CLINICAL RESEARCH PROTOCOL

DRUG:	CAR.B7-H3 T cells
STUDY NUMBER(S):	LCCC 1818-ATL
PROTOCOL(S) TITLE:	A Phase 1 Study of Autologous Activated T-cells Targeting the B7-H3 Antigen in Subjects with Recurrent Epithelial Ovarian Cancer
IND NUMBER:	*19641*
SPONSOR:	Lineberger Comprehensive Cancer Center
ORIGINAL PROTOCOL DATE:	05 January 2020
VERSION NUMBER:	Version 4.0
VERSION DATE:	April 12, 2023

SUMMARY OF CHANGES

Protocol Amendment #3

LCCC1818-ATL: A Phase 1 Study of Autologous Activated T-cells Targeting the B7-H3 Antigen in Subjects with Recurrent Epithelial Ovarian Cancer

AMENDMENT INCORPORATES:
X Editorial, administrative changes
X Scientific changes (IRB approval)
X Therapy changes (IRB approval)
X Eligibility Changes (IRB approval)

AMENDMENT RATIONALE AND SUMMARY: The purpose of this amendment is to amend and consolidate inclusion and exclusion criteria, update the lymphodepletion dose of fludarabine to 30 mg/m^2 for consistency with other similar protocols, and reduce restrictions for the number of prior lines of therapy to improve recruitment. Other changes include 1) updating links to institutional guidelines for CRS and CNS toxicity management, 2) updating storage time for biospecimens, 3) HTLV was removed from the list of prohibited infections in the eligibility criteria, 4) allowing local follow up appointments to occur after 3 months, and 5) updating the Principal Investigator.

Administrative/Editorial Changes:

Throughout	Editorial and formatting changes
Section 9.2	The last sentence in Section 9.2 was removed
APPENDIX I – Names of Study Personnel	Principal Investigator updated to Dr. Van Le

Scientific Changes:

Time & Events Table	• Footnote 1 of the T&E table was corrected to indicate that tocilizumab must be onsite prior to cell infusion. • Footnotes 2 and 3 of the T&E table were amended to clarify that long term follow up visits occurring after the 3 month visit can occur locally. • Footnote 4 of the T&E table amended to allow for ECHO to occur up to 90 days prior to procurement with the caveat that if a cardiac toxic therapy occurs after the ECHO it will need to be repeated prior to procurement. • Footnote 7 amended to remove HTLV testing.

Section 7.2.5		Removed HTLV testing
Section 7.4	Biobank Repository	Biospecimen storage time was updated

Therapy Changes:

Sections 1.4.2, 4.1.4, 4.2, 8.2, 8.2.2.5, and Figure 1	The lymphodepletion dose of fludarabine was increased from 25 to 30 mg/m^2
APPENDIX III – CRS Grading Criteria and Management Guidelines and APPENDIX IV – Management of Neurotoxicity/Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) from CAR-T Therapy	Hyperlinks to management guidelines were updated for both CRS and ICANS

Eligibility Changes:

Section 5.1	Inclusion and exclusion criteria have been combined
Criterion 5.1.1	Amended to include reference to a legally authorized representative
Criterion 0	Criterion relating to prior lines of therapy was removed
Criteria **Error! Reference source not found.** - 5.1.22	These criteria were previously exclusion criteria and were amended to make them part of the eligibility for cell procurement.
Criterion 5.1.22	Amended to remove HTLV
Criterion 5.1.23	Criterion added to clarify that intolerance to fludarabine is not permitted.
Criterion 5.1.25.5	Added (pulse oximetry) to organ function requirements
Section 5.2	Section was reorganized for clarity and some criteria were added for consistency with the cell procurement eligibility (5.2.3 – 5.2.9; 5.2.10.1-5.2.10.3. 5.2.12-5.2.19; 5.2.21-5.2.23).
Section 5.2.11	Organ function prior to lymphodepletion adjusted.

THE ATTACHED VERSION DATED APRIL 12, 2023, INCORPORATES THE ABOVE REVISIONS

SUMMARY OF CHANGES

Protocol Amendment #2

LCCC1818-ATL: A Phase 1 Study of Autologous Activated T-cells Targeting the B7-H3 Antigen in Subjects with Recurrent Epithelial Ovarian Cancer

AMENDMENT INCORPORATES:

X Editorial, administrative changes

X Scientific changes (IRB approval)

Therapy changes (IRB approval)

X Eligibility Changes (IRB approval)

Rationale for amendment: The primary purpose of this amendment is to clarify the stopping rules of the study. Modifications are made to ensure toxicities governing the stopping rules of the study are those toxicities associated with CAR-T cell treatment. Progression was clarified as progressive disease identified post the initial 6 week scan. Progression biopsy was updated to be completed at the discretion of the investigator. If imaging at the initial 6-week follow-up indicate full or partial response and the patient progresses afterward, a progression biopsy will be both clinically and scientifically important. If imaging at 6-week follow up indicate disease progression, the treating investigator may chose not to have a progression biopsy, as it may not be clinically significant and to reduce risk burden on patients who underwent a prior biopsy during catheter removal. The rational for this is that the study utilizes RNA based CAR-T product, which does not persist and expand as DNA based CAR-T products. RNA product is diluted during cellular division and it is unlikely to find RNA-expressing cells post infusion. Progressive disease after the initial 6-week scan most likely present true progression for these patients. Repeat imaging at 8-12 weeks in the case of progressive disease has been removed. The ability to perform a blood draw for procurement has been added; however, leukapheresis remains an option to obtain a sufficient amount of cells for product generation. The use of Bard ports is removed and instead further description of Aspira is added. The CRS and ICANS grading and guidelines are updated. Eligibility was updated to exclude subjects with untreated or symptomatic brain metastases. The data capture system was updated from OnCore to Advarra. The medical monitor was updated.

