemic juvenile idiopathic arthritis in patients 2 years of age and older.

Tocilizumab binds specifically to both soluble and membrane-bound IL-6 receptors (sIL-6R and mIL-6R), and has been shown to inhibit IL-6-mediated signaling through these receptors. IL-6 is a pleiotropic pro-inflammatory cytokine produced by a variety of cell types including T- and B-cells, lymphocytes, monocytes and fibroblasts. IL-6 has been shown to be involved in diverse physiological processes such as T-cell activation, induction of immunoglobulin secretion, initiation of hepatic acute phase protein synthesis, and stimulation of hematopoietic precursor cell proliferation and differentiation. IL-6 is also produced by synovial and endothelial cells leading to local production of IL-6 in joints affected by inflammatory processes such as rheumatoid arthritis.

Recent clinical responses in the field of adoptive cellular immunotherapy related to the effective tumor targeting by engineered T cell therapy have been associated with acute cytokine release syndrome (CRS) which often requires intensive care to manage [37]. In one case of CRS, the use of steroids did not abrogate CRS, though this was subsequently controlled by the anti-cytokine antibodies [37], implying that the antibodies not only provide an adequate alternative to steroids but that they are in fact likely a superior alternative. Furthermore, with the avoidance of steroids, these patients have been able to go on and have good responses to therapy. Although this anti-cytokine agent is associated with immune suppression when dosed repeatedly in the context of disease like arthritis or psoriasis there is little evidence to suggest risk in this on-off dosing context where the immediate benefits may well be life-saving [37].

For this study, Tocilizumab will be used per COH institutional SOPs to manage CRS.

8.3.2 Toxicology:

ADVERSE REACTIONS: Most common adverse reactions (incidence ≥ 5%): upper respiratory tract infections, nasopharyngitis, headache, hypertension, increased ALT.

WARNINGS and PRECAUTIONS:
Serious Infections: Serious and sometimes fatal infections due to bacterial, mycobacterial, invasive fungal, viral, protozoal, or other opportunistic pathogens have been reported in patients receiving immunosuppressive agents including ACTEMRA for rheumatoid arthritis. The most common serious infections included pneumonia, urinary tract infection, cellulitis, herpes zoster, gastroenteritis, diverticulitis, sepsis and bacterial arthritis. Among opportunistic infections, tuberculosis, cryptococcus, aspergillosis, candidiasis, and pneumocystosis were reported with ACTEMRA. Other serious infections, not reported in clinical studies, may also occur (e.g., histoplasmosis, coccidioidomycosis, listeriosis). Patients have presented with disseminated rather than localized disease, and were often taking concomitant immunosuppressants such as methotrexate or corticosteroids which in addition to rheumatoid arthritis may predispose them to infections.

ACTEMRA should not be administered in patients with an active infection, including localized infections. The risks and benefits of treatment should be considered prior to initiating ACTEMRA in patients:

- with chronic or recurrent infection;
- who have been exposed to tuberculosis;
- with a history of serious or an opportunistic infection;
- who have resided or travelled in areas of endemic tuberculosis or endemic mycoses; or
- with underlying conditions that may predispose them to infection.

Patients should be closely monitored for the development of signs and symptoms of infection during and after treatment with ACTEMRA, as signs and symptoms of acute inflammation may be lessened due to suppression of the acute phase reactants.

ACTEMRA should be interrupted if a patient develops a serious infection, an opportunistic infection, or sepsis. A patient who develops a new infection during treatment with ACTEMRA should undergo a prompt and complete diagnostic workup appropriate for an immunocompromised patient, appropriate antimicrobial therapy should be initiated, and the patient should be closely monitored

Tuberculosis: Patients should be evaluated for tuberculosis risk factors and tested for latent infection prior to initiating ACTEMRA. Anti-tuberculosis therapy should also be considered prior to initiation of

ACTEMRA in patients with a past history of latent or active tuberculosis in whom an adequate course of treatment cannot be confirmed, and for patients with a negative test for latent tuberculosis but having risk factors for tuberculosis infection. Consultation with a physician with expertise in the treatment of tuberculosis is recommended to aid in the decision whether initiating anti-tuberculosis therapy is appropriate for an individual patient. Patients should be closely monitored for the development of signs and symptoms of tuberculosis including patients who tested negative for latent tuberculosis infection prior to initiating therapy. It is recommended that patients be screened for latent tuberculosis infection prior to starting ACTEMRA. The incidence of tuberculosis in worldwide clinical development programs is 0.1%. Patients with latent tuberculosis should be treated with standard antimycobacterial therapy before initiating ACTEMRA.

Viral Reactivation: Viral reactivation has been reported with immunosuppressive biologic therapies and cases of herpes zoster exacerbation were observed in clinical studies with ACTEMRA. No cases of Hepatitis B reactivation were observed in the trials; however, patients who screened positive for hepatitis were excluded.

Gastrointestinal Perforations: Events of gastrointestinal perforation have been reported in clinical trials, primarily as complications of diverticulitis. ACTEMRA should be used with caution in patients who may be at increased risk for gastrointestinal perforation. Patients presenting with new onset abdominal symptoms should be evaluated promptly for early identification of gastrointestinal perforation.

Laboratory Parameters

Neutrophils

Treatment with ACTEMRA was associated with a higher incidence of neutropenia. Infections have been uncommonly reported in association with treatment-related neutropenia in long-term extension studies and postmarketing clinical experience. It is not recommended to initiate ACTEMRA treatment in patients with a low neutrophil count i.e., absolute neutrophil count (ANC) <2000/mm^3. In patients who develop an absolute neutrophil count <500/mm^3 treatment is not recommended. Neutrophils should be monitored every 4 to 8 weeks.

Platelets

Treatment with ACTEMRA was associated with a reduction in platelet counts. Treatment-related reduction in platelets was not associated with serious bleeding events in clinical trials. It is not recommended to initiate ACTEMRA treatment in patients with a platelet count below 100,000/mm^3. In patients who develop a platelet count <50,000/mm^3 treatment is not recommended. Platelets should be monitored every 4 to 8 weeks.

Liver Function Tests

Treatment with ACTEMRA was associated with a higher incidence of transaminase elevations. These elevations did not result in apparent permanent or clinically evident hepatic injury in clinical trials. Increased frequency and magnitude of these elevations was observed when potentially hepatotoxic drugs (e.g., MTX) were used in combination with ACTEMRA. In one case, a patient who had received ACTEMRA 8 mg/kg monotherapy without elevations in transaminases experienced elevation in AST to above 10x ULN and elevation in ALT to above 16x ULN when MTX was initiated in combination with
ACTEMRA. Transaminases normalized when both treatments were held, but elevations recurred when MTX and ACTEMRA were restarted at lower doses. Elevations resolved when MTX and ACTEMRA were

discontinued. It is not recommended to initiate ACTEMRA treatment in patients with elevated transaminases ALT or AST > 1.5x ULN. In patients who develop elevated ALT or AST > 5x ULN treatment is not recommended. ALT and AST levels should be monitored every 4 to 8 weeks. When clinically indicated, other liver function tests such as bilirubin should be considered.

Lipids
Treatment with ACTEMRA was associated with increases in lipid parameters such as total cholesterol, triglycerides, LDL cholesterol, and/or HDL cholesterol. Assessment of lipid parameters should be performed approximately 4 to 8 weeks following initiation of ACTEMRA therapy, then at approximately 6-month intervals. Patients should be managed according to clinical guidelines [e.g., National Cholesterol Educational Program (NCEP)] for the management of hyperlipidemia.

Immunosuppression
The impact of treatment with ACTEMRA on the development of malignancies is not known but malignancies were observed in clinical studies. ACTEMRA is an immunosuppressant, and treatment with immunosuppressants may result in an increased risk of malignancies.
Hypersensitivity Reactions
Serious hypersensitivity reactions, including anaphylaxis, have been reported in association with infusion of ACTEMRA. Appropriate medical treatment should be available for immediate use in the event of an anaphylactic reaction during administration of ACTEMRA.

Demyelinating Disorders
The impact of treatment with ACTEMRA on demyelinating disorders is not known, but multiple sclerosis and chronic inflammatory demyelinating polyneuropathy were reported rarely in clinical studies. Patients should be closely monitored for signs and symptoms potentially indicative of demyelinating disorders. Prescribers should exercise caution in considering the use of ACTEMRA in patients with preexisting or recent onset demyelinating disorders.

Active Hepatic Disease and Hepatic Impairment
Treatment with ACTEMRA is not recommended in patients with active hepatic disease or hepatic impairment.

Vaccinations
Live vaccines should not be given concurrently with ACTEMRA as clinical safety has not been established. No data are available on the secondary transmission of infection from persons receiving live vaccines to patients receiving ACTEMRA. No data are available on the effectiveness of vaccination in patients receiving ACTEMRA. Because IL-6 inhibition may interfere with the normal immune response to new antigens, patients should be brought up to date on all recommended vaccinations, except for live vaccines, prior to initiation of therapy with ACTEMRA.