Editorial, Administrative Changes

Section 13	The data capture system was updated from OnCore to Advarra.

Section 4.2 Time and Events Table	Removed the W0 D2 Column.
Section 7.1.6	A duplicated sentence was removed.
Section 18.1, Appendix I	The medical monitor was updated from Jonathan Serody, M.D. to Natalie Grover, M.D.

Scientific changes:

Summary, Schema, footnote #1; Section 4.1.2; Section 4.2, footnote #17	The procurement procedures have been updated to allow for a peripheral blood draw. However, leukapheresis is still allowed if needed.
Section 4.1.3	The use of Bard ports is removed and instead language for Aspira use is added.
Section 4.2 Time and Events Table	Footnote #2, second sentence was updated to indicate the visit being described was for day 3 not day 2.
Section 4.2 Time and Events Table and Section 7.1.6	Footnote 8 of the Time and Events table is updated to remove the repeat imaging at 8-12 weeks in the case of progressive disease. Section 7.1.6 repeats this edit and further clarifies that the subject will be removed from the study.
Section 4.2, footnote 15 and Section 7.3.3	The time of specimen collection was changed from last cellular product administration to the time of intraperitoneal catheter removal. Further, the biopsy at the time of disease progression may occur at the discretion of the treating investigator. This progression biopsy is further clarified as progression after the initial 6-week scan.
Section 11.2	The section is updated to specify that sequential boundaries will be used to monitor Grade ≥ 4 non-hematologic toxicity at least possibly related to CAR-T cells.
Appendix III	Updated CRS Grading Criteria and Management Guidelines with edits and updated hyperlink

Appendix IV	Updated Management of Neurotoxicity/Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) from CAR-T Therapy with updated information and hyperlink.

Eligibility Changes

Section 5.1	Criterion #11 updated to clarify the timing of the biopsy from cell infusion to intraperitoneal catheter removal and to further clarify the timing of the biopsy relative to disease progression with the addition of "if not at their first scan."
Section 5.2	Added criterion #4 to exclude subjects with brain metastases. However, a subject with prior brain metastasis may be considered if they have completed their treatment for brain metastasis at least 4 weeks prior to being screened for eligibility, have been off of corticosteroids for ≥ 2 weeks, and are asymptomatic

THE ATTACHED VERSION DATED SEPTEMBER 27, 2021 INCORPORATES THE ABOVE REVISIONS

SUMMARY OF CHANGES

Protocol Amendment #1

LCCC1818-ATL: A Phase 1 Study of Autologous Activated T-cells Targeting the B7-H3 Antigen in Subjects with Recurrent Epithelial Ovarian Cancer

AMENDMENT INCORPORATES:

X Editorial, administrative changes

X Scientific changes (IRB approval)

Therapy changes (IRB approval)

X Eligibility Changes (IRB approval)

Rationale for amendment: The primary purpose of this amendment is to amend the sample collection timepoints for blood and ascites fluid. This study is using mRNA for the CAR-T product and the expression is transient. If we do not see CAR expression, this may be due to lack of expansion or that the CAR is no longer expressed on the cell. The additional PK timepoint at day 3 of each infusion is necessary to evaluate if they were able to expand considering the transient nature of the CAR expression.

Editorial, administrative changes:

Mechanical edits made throughout.

Scientific changes:

Section 4.2 Time and Events Table and Footnotes	The Time and Events Table was updated to include correlative blood and ascites fluid collections at day 3 after cell infusion. Footnotes 9 and 16 were updated to reflect this change.
Section 4.2 Time and Events Table	Correlative blood sample collection on W0D2 was removed
Section 7.3.4	The section was updated to include the new timepoint (day 3 after cell infusion) for ascites collection.

THE ATTACHED VERSION DATED February 17, 2021 INCORPORATES THE ABOVE REVISIONS

LCCC 1818-ATL

A Phase 1 Study of Autologous Activated T-cells Targeting the B7-H3 Antigen in Subjects with Recurrent Epithelial Ovarian Cancer

CONFIDENTIALITY AND INVESTIGATOR STATEMENT

The information contained in this protocol and all other information relevant to CAR.B7-H3 T cells are the confidential and proprietary information of Lineberger Comprehensive Cancer Center, and except as may be required by federal, state or local laws or regulation, may not be disclosed to others without prior written permission of Lineberger Comprehensive Cancer Center.

I have read the protocol, including all appendices, and I agree that it contains all of the necessary information for me and my staff to conduct this study as described. I will conduct this study as outlined herein, in accordance with the regulations stated in the Federal Code of Regulations for Good Clinical Practices and International Conference on Harmonization guidelines, and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and any amendments, and access to all information provided by Lineberger Comprehensive Cancer Center or specified designees. I will discuss the material with them to ensure that they are fully informed about CAR.B7-H3 T cells and the study.