8.3.3 Pharmacology – Handling, Storage, Dispensing and Disposal

Tocilizumab will be used per COH institutional SOPs (https://cityofhope.my.salesforce.com/069d0000001W24A), especially in the management of severe CRS when (C-Reactive Protein) CRP is >/=20mg/dL[38].

Due to the 1-2 day turn-around time reasonably expected for cytokine results, it is reasonable to consider administration of Tocilizumab based on clinical evidence of CRS, and before having the cytokine data in hand.

9 CORRELATIVE STUDIES

9.1 Biological Sample Collection

The following biological specimens will be collected in this study and will be used to evaluate the association of exploratory systemic and/or tissue specific biomarkers with study drug response including efficacy and/or adverse events. Samples will be tracked using a unique identifier that is assigned to each sample for the study.

9.1.1 Peripheral blood (PB)

One 35 ml blood draw (e.g., in five 10 ml purple-top EDTA tubes, AND one 6 ml red-top tube) is to be collected at least on days prior to lymphodepletion (with the exception of cohorts 1 and -1), days 0, 1, 7, 14, 21, 28, 60, 90, and months 6, 9 and 12. Blood samples will be placed at room temperature for pickup by the Biospecimen Coordinator within ~1 hour of collection. Blood samples should be delivered to the TCTRL approximately 2-3 hours from the time they were drawn.

The blood samples, collected in Streck tubes, from baseline (prior to lymphodepletion, with the exception of cohorts 1 and -1), Day 0 (pre-CAR T Cell infusion) and Day 28 will also be delivered to the Peter Kuhn laboratory at the University of Southern California, Los Angeles.

9.1.2 Tumor biopsies

All tumor tissue biopsies specimens (minimum of 2 cores for ClinPath) will first be sent directly to Clinical Pathology for disease assessment and IHC slide preparation. Once clinical assessments by Clinical Pathology are completed, prepared slides may then be transferred to PathCore for PSCA expression screening. Additionally, 2 cores will be collected and sent to the TCTRL laboratories simultaneously. The PI reserves the right to request and/or retain additionally required archival screening tumor samples for further correlative assessment in accordance with the exploratory research objectives (see Section 1.3) for the patients who provided consent for using their samples for future health research.

Participants will have a major soft-tissue lesion biopsied to screen for PSCA expression by IHC and, when possible, by Flow Cytometry, and to characterize the tumor immune environment prior to start of study therapy (and again between Day 28 and 30 post CAR T cell infusion). For soft tissue lesions, at least 4 biopsy cores will be obtained from a lesion which has not previously undergone radiation [one biopsy core will be fresh, a second core will be frozen, and the other 2 cores will be processed standard (paraffin embedded)]. The fresh and frozen samples should be delivered to the TCTRL within approximately 2-3 hours from the time they were drawn. Frozen or FFPE tissue (pre- and post-infusion) should then be sent to Dr. Huihui Ye's Laboratory at University of California, Los Angeles. Standard samples should be delivered to Clinical Pathology first for disease assessment and IHC slide preparation and then sent to PathCore for PSCA expression screening if applicable.

As bone lesion biopsy IHC screening has not yet been optimized, such samples will be screened for correlative and assay optimization purposes and archived for later correlative analysis, but will not dictate study enrollment. When feasible, it is recommended to select a bone lesion with the most intense uptake

on bone scan (taking into account safety of the biopsy and size) with preference for iliac bone, and at least 4 cores be obtained prior to start of study therapy (and again between Day 28 and 30 post CAR T cell infusion). If the lesion is in the iliac bone, marrow aspirate should be obtained at the same biopsy site. For soft tissue and non-iliac lesions, bone marrow aspirate will not be mandated. Of the 4 bone biopsy cores: one biopsy core will be fresh, a second core will be frozen, and the other 2 cores will be processed standard (paraffin embedded). The fresh and frozen samples should be delivered to the TCTRL within approximately 2-3 hours from the time they were drawn. Standard samples should be delivered to Clinical Pathology first for disease assessment and IHC slide preparation and then sent to PathCore for PSCA expression screening if applicable. When marrow is obtained, a sample should be collected in one 10 ml EDTA tube for the Kuhn lab (if the marrow sample is less than 4 ml, it can be collected in an EDTA tube to avoid dilution), and the remainder of the specimen (if requested) delivered to the TCTRL approximately 2-3 hours from the time they were drawn.

9.1.3 Urine

Urine will be collected in standard urine collection cups in the clinic at the frequency described in **Section 10.1.** A urine preservation reagent will be aliquoted into the urine cup and will be stored at room temperature (ZYMO); they will be stable for up to 1 month. Urine samples will be sent to TCTRL c/o: Saul Priceman Lab for cytokine monitoring.

9.1.4 Other potential biological samples

The PI reserves the right to request correlative sample collection of additionally required biological sample collection intended for the medical care of the research participant while on study (for example, bronchial lavage for respiratory complication, skin punch biopsy for rash, etc.). These samples will be requested as add-ons to standard of care collections and will only be provided to the research team if there is excess sample available following standard of care. Sample collection and immediate handling will be determined by the PI and the research team as needed.

9.2 Summary of Correlative Studies

Correlative studies will include collaborative efforts (not limited to) with Translational Genomics Research Institute (TGen), Dr. Huihui Ye's Laboratory at University of California, Los Angeles, Dr. Lior Pachter's Laboratory at the California Institute of Technology, and Dr. Peter Kuhn's Laboratory at the University of Southern California.

9.2.1 Analysis of CAR T cell persistence

The accumulation, magnitude and duration of persistence of adoptively transferred PSCA-CAR T cells in the PB will be measured by Q-PCR with primers specific for the lentiviral sequence WPRE (woodchuck hepatitis virus post-transcriptional regulatory element). Magnitude and duration of CAR T cell persistence will be evaluated with respect to observed toxicities and/or changes in disease/tumor status. When there is sufficient biological sample, the presence of PSCA-CAR T cells might also be monitored by flow cytometry or immunohistochemistry.

9.2.2 Immunophenotyping/functional analyses

Analyses of endogenous/unmodified immune cells and persisting infused CAR T cells for activation, memory, and/or effector phenotype will be performed on biological samples using multiparameter flow cytometric analyses. Similar analyses will be performed on initial CAR T cell products as

background/reference. In some cases, the cells will be antigen- or tumor-stimulated *ex vivo* prior to flow cytometric analysis. Correlation of initial activation status of infused cells (e.g., expression of markers such as CD25, CD137 and PD1) with *in vivo* T cell persistence and direct *ex vivo* characterization of anti-tumor effector functions of persisting infused cells (e.g., their cytolytic activity and cytokine production upon antigen/tumor stimulation ex vivo) will also be performed. In some cases, fresh tumor biopsy material (when available) might be used as the targets for CAR T cell functional analyses using either *in vitro* assays or *in vivo* tumor xenograft mouse models.

9.2.3 Serum cytokine measurement

Serum cytokine measurements from PB serum (processed from red-top tubes) will be analyzed using the Human Cytokine 30-Plex Luminex assay (Invitrogen), or equivalent kit including cytokines such as GM-CSF, IFN-γ, IL-2, IL-6, IL-10, IL-12, TNF-α, and VEGF. As there is significant evidence in the field that serum cytokine levels are the most appropriate measure of T-cell functional activity that corresponds to associated clinical toxicity [39, 40], these cytokine analyses will assist us in potentially attributing toxicities to the infused CAR T cells.

9.2.4 Tumor analyses

DNA/RNAs from circulating tumor cells (CTC) in PB (collected in Streck tubes at baseline and Day 28, and processed by the Kuhn lab, or similar contract lab) and tumor biopsies prior to and at various time points during treatment will be subjected to RNA-Seq analysis for detection of genetic variations and expression changes before and after treatment. We will also analyze cfDNA (of tumor origin) in PB by whole exome sequencing pre- and post-treatment.

PSCA expression on tumor cells within the biological samples, when available, will be determined by qPCR, immunohistochemistry and/or flow cytometry at various time points after CAR T cell infusion in an effort to detect possible antigen escape. Biopsies may also be analyzed for immune cell infiltrates and changes to the tumor microenvironment by immunohistochemistry. Fresh biopsy material (when available) might also be used to evaluate tumor sensitivity and/or resistance to various therapies in either *in vitro* cytotoxicity assays or *in vivo* tumor xenograft mouse models.

9.2.5 PSCA-CAR immunogenicity

We will assess the immunogenicity of the PSCA-CAR T cells by testing peripheral blood for human anti-CAR antibodies (HACA/HAMA) in an ELISA- or flow cytometry-based assay.

9.2.6 Mathematical Modeling of Cancer Immunity at Single Cell Resolution

Within the Priceman Lab at COH, in collaboration with Dr. Lior Pachter's Laboratory at Caltech, will assess single cell gene expression over the course of CAR T cell therapy. Samples such as the patient's CAR T cell products, peripheral blood, bone marrow aspirate, and/or tumor biopsies will be sent to CalTech for Single Cell analysis which will include, at minimum, quantification of immune cell subsets (e.g., T cells, myeloid, etc.) along with TCR repertoire analysis to understand immune dynamics and T cell clonality following CAR T cell therapy.

9.2.7 Urine Analysis

The accumulation, magnitude, and duration of persistence of adoptively transferred PSCA-CAR T cells in urine will be measured by Q-PCR with primers specific for the lentiviral sequence WPRE (woodchuck hepatitis virus post-transcriptional regulatory element). Magnitude and duration of CAR T cell persistence will be evaluated with respect to observed toxicities and/or changes in disease/tumor status. When there is sufficient biological sample, the presence of PSCA-CAR T cells might also be monitored by flow cytometry or immunohistochemistry.

Cytokine measurements from urine will be analyzed using the Human Cytokine 30-Plex Luminex assay (Invitrogen), or equivalent kit including cytokines such as GM-CSF, IFN-γ, IL-2, IL-6, IL-10, IL-12, TNF-α, and VEGF. As there is significant evidence in the field that urine cytokine levels are the most appropriate measure of T-cell functional activity that corresponds to associated clinical toxicity [39, 40], these cytokine analyses will assist us in potentially attributing toxicities to the infused CAR T cells.

DNA/RNAs from circulating tumor cells (CTC) in urine prior to and at various time points during treatment will be subjected to RNA-Seq analysis for detection of genetic variations and expression changes before and after treatment. We will also analyze cfDNA (of tumor origin) in urine by whole exome sequencing pre- and post-treatment. PSCA expression on tumor cells within the biological samples, when available, will be determined by qPCR, immunohistochemistry and/or flow cytometry at various time points after CAR T cell infusion in an effort to detect possible antigen escape.

We will assess the immunogenicity of the PSCA-CAR T cells by testing urine for human anti-CAR antibodies (HACA/HAMA) in an ELISA- or flow cytometry-based assay.

9.2.8 Other potential analyses

Any biological sample collected according to the Study Calendar (**Section 10**) can be analyzed for any of the tests outlined in the protocol and for any tests necessary to minimize risks to study subjects. This includes testing to ensure analytical methods produce reliable and valid data throughout the course of the study. This can also include, but is not limited to, investigation of unexpected results, incurred sample reanalysis, and analyses for method transfer and comparability. The investigators also reserve the right to adjust the studies to be performed as techniques evolve and new information may become available that is relevant to the study agent and/or study population.

10 STUDY CALENDAR

10.1 Study Activity Calendar

Study Parameters and Calendar		Screening/ Enrollment Activities			Active Treatment										Protocol Follow-Up					
					Study Treatment			Month 1 DLT and Response Evaluations							Short Term Protocol Follow-Up[17]				LTFU[18]	
	Pre-screening	Screening Evaluations[12]	**Leukapheresis**	Prior to Lymphodepletion	Lymphodepletion (except for cohorts 1 and -1)	Prior to CAR T Cell Infusion[13]	**CAR T Cell Infusion (Day 0)[14]**	Within 24° post CAR T cells	Interim evaluations[15]	Day 7 post CAR T Cell (+/- 3 days)	Interim evaluations[15]	Day 14 post T Cell (+/- 3 days)	Day 21 post T Cell (+/- 3 days)	Day 28 post T Cell (+/- 3 days)	Day +60 post T Cell (+/- 7 days)	Day +90 post T Cell (+/- 7 days)	Months 6. 9 and 12 (+/- 14 days)	End of Therapy Assessment (+/- 14 days)	LTFU Evaluation: Yearly post CAR T cells (+/- 2 months)
Screening Consent	X																			
Treatment Consent				X																
Demographics		X																		
Medical History		X	X	X																
Concurrent Meds		X					X		X	X	X	X	X	X	X	X	X	X		
H&P		X				X				X		X	X	X	X	X	X			
Vital Signs, ECOG		X				X		X	X	X	X	X	X	X	X	X	X			
CBC, Differential, ANC		X		X		X		X	X	X	X	X	X	X	X	X	X			
Comprehensive Metabolic Panel, plus LDH, phosphorous, magnesium		X		X		X		X	X	X	X	X	X	X	X	X	X			
Ferritin						X		X	X	X	X	X	X	X						
CRP Levels [7]				X		X		X	X	X	X	X	X	X						

Study Parameters and Calendar	Pre-screening	Screening/ Enrollment Activities			Active Treatment										Protocol Follow-Up				
					Study Treatment			Month 1 DLT and Response Evaluations							Short Term Protocol Follow-Up[17]				LTFU[18]
		Screening Evaluations[12]	**Leukapheresis**	Prior to Lymphodepletion	Lymphodepletion (except for cohorts 1 and -1)	Prior to CAR T Cell Infusion[13]	**CAR T Cell Infusion (Day 0)[14]**	Within 24° post CAR T cells	Interim evaluations[15]	Day 7 post CAR T Cell (+/- 3 days)	Interim evaluations[15]	Day 14 post T Cell (+/- 3 days)	Day 21 post T Cell (+/- 3 days)	Day 28 post T Cell (+/- 3 days)	Day +60 post T Cell (+/- 7 days)	Day +90 post T Cell (+/- 7 days)	Months 6. 9 and 12 (+/- 14 days)	End of Therapy Assessment (+/- 14 days)	LTFU Evaluation: Yearly post CAR T cells (+/- 2 months)
PSCA expression[25]	X			X										X					
PSA		X		X		X						X		X	X	X	X	X	
Uric Acid		X		X		X		X	X	X	X	X	X	X	X	X	X		
Calculated CrCl[1]		X																	
Urine analysis		X		X			X		--------X----------[30]										
QuantiFERON-TB Gold or equivalent		X																	
HIV Testing[2]		X[33]														X	X[16]		X
Hepatitis A		X																	
Hepatitis B Panel[3]		X																	
Hepatitis C total antibody[4]		X																	
ECHO scan		X																	
EKG		X																	
Bone scan (Technetium-99)		X												X		X	X		

Study Parameters and Calendar		Pre-screening	Screening/ Enrollment Activities			Active Treatment									Protocol Follow-Up				
						Study Treatment			Month 1 DLT and Response Evaluations						Short Term Protocol Follow-Up[17]				LTFU[18]
			Screening Evaluations[12]	**Leukapheresis**	Prior to Lymphodepletion	Lymphodepletion (except for cohorts 1 and -1)	Prior to CAR T Cell Infusion[13]	**CAR T Cell Infusion (Day 0)[14]**	Within 24° post CAR T cells	Interim evaluations[15]	Day 7 post CAR T Cell (+/- 3 days)	Interim evaluations[15]	Day 14 post T Cell (+/- 3 days)	Day 21 post T Cell (+/- 3 days)	Day 28 post T Cell (+/- 3 days)	Day +60 post T Cell (+/- 7 days)	Day +90 post T Cell (+/- 7 days)	Months 6. 9 and 12 (+/- 14 days)	End of Therapy Assessment (+/- 14 days)	LTFU Evaluation: Yearly post CAR T cells (+/- 2 months)
Chest/Abdomen/Pelvis CT with contrast[23]			X												X		X	X		
CT/ultrasound guided biopsy[24]					X										X					
MRI of target lesion[9]					X										X		X			
Apheresis				X																
AE Evaluation[5,6]				X		---------- X ----------										X	X	X	X	X
Toxicity Assessment	Neurological assessment[22]				X	---------- X[8, 10] ----------														
	3-4 cc Peripheral Blood Draw[20]					----------X----------[21]														

Study Parameters and Calendar		Pre-screening	Screening/ Enrollment Activities			Active Treatment									Protocol Follow-Up				
						Study Treatment			Month 1 DLT and Response Evaluations						Short Term Protocol Follow-Up[17]				LTFU[18]
			Screening Evaluations[12]	**Leukapheresis**	Prior to Lymphodepletion	Lymphodepletion (except for cohorts 1 and -1)	Prior to CAR T Cell Infusion[13]	**CAR T Cell Infusion (Day 0)[14]**	Within 24° post CAR T cells	Interim evaluations[15]	Day 7 post CAR T Cell (+/- 3 days)	Interim evaluations[15]	Day 14 post T Cell (+/- 3 days)	Day 21 post T Cell (+/- 3 days)	Day 28 post T Cell (+/- 3 days)	Day +60 post T Cell (+/- 7 days)	Day +90 post T Cell (+/- 7 days)	Months 6. 9 and 12 (+/- 14 days)	End of Therapy Assessment (+/- 14 days)	LTFU Evaluation: Yearly post CAR T cells (+/- 2 months)
Correlatives[11]	5-10 cc Peripheral Blood Draw[26]				X			X							X					
	35 cc Peripheral Blood Draw[34]				X			X	X[19]	X	X	X	X	X	X	X[28]	X[28]	X[28]		
	Urine[35]				X		X				X		X		X					
	Tumor Biopsy + bone marrow aspirate[27]				X										X[29]					
Lymphodepleting Regimen *(except cohorts 1 and -1)*[36]						X														
Study Agent PSCA(ΔCH2)BBζ CAR T cells[36]								X[36]												
Dexamethasone Administration[31]									X		X									