Principal Investigator Name (printed) Signature

_____ _____

Date

_____

Study Summary

Title:	A Phase 1 Study of Autologous Activated T-cells Targeting the B7-H3 Antigen in Subjects with Recurrent Epithelial Ovarian Cancer
Rationale:	There is a critical need to identify new treatment options for recurrent ovarian cancer. Preclinical observations support B7-H3 as a viable target of CAR-T therapy. Based on these observations, we will conduct a phase 1 study of CAR.B7-H3 T cells in subjects with recurrent epithelial ovarian cancer to determine the safety and tolerability of this treatment modality and identify a recommended phase 2 dose for further study.
Target Population:	Women with relapsed or refractory platinum resistant epithelial ovarian cancer after up to 4 lines of prior therapy
Number of Subjects:	21
Primary Objective:	To determine the safety and tolerability of autologous CAR.B7-H3 T cell product administered intraperitoneally after lymphodepletion with cyclophosphamide and fludarabine to subjects with relapsed or refractory platinum-resistant epithelial ovarian cancer.
Secondary Objectives:	To determine the disease control rate (DCR) mediated by autologous CAR.B7-H3 T cell product administered to subjects with relapsed or refractory platinum-resistant epithelial ovarian cancer. To determine the progression free survival (PFS) after lymphodepletion with cyclophosphamide and fludarabine and administration of the CAR.B7-H3 T cell product in subjects with relapsed or refractory platinum-resistant epithelial ovarian cancer. To determine the overall survival (OS) in adult subjects with relapsed or refractory platinum-resistant epithelial ovarian cancer following lymphodepletion with cyclophosphamide and fludarabine and infusion with autologous CAR.B7-H3 T cells.
Study Design:	This is single center, open-label phase 1 dose escalation trial that uses modified 3+3 design to identify a recommended phase 2 dose (RP2D) of CAR.B7-H3 T cell product. An expansion cohort will enroll additional subjects at the RP2D for a total enrollment of up to 21 subjects on the protocol.
Primary Endpoint:	Toxicity will be classified and graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE), version 5.0, CRS Grading Criteria (APPENDIX III – CRS Grading Criteria

and Management Guidelines) and ICANS Grading Criteria (APPENDIX IV – Management of Neurotoxicity/Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) from CAR-T Therapy).

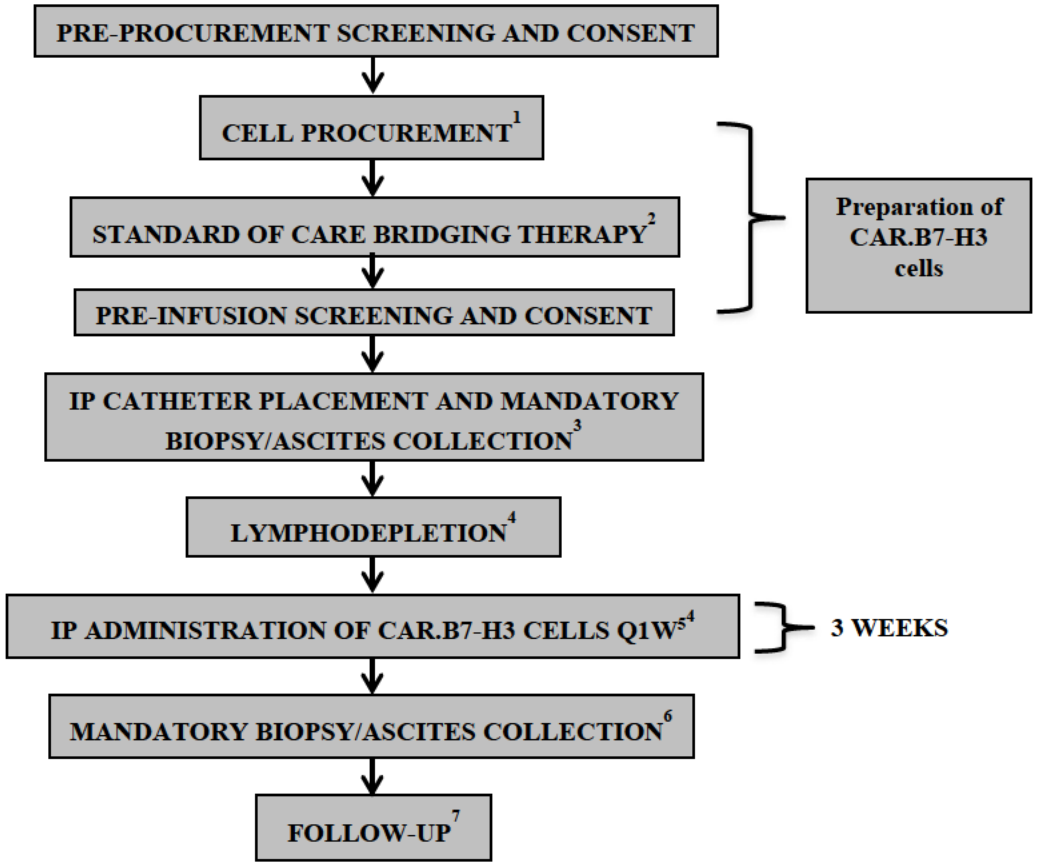
Secondary Endpoints:

Disease control rate will be defined as the percentage of subjects with [complete response (CR) + partial response (PR) + stable disease] at 6 months per RECIST 1.1 (APPENDIX II – Tumor Measurement Based on RECIST 1.1) criteria.