Study Parameters and Calendar	Pre-screening	Screening/ Enrollment Activities			Active Treatment										Protocol Follow-Up					
					Study Treatment			Month 1 DLT and Response Evaluations							Short Term Protocol Follow-Up[17]				LTFU[18]	
		Screening Evaluations[12]	**Leukapheresis**	Prior to Lymphodepletion	Lymphodepletion (except for cohorts 1 and -1)	Prior to CAR T Cell Infusion[13]	**CAR T Cell Infusion (Day 0)[14]**	Within 24° post CAR T cells	Interim evaluations[15]	Day 7 post CAR T Cell (+/- 3 days)	Interim evaluations[15]	Day 14 post T Cell (+/- 3 days)	Day 21 post T Cell (+/- 3 days)	Day 28 post T Cell (+/- 3 days)	Day +60 post T Cell (+/- 7 days)	Day +90 post T Cell (+/- 7 days)	Months 6. 9 and 12 (+/- 14 days)	End of Therapy Assessment (+/- 14 days)	LTFU Evaluation: Yearly post CAR T cells (+/- 2 months)	
Disease Status[32]																			X	

1 Calculated by Cockroft Gault
2 Please order the Abbot Realtime HIV viral load assay for quantification of HIV-1 (Abbot) or APTIMA HIV-1RNA Qualitative Assay (from Gen-probe, Inc) which is ordered as "Misc send out test" (LabCorp Order Code: 550880). Please note that the Cobras AmpliPrep/COBRA TaqMan Quantitative HIV test should NOT be used.
3 Hepatitis B surface antigen, core antibody and surface antibody
4 If positive, Hepatitis C RNA quantitation
5 In the event of toxicities, additional specimen samples may be collected at the time of SOC and sent to TCTRL for investigation/assessments.
6 AE evaluations to begin during study driven procedures (leukapheresis, then lymphodepleting regimen or CAR T cell infusion based on assigned treatment plan).For AE collection guidance, please refer **to Section 5.8.3**.
7 Daily CRP levels should be taken if a patient experiences persistent fevers ($\geq 38^0$C) and clinical indications of toxicity
8 MRI is an acceptable substitute (or preferred substitute for anyone with neurological abnormality at baseline)
9 MRI is required for bone lesion.
10 In the event a patient experiences neurological AEs (such as change in mental status) after receiving CAR+ T cell infusion a neurological consultation may be conducted as clinically indicated.
11 Correlative samples may be drawn at the time of SOC procedure per PI discretion. On all correlative sample draw days ask PI if a CBC/Diff should also be drawn (e.g., typical for all PB draw days)
12 Tests resulted within 6 weeks of signing the consent do not need to be repeated.
13 Evaluations may be performed up to 7 days prior to therapy
14 CAR T cell Infusion may be delayed up to 14 days following the last dose of lymphodepleting regimen.
15 Toxicity evaluations will be performed at least every two days up to a minimum of 14 days post CAR T cell infusion.
16 HIV testing and imaging to be done ONLY for Months 6 and 12
17 Follow-up evaluations per modified **Calendar 10.2**
18 Reference **Section 5.8.2** (Long-Term Follow-Up). Positive results may require additional testing, and expedited FDA reporting.
19 Correlative blood draws may be performed between 18-72 hours post CAR T cell infusion
20 Up to 3 additional 3-4 ml peripheral blood draws MAY be drawn per week for serum analysis/profiling
21 Evaluations only required if clinically indicated, per PI/designee discretion
22 Neurological assessments may include and are not limited to a Brain CT/MRI, CSF assessment and/or a neurological consult. In the event a patient experiences neurological AEs (such as change in mental status) before and/or after receiving CAR+ T cell infusion, additional assessments including a lumbar puncture may be conducted as clinically indicated.
23 CT without contrast for participants who have impaired renal function and/or history of severe allergies.

24 Correlative marrow samples may be drawn at the time of procedure per PI discretion.
25 Eligibility screening must be performed of soft-tissue tumor specimens, all other specimens will be for correlative and assay optimization purposes only. Additional testing may be performed at any SOC time-point, at the discretion of the PI.
26 Samples to be collected in Streck tubes and delivered to the Peter Kuhn laboratory at the University of Southern California, Los Angeles.
27 When marrow is obtained, a sample should be collected in Streck tubes for the Kuhn lab, and the remainder of the specimen delivered to the TCTRL approximately 2-3 hours from the time they were drawn. See **Section 9.1.2** for reference.
28 Participants who do not have a local lab/oncologist office to assist with mailing kits to COH do not require correlative blood draw to be collected UNLESS they are reported to have a suspected AE theoretically related to the CAR T cell product.
29 The allowed window for this sample collection is -3 days to + 7 days for the D28 timepoint
30 Urinalysis for toxicity assessment may be performed every other day starting at Day 4 through appropriately Day 14. If WBC and/or RBC are noted to increase by 1 level, implement cystitis mitigation strategies (reference **Section 5.10.5)**.
31 In the event subjects have adverse toxicities, such as cystitis, related to study therapy (lymphodepletion and/or CAR T cells), the PI may give dexamethasone by intravesical or IV delivery. Reference **Section 5.10.5** for cystitis mitigation
32 Up to first progression post CAR T treatment
33 HIV testing at screening may be done either by Ag/Ab combo assay or qPCR.
34 Correlative blood draws are 35cc (e.g., five 10 mL purple-top EDTA tubes, AND one 6 mL red-top tube). Samples will be sent to TCTRL c/o: Saul Priceman Lab.
35 Correlative urine samples will be collected at pre-LD, pre-CAR T cell infusion, and up to three times during the first 28 days after receiving CAR T cell treatment (day 7, 14, and 28, +/- 3 days). Correlative urine collection will be sent to TCTRL c/o: Saul Priceman Lab for cytokine monitoring.
36 Participants may receive a second infusion that will not exceed the initial dose assigned and will be administered not less than 28 days post the initial infusion. Additional CAR T cell infusions after a second infusion will be allowed not less than 28 days apart following the same rules as the second infusion. Additional lymphodepletion prior to second and any subsequent doses will be optional and will be given at a dose not greater than the initial lymphodepletion. Toxicity evaluation and disease response evaluations will follow as described for the initial T cell infusion.

10.2 Modified Study Calendar for LTFU Purposes (for subjects who relapse or start new therapy)

Study Parameters and Calendar		Post CAR T Cell Infusion Evaluations				
		Month 2 (+/- 7 days)	Month 3 (+/- 7 days)	Month 6 (+/- 14 days)	Month 12 (+/- 14 days)	LTFU Evaluation: Yearly post T cells
H&P, Vital Signs, ECOG, Disease Status[3]		X	X	X	X	
LTFU Adverse Event Evaluation	Assessment for Reproductive Risk and Risk to Fetus	X	X	X	X	X
	Assessment for CAR T cell and Gene Therapy-related Toxicities	X	X	X	X	X
	Assessment for Secondary Malignancies	X	X	X	X	X
	Assessment for new Autoimmune Disorder	X	X	X	X	X
CBC, Differential		X	X	X	X	
Comprehensive Metabolic Panel, LDH		X	X	X	X	
HIV qPCR Assay[2]			X	X	X	X
Evaluation of CAR T cell Persistence (WPRE)[1]		X	X	X	X	X

[1] No longer applicable for participants who have at least 3 documented evaluations without detectable CAR T cell persistence and whose B cells have returned to normal range.
[2] Please order the Abbot Realtime HIV viral load assay for quantification of HIV-1 (Abbot) or APTIMA HIV-1RNA Qualitative Assay (from Gen-probe, Inc). Please note that the Cobras AmpliPrep/COBRA TaqMan Quantitative HIV test should NOT be used. Reference **Section 5.8.2** (Long-Term Follow-Up). Positive results may require additional testing, and expedited FDA reporting.
[3] Up to confirmed first progression post CAR T treatment

11 ENDPOINT EVALUATION CRITERIA/MEASUREMENT OF EFFECT

11.1 Primary Endpoints:

- Full toxicity profile as assessed by the NCI CTCAE v5 and modified CRS grading as applicable (reference is made to **Appendix A**) post CAR T cell infusion
- Dose-limiting toxicity is defined by any grade 3 or NCI CTCAE and modified CRS grading as applicable (reference is made to **Appendix A**), toxicities that are not included on the anticipated toxicities list as "allowable" and resolved within the timeframe descibed in **Section 6**.