PFS will be measured from the time of lymphodepletion prior to infusion with CAR.B7-H3 to progression (as defined per RECIST 1.1 (APPENDIX II – Tumor Measurement Based on RECIST 1.1) or death.

OS will be measured from the date of lymphodepletion prior to CAR.B7-H3 T cell product administration to the date of death.

Figure 1 Study Schema for Dosing with Prior Lymphodepletion



1. Peripheral blood, up to 300 mL (in up to 3 collections) will be obtained from subjects for cell procurement. In subjects with inadequate lymphocyte count in the peripheral blood, a leukapheresis may be performed to isolate sufficient T cells. The parameters for apheresis will be up to 2 blood volumes.

2. Subjects will be allowed to receive standard of care therapy e.g., chemotherapy or radiation therapy to stabilize their disease following cell procurement if the treating physician feels that it is in the subject's best interest.

3. The intraperitoneal catheter will be placed as described in Section 4.1.3 in subjects who are deemed otherwise eligible to receive lymphodepletion prior to the CAR.B7-H3 infusion. During the placement, the drainage of ascites fluid will be performed. If the subject consents to ascites collection, this fluid will be used for correlative studies as described in Section 7.3.4. Additionally, a tumor sample will be collected at this time point for correlative studies as described in Section 7.3.3. As the subject will require placement of the intraperitoneal catheter by Vascular Interventional Radiology (VIR) or in the operating room, this sample will be collected at the time of catheter insertion.

4. Subjects will receive lymphodepleting chemotherapy consisting of cyclophosphamide 300 mg/m^2 IV and fludarabine 30 mg/m^2 over 3 consecutive days administered 2-14 days prior to initial cellular product administration.

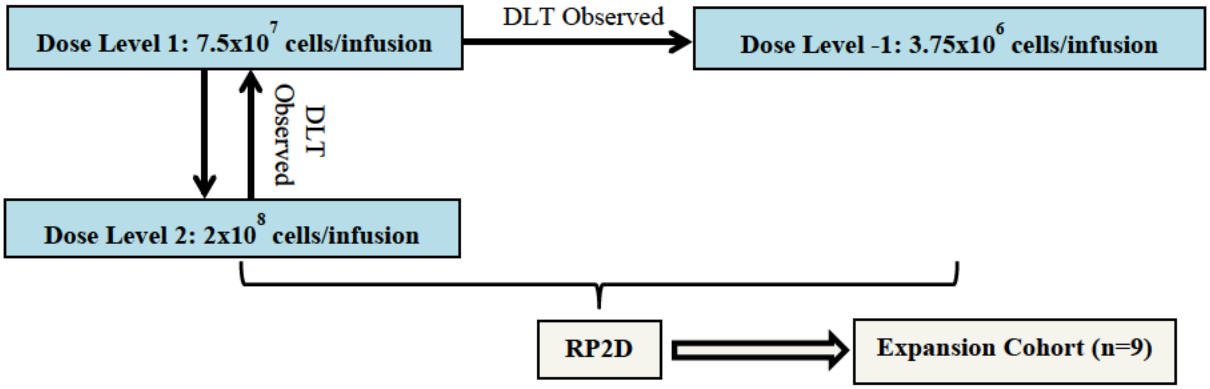
5. Prior to CAR.B7-H3 administration, in the absence of ascites, 100-200 mL of saline will be infused into subjects intraperitoneally via an IP port/catheter. CAR.B7-H3 cells will then be administered intraperitoneally over 5-10

minutes via an IP port/catheter. After the cellular product administration IP catheter should be flushed normal saline (up to 2x the volume of the CAR.B7-H3 infusion). The subject will then be rolled per institutional intraperitoneal chemotherapy protocols to distribute the intraperitoneally infused product. Subjects will receive 3 weekly doses of CAR.B7-H3 T cell product infusions at the same dose as dictated by the modified 3+3 dose escalation design.

6. Subjects with measurable disease per RECIST 1.1 will have their tissue sample collected on W2D0 ±3 days via CT-guided or ultrasound-guided biopsy. This may be collected at the time of IP catheter removal.

7. Subjects will be followed for DLTs from the time of the first infusion until 4 weeks following the final (third) cell infusion. Additionally, subjects will be followed for up to 5 years or until disease progression or death. Imaging will be performed every 3 months until disease progression.

Figure 2 Dosing Schema



Subjects will be assigned in cohorts of 3 at escalating doses (DL1, DL2) with prior lymphodepletion. A 3+3 design will be used to evaluate DL1 and DL2. If dose level 1 is not tolerated, the trial will be suspended momentarily to evaluate safety and reach a decision on whether to evaluate a dose that is at least 50% lower than the proposed starting dose of 7.5×10^7 cells. If enough cells for 3 infusions are not generated, then a subject may be treated at a previously safely completed dose level on which they would have enough cells to receive three infusions. Their DLT data will be incorporated into the dose escalation/de-escalation algorithm. Any dose level may be expanded to allow enrollment of 4-9 subjects to obtain more data at that dose. If $\geq$0.33 of the total number of subjects on a dose level experience a DLT, the study would not escalate to the next highest dose level and the MTD would be exceeded. Recommended phase 2 tolerable cell dose (RP2D) will be decided based on the maximum tolerated cell dose and additional factors.

TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

LIST OF ABBREVIATIONS

AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
ATL	Autologous T Lymphocyte – Chimeric Antigen Receptor
β-HCG	Beta-Human Chorionic Gonadotropin
BUN	Blood Urea Nitrogen
CAP	College of American Pathologists
CAR-T	Chimeric Antigen Receptor T Cell
CBCD	Complete Blood Count with Differential
cfDNA	Circulating Free Deoxyribonucleic Acid
CLIA	Clinical Laboratory Improvement Amendments
CMP	Comprehensive Metabolic Panel
CNS	Central Nervous System
CoA	Certificate of Analysis
CPO	Clinical Protocol Office
CR	Complete Response
CRS	Cytokine Release Syndrome
CT	Computer Tomography
DCR	Disease Control Rate
DL	Dose Level

DLT	Dose Limiting Toxicity
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
DSMC	Data Safety Monitoring Committee
ECHO	Echocardiography
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EFS	Event-Free Survival
ELISPOT	Enzyme-Linked ImmunoSpot
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GeMCRIS	Genetic Modification Clinical Research Information System
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GMP	Good Manufacturing Practices
HAMA	Human Anti-Mouse Antibodies
HBs-Ag	Hepatitis B surface Antigen
HBc	Hepatitis B Core Antigen
HBV	Hepatitis B Virus
HBC	Hepatitis C Virus
HIPAA	Health Insurance Portability and Accountability Act
HIPEC	Hyperthermic Intraperitoneal Chemotherapy
HIV	Human Immunodeficiency Virus
HLH	Hemophagocytic Lymphohistiocytosis
HTLV	Human T-Lymphotropic Virus

IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICANS	Immune effector cell-associated neurotoxicity syndrome
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDS	Investigational Drug Service
IEC	Independent Ethics Committee
IFN	Interferon
Ig	Immunoglobulin
IHC	Immunohistochemistry
IL	Interleukin
INR	International Normalized Ratio
IRB	Institutional Review Board
IV	Intravenous
kg	Kilogram
L	Liters
LCCC	Lineberger Comprehensive Cancer Center
LDH	Lactate Dehydrogenase
LVEF	Left Ventricular Ejection Fraction
mAb	Monoclonal Antibody
MAS	Macrophage Activation Syndrome
mg	Milligram
min	Minute
MRI	Magnetic Resonance Imaging

MTD	Maximum Tolerated Dose
NCI-CTCAE	National Cancer Institute – Common Terminology Criteria for Adverse Events
NIH	National Institute of Health
NK	Natural killer
OC	Ovarian Cancer
OHRE	Office of Human Research Ethics
ORR	Objective Response Rate
OS	Overall Survival
OSP	NIH Office of Science Policy
PARP	Poly ADP Ribose Polymerase
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PD	Progressive Disease
PET	Positron Emission Tomography
PFS	Progression Free Survival
PI	Principal Investigator
PR	Partial Response
PRC	Protocol Review Committee
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
qPCR	Quantitative Polymerase Chain Reaction
RAC	NIH Recombinant DNA Advisory Committee
RECIST	Response Evaluation Criteria in Solid Tumors

RFS	Relapse-Free Survival
RNA	Ribonucleic Acid
RP2D	Recommended Phase 2 Dose
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCD	Tolerable Cell Dose
TLS	Tumor Lysis Syndrome
ULN	Upper Limit of Normal
UNC	University of North Carolina
USP	United States Pharmacopeia

1 INTRODUCTION AND RATIONALE

1.1 Background

There were approximately 22,240 new cases of and 14,070 deaths from ovarian cancer in the United States in 2018. The majority of women (approximately 80%) are diagnosed with stage III or IV disease at the time of presentation [1, 2]. For most of them, standard therapy involves a combination of chemotherapy and surgery. Most women undergo cytoreductive surgery followed by chemotherapy, which can be intravenous, intraperitoneal or a combination of both [3-6]. However, some women undergo neoadjuvant chemotherapy followed by surgery. These therapies result in response rates of 70-80%, depending on if the patient is optimally (< 1 cm residual disease) or suboptimally (> 1 cm residual disease) cytoreduced [6, 7]. Despite these excellent response rates, the majority of patients will have a disease recurrence. At disease recurrence prognosis depends on when recurrence occurred in relation to the last cycle of a platinum-based regimen. Women who are platinum-sensitive (> 12 months after completion of prior platinum before recurrence) have a 50 – 65% response rate to subsequent platinum therapy. However, these women ultimately develop platinum-resistant disease. For women who are either platinum-resistant (recurrence < 6 months) or potentially platinum-sensitive (recurrence 6 – 12 months) after completion of initial chemotherapy, there are treatment options available, but most are not curative [8]. Therefore, there is a critical need to identify new treatment options for women with recurrent ovarian cancer.

1.2 Current Standard of Care

National Comprehensive Cancer Network (NCCN) guidelines outline a variety of second- and third-line agents that are available for the treatment of recurrent epithelial ovarian cancer. While these have traditionally involved other chemotherapeutic agents (gemcitabine, liposomal doxorubicin, topotecan, etc.) more recently other agents such as bevacizumab and poly ADP ribose polymerase (PARP) inhibitors have been approved. In women with platinum-resistant disease, response rates range from 10-25% and duration of response is generally less than 6 months with the most usual chemotherapeutic agents [8-14]. Treatment in the setting of relapsed disease is typically not curative and is given with the intent of prolonging life or palliation of symptoms. Bevacizumab in combination with chemotherapy has been shown to improve progression free survival by 4 months (AURELIA trial) and as a single agent, response rates range from 13-16% [15]. PARP inhibitors have recently been approved for use as both maintenance agents after first line therapy and for treatment of recurrent ovarian cancer [7, 16-21]. Some of the medications are reserved for women who have either a germline or a somatic mutation in BRCA, however, niraparib has been recently approved for women who do not harbor a mutation in either their germline or in their tumor and are platinum-sensitive after their first recurrence [19].