11.2 Secondary Endpoints

- Persistence of CAR T cells through 28 days post infusion (defined as CAR T cells >0.1% of total CD3 cells by flow-cytometry).
- Expansion of CAR T cells (Max log10 copies/µg of genomic DNA).
- Disease response
    - RECIST
    - Prostate Cancer Working Group (PCWG3) criteria
    - Changes in PSA
- Survival
    - Overall survival (death from all causes from date of CAR T cell infusion)
    - Progression-free survival (PFS) time defined as survival without radiographic evidence of disease progression based on RECIST starting at the date of CAR T cell infusion or lymphodepletion.
- PSCA expression on tumor cells by IHC and/or flow cytometry
- Serum cytokine profile before and after CAR T cell infusion: to assess potential CRS toxicity and CAR T cell effector function, sequential serum samples will be analyzed for Th1/ Th2 cytokines (e.g., IL-2, IFNγ, TNFα, IL-10, GMCSF, IL-6, MIP-1α) by bead array

11.3 Exploratory Endpoints

- Phenotypes and frequencies of immune cell subsets in the peripheral blood pre- and post-therapy: analysis will include CD4:CD8 ratios, differentiation status (CD62L, CD27, CD45 RA/RO), and exhaustion markers (PD1, Tim3, LAG3),trafficking (CCR7, α4β7), proliferation markers (ki67) and effector functions (cytotoxicity, Th1/Th2 cytokines, and CD107a degranulation) on endogenous and CAR+ T cells
- Phenotype of tumor-infiltrating lymphocytes
- Gene expression (by RNA-seq) of CTCs.
- cfDNA in peripheral blood by whole exome sequencing
- CAR immunogenicity based on the presence of anti-PSCA CAR antibodies or T cell mediated immune responses

11.4 Definitions of Response and Progression

11.4.1 RECIST 1.1

RECIST 1.1 will be used to evaluate for soft tissue response/progression.

Measurable disease – Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm with conventional techniques (CT, MRI, x-ray) or as >10 mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Previously irradiated lesions are non-measurable except in cases of documented progression of the lesion since the completion of radiation therapy.

Non-measurable disease – All other lesions (or sites of disease), including small lesions (longest diameter <20 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Target lesions – All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

Malignant lymph nodes – To be considered pathologically enlarged and measurable, a lymph node must be >15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-target lesions – All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

Evaluation of target lesions

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

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

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

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

Evaluation of non-target lesions

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

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

Progressive Disease (PD): Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

11.4.2 Bone Scan evaluation

Prostate Cancer Working Group 3 definitions [2] will be used for bone scan evaluation.

Appearance of a new bone lesion on bone scan can be related to "flare" and thus can represent response rather than progression. For this reason, appearance of new lesions on the first interval scan will require confirmation with *additional new lesions* on the 2nd scan; if there are no additional new lesions this is not considered progression. If there are additional new lesions, the patient will be considered to have confirmed disease progression, dated to the date of the first scan. If new lesions are detected on a scan after the first restaging scan, this constitutes progression.

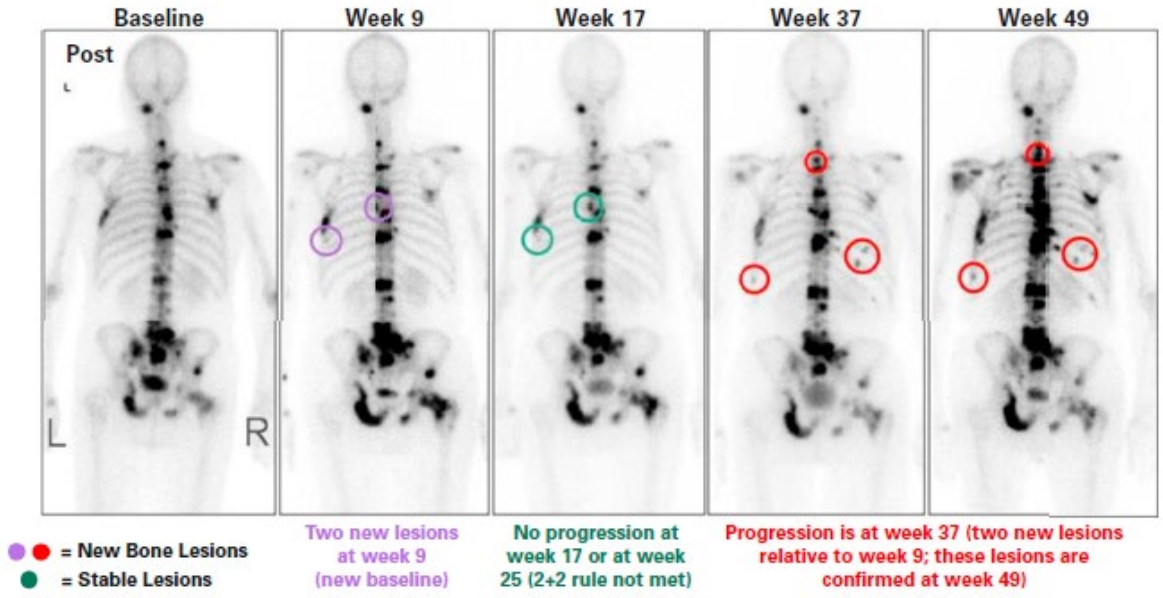


Figure 4. Pictorial depiction of "2+2" definition for bone scan progression

12 STATISTICAL CONSIDERATIONS

12.1 Study Design

This is a phase I dose escalation trial of adoptive CAR T cell therapy. The goal of the trial is to determine the RP2D.

Tables 12.1a and 12.1b: PSCA-CAR T cell dose schedule. The first nine study participants were treated according to **Table 12.1a** (3 participants at Dose 1, and 6 participants at Dose 1b).

As of February 2021, Doses 1 and 1b (shaded in blue) have been completed.

Table 12.1a. CAR+ Cell Dose Schedule

Dose Level	Lymphodepletion (Standard Flu/Cy)	#CAR+ cells
-1	No	50 M
-1a*	Yes	50 M
Starting Dose 1	No	100 M
1b	Yes	100 M
2	Yes	300 M
3	Yes	600 M

^Dose range allows for -20% of listed dose. Listed dose is the upper limit of the dose cohort
*Dose level -1a should be considered a ½ step above dose level -1. We get to -1a if the toxicity at dose level -1 is acceptable and the toxicity level at dose level 1 is too toxic.

Table 12.1b.^ Revised CAR+ Cell Dose Schedule (initiated at protocol V06)

Dose Level	Lymphodepletion (Modified Flu/Cy)	#CAR+ cells[+]
1c*	No (Cy alone)	100 M
Starting Dose 1d	Yes	100 M
2	Yes	300 M

*Dose 1c will be used as a de-escalation dose if 1d is too toxic
^ Cystitis mitigation plan in place for subjects enrolling in Table 12.1.b cohorts per V06 of the protocol
[+]Initiated on protocol V07: Participants may receive additional infusions that will not exceed the initial dose assigned and will be administered not less than 28 days post the prior infusion.

The RP2D will be based on the maximum tolerated dose (MTD) and additional toxicity and activity data. The toxicity equivalence range (TEQR) design of Blanchard and Longmate (2011)[1] will be used to determine the MTD. The CAR T+ cell dose schedule is provided in **Tables 12.1a** and **b** (Table 12.1a original schedule, 12.1b revised schedule). The decision to escalate to a higher dose, stay at the same dose, or de-escalate to a lower dose is determined by the portion of the toxicity probability scale (0-1) in which our toxicity rate lands. The toxicity probability scale is portioned into three pieces based on the target toxicity probability (pT) and the identified range of equivalence (pT-ε1 to pT+ ε2). If the study DLT rate is in the 0 to pT- ε1 range the guideline indicates escalate, if it is in the pT-ε1 to pT+ ε2 range the guideline indicates

stay, and if the rate is in the pT+ ε2 to 1 range the guideline indicates de-escalate. Further we also implement the additional rules: 1) terminate the trial if dose schedule 1c is too toxic, and 2) if the current dose is safe but based on the data the next dose is deemed too toxic, stay at the current dose.

The TEQR can be viewed as a minimal elaboration of the 3+3 design to include an explicit toxicity target range, and permit intuitive specification of a too toxic level for closing a dose level. In this implementation of the TEQR design, we define the target equivalence range of DLT as 0.20-0.35. Toxicity levels of 0.51 or higher will be considered too toxic and doses that achieve that level will not be revisited. If the rate of participants that experience a DLT is below 0.20 we will escalate, if the rate is above 0.35 we will de-escalate and if it is between 0.20-0.35 we will stay at the current dose. Participants will enter the protocol in cohorts of 3. This dose escalation portion of this study will end when 6 research participants are studied at a single dose level with a toxicity level below 0.51. The MTD will be the dose closest to target of 0.25 below 0.51 based on isotonic regression.

Note in the case that the cell sample manufactured is smaller than the assigned dose, but is within the dose schedule, we will allow the research participant to enter the study at the lower CAR T cell dose level. Similarly, in the case that a research participant who has a manufactured product and is assigned to the higher dose cohort but needs to go for treatment before the next cohort will be open, will be allowed to enter at the lower dose that was studied and deemed to be safe. The dose escalation/de-escalation decisions guidelines will be based on the sample size for that cohort. This data will be included in the calculation of the RP2D using isotonic regression at the end of the study.

Table 12.2: Dose escalation/de-escalation guidelines
(E= escalate, D=de-escalate, S=stay, DU=de-escalate and do not return to this dose).