There are currently several trials evaluating novel combinations of approved or investigational agents, new immunotherapy trials, and targeted agents. Vaccine trials

including dendritic cell vaccines, vaccines directed at antigens enriched in tumor cells and patient-specific autologous tumor cell vaccines. Despite all of these agents and strategies, many women will have limited treatment options after 2 – 3 regimens for the treatment of their recurrent epithelial ovarian cancer.

1.3 Investigational Treatment: B7-H3 CAR-T Therapy for Ovarian Cancer

Chimeric antigen receptor T (CAR-T) cells are immune cells that are isolated from patients and then genetically modified in order to make them able to identify and eliminate cancer cells based on the proteins that are expressed on the tumor cell surface. Impressive clinical responses have been reported in B-cell malignancies using CAR-T cells specific for the CD-19 antigen [22-24]. However, adapting this strategy to solid tumors has been more difficult because tumor-associated antigens which are expressed on the tumor surface are also often shared with normal tissues. These are also often characterized by both inter- and intratumor heterogeneity and are often not expressed across tumor types [25-27].

B7-H3 is a type I transmembrane protein that belongs to the B7 and CD28 families [28, 29]. B7-H3 protein is not overly expressed in normal tissue, such as breast, prostate, liver, and colon [30, 31], but it is aberrantly expressed in 60% to 93% of tumor cells in several cancer types including melanoma, leukemia, breast, prostate and ovarian cancer [31-35]. In addition, B7-H3 is found overexpressed by the tumor-associated vasculature and stroma fibroblasts [31, 32]. Overexpression of B7-H3 on tumor cells frequently correlates with fewer tumor-infiltrating lymphocytes, faster cancer progression, and poor clinical outcome in several malignancies, such as prostate cancer, ovarian cancer, lung cancer and clear cell renal carcinoma [32-42].

1.3.1 *Clinical Experience with B7-H3 Monoclonal Antibodies*

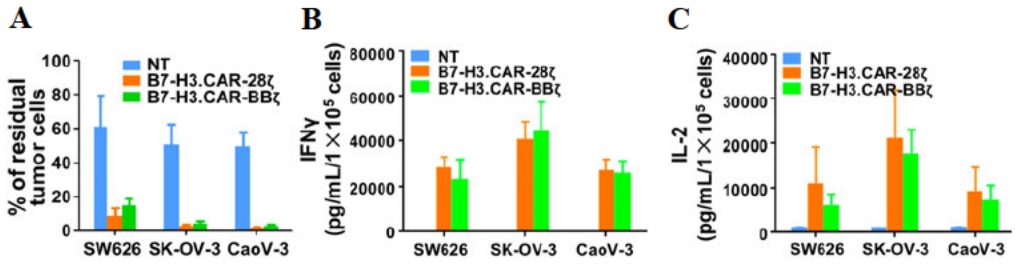
B7-H3-specifc monoclonal antibodies (mAbs) showed antitumor activity against B7-H3+ tumor cells in preclinical xenograft mouse models, and phase 1 clinical trials are currently ongoing to test safety and antitumor activity (NCT01099644, NCT01502917, NCT02381314 and NCT02982941) [31, 43-46]. Preliminary phase 1 data with the B7-H3-specific MGA271 (enoblituzumab) antibody did not show toxicity (STIC 2015). This study was a dose escalation study of MGA271 in patients with a variety of tumors; for cycle 1 the patients received weekly dosing for 4 weeks and then off for 4 weeks. After cycle 2, they were dosed weekly for three weeks followed by one week off prior to repeating the cycle. In this patient cohort, the antibody was considered safe in that no treatment related discontinuations or severe immune-mediated toxicity was observed. Of the 116 patients, only 5 (4%) had any grade 3 – 4 adverse events and only 1 (1%) had a grade 3-4 infusion-related reaction/cytokine release syndrome [36]. At the time of presentation, 88 patients had evaluable disease and the interim results supported continued evaluation of MGA271 as monotherapy and in combination with checkpoint inhibitors.