		Number of research participants treated on the current dose level (Standard sample size based on a cohort size =3 are bolded.)									
		3	4	5	**6**	7	8	**9**	10	11	**12**
Number of Research Participants Experiencing a DLT	0	**E**	E	E	**E**	E	E	**E**	E	E	**E**
	1	**S**	S	S	**E**	E	E	**E**	E	E	**E**
	2	**DU**	D	D	**S**	S	S	**S**	S	E	**E**
	3	**DU**	DU	DU	**D**	D	D	**S**	S	S	**S**
	4				**DU**	DU	D	**D**	D	D	**S**
	5						DU	**DU**	D	D	**D**
	6								DU	DU	**D**
	7										**DU**

Table 12.2 provides the dose escalation de-escalation guidelines for a target toxicity of 25% and ε_1=5% ε_2= 10% giving an equivalence range of 20%-35%. The numbers of research participants treated at the current dose is provided in the columns and the number of research participants experiencing a DLT in the rows. Note that this design is similar to a 3+3 design for the first 3 patients seen at a dose as it escalates if 0 study participants experience DLTs, holds if 1 experiences a DLT (33%), and de-escalates if 2 experience a DLT, differing from a 3+3 at sample sizes of 6 and above as it holds at 33% toxicity and de-escalates 36% toxicity or above. The Phase I portion of the study will end when 6 research participants are studied at a single dose level. Toxicity levels of 0.51 or higher are considered too toxic and doses that achieve that level will not be revisited. The MTD will be the dose closest to target of 0.25 below 0.51 based on isotonic regression.

Table 3: Operating Characteristics for 3 Dose Levels and 4Toxicity Probability Scenarios (Starting at Dose 1d)				
	Dose levels			
	1c	**1d**	**2**	**No MTD**
Scenario 1	**0.05**	**0.15**	**0.23**	
MTD rate	**.07**	**.41**	**.52**	**0.00**
Ave DLT Rate at the MTD				**0.18**
Proportion of Times that MTD Dose Achieved N=6 Participants				**1.00**
Scenario 2	**0.10**	**0.20**	**0.40**	
MTD rate	**0.11**	**0.57**	**0.31**	**0.0**
Ave DLT Rate at the MTD				**0.22**
Proportion of Times that MTD Dose Achieved N=6 Participants				**1.00**
Scenario 3	**0.20**	**0.40**	**0.60**	
MTD rate	**0.32**	**0.56**	**0.06**	**0.06**
Ave DLT Rate at the MTD				**0.26**
Proportion of Times that MTD Dose Achieved N=6 Participants				**0.94**
Scenario 4	**0.025**	**0.05**	**0.20**	
MTD rate	**0.01**	**0.24**	**0.76**	**0**
Ave DLT Rate at the MTD				**0.16**
Proportion of Times that MTD Dose Achieved N=6 Participants				**1.00**

Note **Table 12.3** provides the rates at which each dose level for each schedule was chosen as the MTD (MTD rate), the average DLT rate at the MTD, and the percentage of trials with N=6 at the MTD.

Expansion: After following 6 participants at the MTD dose, if less than 2 of 6 patients experience activity, defined as confirmed stable disease, the protocol will stop accrual as there will be a less than 5% chance of 60% of the patient population experiencing activity (Gehan,1961 [2]). If 2 or more patients experience activity, we plan to add on an additional 6 participants, for a total of 12 participants at MTD. If additional DLTs are seen in the expansion cohort the dose escalation guidelines for de-escalation (Table 2) will be used as a guide to indicate a need for data review for toxicity.

12.2 Study Staggering Rules

The first 3 research participants in the first cohort without lymphodepletion (cohort 1) and the first cohort with lymphodepletion (either cohort 1b or -1a) will be treated and followed one at a time through the DLT period for a minimum of 28 days, while all further research participants will be treated in cohorts of 3. Dose escalation to the next dose or to add in a cohort of 3 participants will not take place until at least 3 participants have completed the current 28-day DLT period.

12.3 Study Stopping Rules

The study will hold accrual, and the data will be reviewed if:

- A grade 5 toxicity is observed which has an attribution of possibly, probably or definitely related to treatment with PSCA-CAR T cells (study agent). Note only deaths occurring < 30 days from the patient's last dose of CAR T cells will be considered for the purposes of defining conditions for discontinuation of the study.
- Replication-competent lentivirus (RCL) is detected.
- The study will stop for feasibility problems if we reach a rate of 50% inability to achieve the needed number of cells for the dose level under study, after at least 6 research participants have been enrolled and are eligible for CAR T cell infusion. Patients will be monitored sequentially after 6 patients have been accrued.

12.4 Sample Size and Accrual

As of February 8th 2021, nine participants have been treated on this trial.

Three participants (UPNs 375, 376, 393) have been treated on dose level 1 (100M). There were no DLTs. The PMT team approved the dose escalation to dose level 1b.

Six participants (UPNs 388, 406, 403, 455, 454, 504) have been treated on dose level 1b (standard lymphodepletion followed by 100M). Two participants experienced a DLT including Gr3 cystitis. The protocol management team met and decided that rather than treat 3 more participants at dose level 1b, we would update the dose schedule to allow for a modified (i.e., reduce) lymphodepletion dose schedule with the possible addition of a mitigating treatment plan for cystitis. These changes are included in **Table 12.1b.**

For the dose escalation the median sample size for the updated schedule based on simulations of 2000 similar trials is 9 research participants with a minimum of 6 and a maximum of 12. Adding 3 for replacement of unevaluable research participants, and adding in the participants previously treated on schedule **12.1a**, the sample size for the dose escalation should not exceed 24 total participants. If we see activity the expansion will add 6 for a total of up to 30 participants.

Accrual is expected to be 6 evaluable research participants per year, where participants evaluable for dose escalation need to have received $\geq$80% of the assigned CAR T cell infusion dose (on dose levels including lymphodepletion the participants will also need to receive $\geq$80% of the lymphodepletion regimen), and be followed for 28 days after the CAR T cell infusion dose or have experienced a DLT, otherwise the participant will need to be replaced. Thus, we expect to complete accrual for the dose escalation in ~3 years and adding a 4th year for expansion if we see activity.

A sample size of 6 will give a maximum margin of error for 90% confidence limits on DLT and disease response rates of 0.35 and allow us to detect toxicity with a true rate of 0.20 in 73 of 100 trials. A sample

size of 12 at the MTD will provide us with *i)* maximum margin of error of 0.25 for a 90% confidence interval (90% CI) for the DLT rate, and *ii)* to detect a toxicity with a true rate of 0.20 in 93% of trials.

12.5 Analysis Plan

RP2D will be based on the MTD as well as the available activity and correlative data. Rates and associated 90% Clopper and Pearson binomial confidence limits will be estimated for *i)* DLTs within 28 days of CAR T cell infusion at the RP2D, and *ii)* disease response. Tables will be created to summarize all toxicities and side effects by attribution to treatment, dose, organ, and severity. Maximum persistence (in days) and peak expansion (log10 copies/ug of genomic DNA) will be described. Kaplan Meier methods will be used to estimate median PFS and OS and graph the results. Statistical and graphical methods will be used to describe persistence and expansion of the CAR T cells (peripheral blood), cytokine levels (peripheral blood) and PSA levels over the study period. Linear regression will be used to assess the relationship between PSCA expression and disease response, and toxicities experienced. We will provide descriptive statistics for patient demographics and exploratory studies.

13 DATA HANDLING, DATA MANAGEMENT, RECORD KEEPING

13.1 Source Documents

Source documents are original documents, data, and records (e.g., medical records, pharmacy dispensing records, recorded data from automated instruments, laboratory data) that are relevant to the clinical trial. The investigator or their designee will prepare and maintain adequate and accurate source documents. These documents are designed to record all observations and other pertinent data for each patient enrolled in this clinical trial. Source documents must be adequate to reconstruct all data transcribed onto the case report forms.

13.2 Data Capture Methods and Management

Data for this trial will be collected using Medidata Rave.

13.3 Case Report Forms/Data Submission Schedule

Study personnel will enter data from source documents corresponding to a participant's visit into the protocol-specific electronic Case Report Form (eCRF) when the information corresponding to that visit is available.

The investigator is responsible for all information collected on participants enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the investigator. All case report forms must be completed by designated study personnel. The completed case report forms must be reviewed, signed and dated by the Investigator or designee in a timely fashion.

All data will be collected using electronic data collection, stored as indicated in **Section 13.2**, and will be submitted according to the timelines indicated in **Table 13.3**.

Table 13.3 Data Submission Schedule

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 14 calendar days of registration
Baseline Assessment Forms	Within 14 calendar days of registration
Treatment Forms	Within 14 calendar days of treatment administration
Adverse Event Report Forms	**Phase 1/ Safety Lead-in**: Within 7 calendar days of the assessment/notification
Response Assessment Forms	Within 10 calendar days of the response assessment
Other Assessment Forms	Within 10 calendar days of the assessment
Off Treatment/Off Study Forms	Within 10 calendar days of completing treatment or being taken off study for any reason
Follow up/ Survival Forms	Within 14 calendar days of the protocol defined follow up visit date or call

13.4 Regulatory Records

The investigator will maintain regulatory records, including updating records in accordance with Good Clinical Practice guidelines and FDA regulations.

14 ADVERSE EVENTS AND UNANTICIPATED PROBLEMS

The research team is responsible for classifying AEs and UPs as defined in the relevant regulations and reporting to all applicable parties, including but not limited to the COH IRB, DSMC, Food and Drug Administration (FDA), National Institutes of Health (NIH) and other collaborators, e.g., pharmaceutical companies. The research team is responsible for the continued monitoring and tracking of all AEs in order to ensure non-reportable events are reviewed and monitored and do not rise to a reporting level.

14.1 Assessment of Adverse Events

The site Investigator will be responsible for determining the event name, and assessing the severity (i.e. grade), expectedness, and attribution of all adverse events as applicable per the City of Hope Clinical Research Adverse Event and Unanticipated Problem policy. Adverse events, with the exception of CRS*, will be characterized using the descriptions and grading scales found in version 05 of CTCAE. A copy of the scale can be found at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

The following definitions will be used to determine the causality (attribution) of the event to the study agent or study procedure.

- **Unrelated –** The event is clearly NOT related to study treatment, and is clearly related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant medications administered to the participant.
- **Unlikely –** The event is unlikely related to the study treatment, and is most likely related to other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible –** The event may be related to study treatment, as it follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Probable –** The event is most likely related to the study treatment, as it follows a reasonable temporal sequence from the time of drug administration and a known response pattern to the study drug, and is unlikely related to the participant's clinical state, other therapeutic interventions, or concomitant drugs.
- **Definite –** The event is clearly related to the study treatment, as it follows a reasonable temporal sequence from the time of drug administration and a known response pattern to the study drug, and is not reasonably explained by other factors such as the participant's condition, therapeutic interventions, or concomitant drugs.

*CRS adverse events will be characterized using the descriptions and grading scales found in the Lee, *et. al.* publication 'Current concepts in the diagnosis and management of cytokine release syndrome, Blood, 124(2):188-95, 2014.)'. A copy of the definitions and scale can be found in **Appendix A**.

14.2 Reporting of Adverse Events

14.2.1 Routine Recording of Non-Serious Adverse Events

Routine AE recording will occur via data entry into the study eCRF. Recording of adverse events will begin once the patient has initiated lymphodepletion (CAR T cell infusion day ONLY for cohorts 1 and -1), while

only serious adverse events attributed to protocol-related procedures (such as leukapheresis) will be collected and reported prior to the start of lymphodepletion (CAR T cell infusion for the first cohort of patients). Upon initiation of lymphodepletion, all AEs will be recorded and reported regardless of attribution until the subject (1) is taken off protocol therapy (either due to starting contraindicated drugs/therapy, or has progressed) or has withdrawn from the study (reference **Section 5.8.3**). Adverse events will be monitored by the Protocol Management Team (PMT). Adverse events that do not meet the criteria of serious OR are not unanticipated problems do not require expedited reporting. AEs reported through expedited processes (i.e. reported to the IRB, DSMC, FDA, etc.) must also be reported in routine study data submissions.

14.2.2 Expedited Reporting Requirements of SAEs and Ups to the COH Regulatory Committees

Adverse events that meet the criteria of serious OR are unanticipated problems will be reported according to the approved City of Hope Clinical Research Adverse Event and Unanticipated Problem policy. Reportable SAEs must be followed until the event is resolved, stabilized, or determined to be irreversible by the investigator. Follow-up SAE reports must be submitted for all events that require expedited reporting when the status of the event changes and until the resolution or stabilization of the event.

Events that DO NOT Meet the Definition of Serious Adverse Events

The following events do not meet the definition of serious adverse events and do not require expedited reporting to the COH regulatory committees via iRIS:

- Elective surgery or other scheduled hospitalization periods that were planned before the patient was included in this trial are not to be recorded as SAEs, unless an outcome of the surgery/hospitalization was considered serious.
- Hospitalization for observation or convenience prior to or following investigational product infusions without an SAE occurring should not be recorded as an SAE, e.g., if a patient is hospitalized merely for observation, or if a patient begins or finalizes the infusion at a time of day requiring a convenience overnight stay in the hospital.
- Procedures to support the treatment regimen that require hospitalization should not be recorded as SAEs; however, in cases where a procedure results in complications requiring/prolonging hospitalization, this must be recorded and reported as an SAE.

14.2.3 Adverse Events of Special Interest (AESI)

Specific adverse events, or groups of adverse events, will be followed as part of standard safety monitoring activities. These events, regardless of seriousness, will be reported.

14.2.3.1 CAR T-related AEs

CAR T cell related adverse events of special interest are those associated with cytokine release syndrome (CRS) and neurotoxicity. Appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other etiologic causes of such CAR T cell related AESIs.

14.2.3.2 Secondary Malignancy

A secondary malignancy is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

The investigator must immediately notify the Study PI via an expedited report.

14.2.4 Additional AE Reporting Requirements

14.2.4.1 Reporting to the FDA

The study PI (or designee) will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA in accordance with the City of Hope Clinical Research Adverse Event and Unanticipated Problem policy.

Serious Adverse Events meeting the requirements for expedited reporting to the Food and Drug Administration (FDA), as defined in 21 CFR 312.32, will be reported as an IND safety report using the MedWatch Form FDA 3500A for Mandatory Reporting.

The criteria that require reporting using the Medwatch 3500A are:

- Any unexpected fatal or life threatening adverse experience associated with use of the drug must be reported to the FDA no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]
- Any adverse experience associated with use of the drug that is both serious and unexpected must be submitted no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]
- Any follow-up information to a study report shall be reported as soon as the relevant information becomes available. [21 CFR 312.32(d)(3)]

In addition, the study PI will submit annually within 60 days (via COH OIDRA) of the anniversary date of when the IND went into effect, an annual report to the FDA which is to include a narrative summary and analysis of the information of all FDA reports within the reporting interval, a summary report of adverse drug experiences, and history of actions taken since the last report because of adverse drug experiences.

14.2.4.2 Reporting to the Institutional Biosafety Committee (IBC)

All clinical trials involving recombinant DNA require Institutional Biosafety Committee (IBC) review and approval. As such, SAEs occurring in human gene transfer studies conducted under COH-held INDs meeting the criteria of expedited reporting and that are attributed by the PI to be at least possibly related to the human gene transfer product will be submitted to the IBC using the IBC Communication Form in iRIS. Non-serious adverse events will be reported annually in the IBC annual report, unless otherwise specified by the IBC.

15 PROTOCOL DEVIATIONS

Deviations from the protocol should be avoided, except when necessary to eliminate immediate hazard(s) for the protection, safety, and well-being of a research participant. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly. All protocol deviations and planned protocol deviations will be reported in accordance with the Clinical Research Protocol Deviation policy.

In addition, if contractually obligated, the industry partner/funding source must also approve planned deviations, as necessary.

16 STUDY OVERSIGHT, QUALITY ASSURANCE, AND DATA & SAFETY MONITORING

16.1 All Investigator Responsibilities

An investigator is responsible for ensuring that an investigation is conducted according to the signed investigator statement, the investigational plan, and applicable regulations; for protecting the rights, safety, and welfare of subjects under the investigator's care; and for the control of drugs under investigation.

16.2 Study Principal Investigator Responsibilities

The Study Principal Investigator is responsible for the conduct of the clinical trial, including overseeing that sponsor responsibilities are executed in accordance with federal regulations.

16.3 Protocol Management Team (PMT)

The Protocol Management Team (PMT), minimally consisting of the study PI, collaborating investigators, research nurse, clinical research associate/coordinator, and the study biostatistician, is responsible for ongoing monitoring of the data and safety of this study, including implementation of the stopping rules for safety/toxicity.

The PMT is recommended to meet (in person or via teleconference) to review study status. The meeting is a forum to discuss study related issues including accrual, SAE/AE/UPs experienced, study response, deviations/violations, and study management issues. The appropriateness of further subject enrollment and the specific intervention for subsequent subject enrollment are addressed.

16.4 Quality Assurance

Clinical site monitoring is conducted to ensure that the rights of human subjects are protected, that the study is implemented in accordance with the protocol and regulatory requirements, and that the quality and integrity of study data and data collection methods are maintained. Monitoring for this study will be performed by the City of Hope Office of Clinical Trials Monitoring (OCTM), within City of Hope's Office for Safety and Data Quality.

Details of clinical site monitoring are documented in the OCTM SOP and the Risk Based Monitoring (RBM) plan. These documents specifies the frequency of monitoring, monitoring procedures, the amount of subject data to be reviewed, and the distribution of monitoring reports to the study team and the COH DSMC.

16.5 Risk Determination

This is a high risk study, as defined in the City of Hope Institutional DSMP. This determination was made because the study involves a COH held IND.

16.6 City of Hope Data and Safety Monitoring Committee (DSMC)

The COH Data and Safety Monitoring Committee (DSMC) will review and monitor study progress, compliance, toxicity, safety, and accrual data from this trial via the PMT Progress Report (submitted by the Study Principal Investigator according to the frequency outlined in the City of Hope Institutional DSMP). The DSMC is composed of clinical specialists who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Protocol Management Team.

17 ETHICAL AND REGULATORY CONSIDERATIONS

17.1 Ethical Standard

This study will be conducted in conformance with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research (US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, April 18, 1979) and the Declaration of Helsinki.