1.3.2 *Experience with Regional CAR-T Cell Product Administration*

Ovarian cancer is characterized with metastatic spread in the peritoneal cavity. While extra-peritoneal recurrences can occur (retroperitoneal nodes, chest, brain, etc.), the majority of women will recur with peritoneal based disease and will succumb to the effects of complications related to this, which can include progressive bowel obstruction. Intraperitoneal therapy has been a hallmark of ovarian cancer treatment for decades and new advances continue supporting this strategy. In 2006, the NCI released a clinical announcement in support of the benefits of intraperitoneal chemotherapy in women with optimally debulked ovarian cancer [47]. Despite the survival advantage reported from these regimens [48], there has been slow adoption of this treatment strategy in part due to catheter-related complication, treatment toxicity and increased number of visits for patients. The most recent iteration of this strategy has involved intraperitoneal chemotherapy during surgery delivered under hyperthermic conditions (HIPEC-hyperthermic intraperitoneal chemotherapy). The strategy behind this treatment includes that the hyperthermia increases the penetration of chemotherapy at the peritoneal level, that it increases the sensitivity of the cancer to the chemotherapeutic agents, and that it induces apoptosis, inhibits angiogenesis, and has additional effects [49]. A recent randomized phase III trial of HIPEC in patients undergoing interval cytoreduction after neoadjuvant chemotherapy was recently published. In this study, the addition of HIPEC led to a hazard ratio for recurrence or death of 0.66 (95% CI, 0.50 to 0.87, p=0.003). Additionally, the percentage of patients who had grade 3 – 4 toxicity was no different between the two treatment arms (25% v 27%, p=0.76). Both after initial cytoreductive surgery in the absence of neoadjuvant chemotherapy and in the setting of interval cytoreductive surgery, intraperitoneal therapy appears to improve outcomes for women with advanced ovarian cancer. This has also been shown in colorectal cancer in which patients who underwent cytoreductive surgery and HIPEC had an improved overall survival as compared to those who underwent intravenous chemotherapy [50, 51]. Therefore, it is reasonable to explore intraperitoneal therapy in the recurrent setting.

Our group (Dotti et al.) investigated the antitumor effects of CAR.B7-H3 T cells in ovarian cancer models. Three human OC cell lines (SW626, SK-OV-3 and CaoV-3) which showed B7-H3 expression were co-cultured with CAR.CD19 T cells, B7-H3.CAR-28ζ-Ts or B7-H3.CAR-BBζ-Ts at T cell to tumor cell ratio of 1:5. As shown in Figure 3, both B7-H3.CAR-28ζ-Ts and B7-H3.CAR-BBζ-Ts effectively controlled [52]the growth of OC cell lines and led to released cytokines (IFN-γ and IL-2) in the culture supernatants.

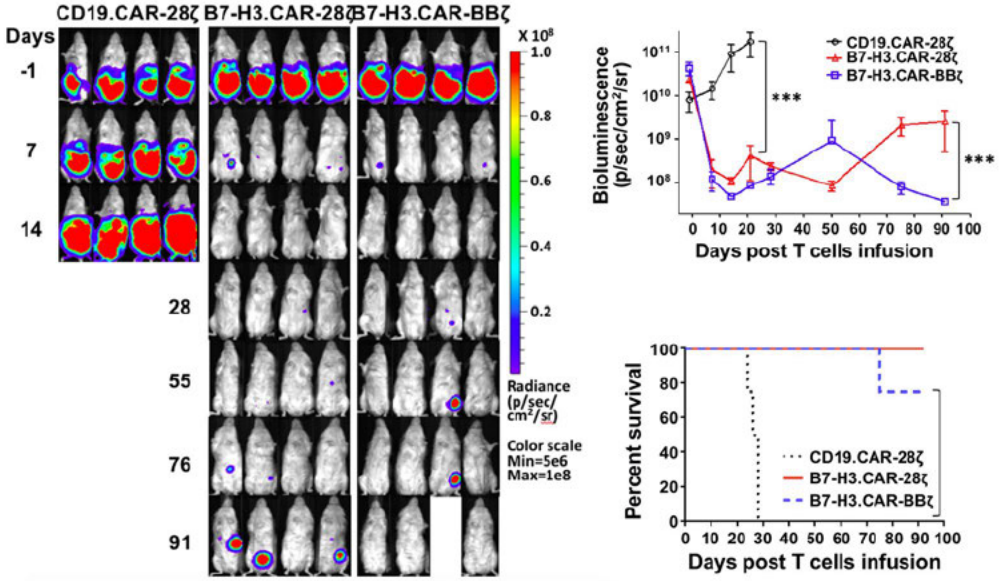
Figure 3 The effects of BY-H3.CAR-28ζ-Ts and B7-H3.CAR-BBζ-Ts on the growth of OC cell lines SW626, SK-0V-3 and CaoV-3



(2) Summary of residual tumor cells in culture on day 5 of co-culture of control (NT), B7-H3.CAR-28ζ-Ts and B7-H3.CAR-BBζ-Ts with ovarian cancer (OC) cell lines at T cell to tumor cell ratio of 1 to 5 (n=5). Error bars denote standard deviation (SD). (B and C) Summary of IFNγ (B) and IL-2 (C) released by NT ByH3.CAR-28ζ-Ts and By-H3.CAR-BBζ-Ts in the co-culture supernatant collected after 24 hours and measured by ELISA (n=5). Error bars denote SD.

For the *in vivo* validation, the Ffluc-transduced SK-OV-3 tumor cell line was implanted intraperitoneally (IP) into NSG mice to create an intraperitoneal metastatic OC model. Two weeks post tumor implantation, mice received CAR.CD19 T cells, B7-H3.CAR-28ζ-Ts or B7-H3.CAR-BBζ-Ts IP since this type of CAR-T delivery is currently considered in clinical trials (NCT02498912). As shown in Figure 4, CAR.B7-H3 T cells significantly controlled tumor growth and extended the overall survival. Antitumor activity of CAR.B7-H3 T cells was also observed when T cells were infused IV, although the IP inoculation was more effective.