17.2 Regulatory Compliance

This study is to be conducted in compliance with the IRB approved protocol and according to the following considerations:

- US Code of Federal Regulations (CFR) governing clinical study conduct
  - Title 21 Part 11 – Electronic Records; Electronic Signatures
  - Title 21 Part 50 – Protection of Human Subjects
  - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
  - Title 21 Part 56 – Institutional Review Boards
  - Title 21 Part 58 – Good Laboratory Practice for Nonclinical Laboratory Studies
  - Title 21 Part 312 – Investigational New Drug Application
  - Title 45 Part 46 – Protection of Human Subjects
- US Federal legislation, including but not limited to
  - Health Insurance Portability and Accountability Act of 1996
  - Section 801 of the Food and Drug Administration Amendments Act
- Applicable state and local laws. For research occurring in California, this includes but is not limited to State of California Health and Safety Code, Title 17
- Applicable institutional research policies and procedures

17.3 Institutional Review Board

In accordance with City of Hope policies, an Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB's written unconditional approval of the study protocol and the informed consent document will be in the possession of the investigator before the study is initiated.

The IRB will be informed of revisions to other documents originally submitted for review; serious unexpected or unanticipated adverse experiences occurring during the study, and any additional adverse experiences in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

Any amendment to the protocol document and accompanying informed consent document/template, as developed and provided by the PI, will require review and approval by the COH IRB before the changes are implemented in the study. Additionally, all protocol changes (including protocol amendments and

changes in location of administration) should be reported to the IBC in iRIS using the IBC Amendment Form.

17.4 Informed Consent

The Principal Investigator or IRB approved named designate will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the HIPAA research authorization form. Prospective participants will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or any relationship they have with City of Hope. Prospective participants will be afforded sufficient time to consider whether or not to participate in the research.

After the study has been fully explained, written informed consent will be obtained from either the prospective participant or his/her guardian or legal representative before study participation. The method of obtaining and documenting the informed consent and the contents of the consent must comply with the ICH-GCP and all applicable regulatory requirements.

Before implementing any study procedure, informed consent shall be documented by the use of a written consent form approved by the IRB and signed and dated by the prospective participant or his/her legally authorized representative at the time of consent. A copy of the signed informed consent will be given to the participant or his/her legally authorized representative. The original signed consent must be maintained by the investigator and available for inspection sponsor designated representatives, or regulatory authority at any time.

17.5 Participant Withdrawal

Participants may withdraw from the study at any time and for any reason without prejudice. The withdrawal must be documented per institutional policies.

Participant withdrawal may consist of any of the following with regard to study procedures and data collection:

- Withdrawal from study treatment, but agreement to continue with active study procedures and chart review and survival follow-up.
- Withdrawal from study treatment and all active procedures, but agreement for chart review and survival follow-up.
- Withdrawal from study treatment, all active procedures, and any future data collection.

Participants who agreed to the collection of research blood samples may withdraw consent to use their specimens, if they are not yet processed as detailed in the consent form. Once the PI and site PI is notified of this withdrawal of informed consent, the research specimens will not be used in any research. At that time, any of the existing specimens will be destroyed.

17.6 Special and Vulnerable Populations

17.6.1 Inclusion of Women and Minorities

The study is open anyone regardless of gender, race or ethnicity. Efforts will be made to extend the accrual to a representative population. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

17.6.2 Exclusion of Pediatric Population

Pediatric participants (< 18 years old of age) are excluded from this study since safety and effectiveness of protocol therapy has not been defined for the study population. Additional studies may be performed in the pediatric population once safety and effectiveness of protocol therapy is defined in the adult study population.

Also, the incidence of MCL is rare in the pediatric population. Its prevalence is estimated at about 1/25,000 in the adult population, with the median age of 65 years (range 35-85 years)

17.6.3 Inclusion of HIV Positive Individuals

Participants with HIV are excluded due to concerns about inadvertent augmentation of infectious and/or inflammatory activity.

17.6.4 Vulnerable Populations

This study does not include any vulnerable populations defined per 45 CFR §46.111 (a)(3) and 45 CFR §46, Subparts B-D identifies children, prisoners, pregnant women, mentally incapacitated persons, or economically or educationally disadvantaged persons as vulnerable populations.

17.7 Participant Confidentiality

Participant confidentiality is strictly held in trust by the investigators, study staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to any study information relating to participants.

This research will be conducted in compliance with federal and state requirements relating to protected health information (PHI), including the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). HIPAA regulations require a signed subject authorization informing the subject of the nature of the PHI to be collected, who will have access to that information and why, who will use or disclose that information, ant the rights of a research participant to revoke their authorization for use of their PHI. In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Release of research results should preserve the privacy of medical information and must be carried out in accordance with Department of Health and Human Services Standards for Privacy of Individually Identifiable Health Information, 45 CFR 164.508. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed and no identifiers will be used.

Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. Data will be entered, analyzed and stored in encrypted, password protected, secure computers that meet all HIPAA requirements. All data capture records, drug accountability records, study reports and communications will identify the patient by initials and the assigned patient number.

The investigator/institution will permit direct access to source data and documents by sponsor representatives, the FDA, and other applicable regulatory authorities. The access may consist of trial-related monitoring, including remote monitoring, audits, IRB/IEC reviews, and FDA/regulatory authority

inspections. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

Participant specimens will be de-identified (coded) prior to submission to research laboratories. The specimens will be labeled with the study number, subject ID, date and timepoint of collection. The key to the code will be maintained in the COH clinical trials management system which is a secure environment.

17.8 Use of Unused (Leftover) Specimens Collected for this Trial

In the informed consent, the participants have an option to allow any remaining unused samples to be stored for future research use. The options include: (a) placing the samples in a COH IRB approved CTCL biorepository (COH Protocol IRB #TBD) with some clinical information and potentially PHI attached, (b) fully de-identifying the samples (prior to study completion) so that they may be used for research that is exempt from IRB review, or (c) having the samples destroyed.

While it is the intent to achieve the selected option, to best protect the participant, if option of "a" or "b" is selected and is not feasible at the time of study completion, the participant's leftover samples will be destroyed before completion to best protect their interest.

17.9 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study Sponsor (City of Hope) prior to participation in this study. All City of Hope investigators will follow the City of Hope conflict of interest policy.

17.10 Financial Obligations, Compensation, and Reimbursement of Participants

CAR T cells will be manufactured at City of Hope and provided free of charge to participants.

Neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study.

Standard of care drugs or procedures provided during the course of study participation will be the responsibility of the research participant and/or the insurance carrier. The participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the participant were not in a research study.

In the event of physical injury to a participant resulting from research procedures, appropriate medical treatment will be available at City of Hope to the injured participant. There are no plans for City of Hope to provide financial compensation in the event of physical injury to a participant.

The research participant will not receive reimbursement or payment for taking part in this study.

17.11 Publication/ Data Sharing

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by City of Hope for the purposes of performing the study, will be published or passed on to any third party without the written approval of the Study PI. Any investigator involved with this study is obligated to provide City of Hope with complete test results and all data derived from the study.

The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

In accordance with the U.S. Public Law 110-85 (Food and Drug Administration Amendments Act of 2007 or FDAAA), Title VIII, Section 801, this trial will be registered onto ClinicalTrials.gov and results will be reported on ClinicalTrials.gov within 12 months of the estimated or actual completion date of the trial, whichever date is earlier.

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APPENDIX A: CYTOKINE RELEASE SYNDROME (CRS) IN THE CONTEXT OF CELLULAR IMMUNOTHERAPY

Table A1. Clinical signs and symptoms associated with CRS

Organ System	Symptoms
Constitutional	Fever ± rigors, malaise, fatigue, anorexia, myalgias, arthalgias, nausea, vomiting, headache
Skin	Rash
Gastrointestinal	Nausea, vomiting, diarrhea
Respiratory	Tachypnea, hypoxemia
Cardiovascular	Tachycardia, widened pulse pressure, hypotension, increased cardiac output (early), potentially diminished cardiac output (late)
Coagulation	Elevated D-dimer, hypofibrinogenemia ± bleeding
Renal	Azotemia
Hepatic	Transaminitis, hyperbilirubinemia
Neurologic	Headache, mental status changes, confusion, delirium, word finding difficulty or frank aphasia, hallucinations, tremor, dymetria, altered gait, seizures

Table A2. Revised CRS Grading System

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

*Grades 2-4 refer to CTCAE v5.0 grading

Table A3. High-dose vasopressors (all doses are required for > 3 hours)

Pressor	Dose
Norepinephrine monotherapy	≥20 µg/min
Dopamine monotherapy	≥10 µg/kg/min
Phenylephrine monotherapy	≥200 µg/min
Epinephrine monotherapy	≥10 µg/min
If on vasopressin	Vasopressin + norepinephrine equivalent of ≥10 µg/min*
If on combination vasopressors (not vasopressin)	Norepinephrine equivalent of ≥20 µg/min*

* VASST Trial vasopressor equivalent equation: norepinephrine equivalent dose = [norepinephrine (µg/min)] + [dopamine (µg/kg/min) ÷ 2] + [epinephrine (µg/min)] + [phenylephrine (µg/min) ÷10].