Figure 4 The effect of CAR.B7-H3 on tumor growth *in vivo*



(A and B) Representative bioluminescence images (A) and bioluminescence kinetics (B) of Fflu-SK-OV-3 tumor growth upon IP inoculation into NSG mice ($5x10^5$ cells/mouse) and treatment 14 days later with IP inoculation of CD19.CAR-Ts, B7-H3.CAR-28ζ-Ts and B7-H3.CAR-BBζ-Ts ($5x10^6$ cells/mouse). Error bars denote SD, ***p<0.0001, Chi-square test. (C) Kaplan-Meier survival curve of mice developing OC (5 mice/group), **p=0.0091, Chi-square test.

Katz et al, published similar findings in a colorectal cancer model leading to the conclusion that this data supported the development of IP CAR-T therapy in patients with peritoneal malignancies [52].

1.3.3 *Toxicities Associated with CAR-T Cells*

The four most common toxicities seen with CAR T cells are cross reactivity, cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS) and macrophage activation syndrome [53, 54].

Cross reactivity is due to the expression of the targeted antigen in normal tissues in addition to tumor cells [55]. Depending on the normal tissues targeted, side effects can be manageable, such as the B cell aplasia observed in patients treated with CD19-specific CAR-T cells, or lethal if organs such as heart, lung or liver are affected [53, 56-59]. However, this on-target off-tumor toxicity, can be a major obstacle to the use of CAR-T cells engineered for the treatment of solid malignancies such as ovarian cancer [60]. As a result, in this study mRNA-engineered T cells will be used evaluate the safety of CAR-T cells targeting antigens on solid tumors in a more controlled and transient manner as previously performed by Beatty *et. Al*. to demonstrate initial safety of CAR-T cells targeting mesothelin in patients with mesothelin-expressing solid tumors [60]. RNA based CAR-T cells will not persist long term as the RNA is diluted during cellular division and thus it is unlikely to find RNA-expressing cells more than 10 days after treatment, thereby mitigating the on-target, off tumor toxicities.

CRS symptoms can vary from mild flu-like symptoms to more severe toxicities such as vascular leak, hypotension, coagulopathy, pulmonary edema and multi-organ failure [56-59]. CRS-associated toxicities, when severe, require intensive medical management including vasoactive agents for hemodynamic support, mechanical ventilation, anti-epileptics, and antipyretics [53]. Cytokine elevations are detectable in most subjects; however, the degree of elevation does not correlate with the severity of CRS or response to therapy. The management of CRS to date involves supportive care measures and targeted therapy with tocilizumab (monoclonal antibody that blocks the IL-6 receptor) administered with or without corticosteroid therapy to suppress the immune response. Currently, the use of tocilizumab appears to be effective in most patients with limited inherent toxicity. As a result, with the initial approval of Kymriah™, the first FDA approved CAR-T cell product, tocilizumab was approved for the treatment of severe or life-threatening CAR-T cell inducted CRS in adult and pediatric subjects [61]. In some cases, concomitant administration of corticosteroids with tocilizumab may be required to alleviate symptoms related to CRS [62].

Neurological toxicities such as progressive confusion, aphasia, word finding difficulty, obtunded states with airway compromise and encephalopathy have been described in subjects experiencing ICANS [54, 63]. ICANS can occur on its own or concurrently with or after CRS.

Although rare, hemophagocytic lymphohistiocytosis (HLH) / macrophage activation syndrome, and sepsis have also been described as CAR-T-cell-related safety concerns [64]. Macrophage activation syndrome is characterized by pancytopenia, liver insufficiency, coagulopathy and neurological symptoms and is thought to be mediated by uncontrolled proliferation and activation of T cells leading to macrophage activation and differentiation and cytokine production with hemophagocytosis.

Clinical experience with these toxicities has evolved and continues to grow. A recent review by Lee *et al*. provides guidance for the management of CAR-T-cell-related toxicities [54]. In contrast to the delayed onset of autoimmune toxicities associated with immune checkpoint inhibitor therapies, toxicities intimately associated with CAR-T-cell therapy tend to be acute, less diverse, and more predictable. Importantly, intensive monitoring and prompt management of toxicities is essential to minimize the morbidity and mortality associated with CAR-T cell therapy.

1.4 Rationale for LCCC 1818-ATL Study

The preclinical observations summarized in Section 1.3 support B7-H3 as a viable target of CAR-T therapy for ovarian cancer. Based on these observations, we will conduct a phase 1 study of CAR.B7-H3 T cell product in subjects with recurrent epithelial ovarian cancer. Escalating doses of CAR.B7-H3 T cells ranging from 7.5×10^7 cells/infusion to 2×10^8 transduced cells/infusion will be evaluated in up to 12 subjects to determine the safety and tolerability of this treatment modality and identify a recommended phase 2 dose for further study based on 3+3 dose finding rules. After dose-finding is completed, an expansion cohort will enroll up to 12 subjects at the estimated RP2D to further assess the safety and efficacy of CAR.B7-H3 T cells. Additionally, disease control rates (DCR), and progression will be determined based on imaging data and RECIST 1.1 criteria. The trial will also evaluate cytokine profiles and the expansion and persistence of CAR.B7-H3 T cells in peripheral blood samples after infusion. We anticipate the maximum expansion of CAR.B7-H3 T cells to occur within 7 days following cellular product administration.

1.4.1 *CAR.B7-H3 T cell Dose Rationale*

Other phase I trials in women with ovarian cancer have had starting doses of 3×10^5/kg (MUC-16ecto) and $3\times10^7/m^2$ (Mov19-BBZ) (NCT03585764) [65]. However, for this trial, mRNA electroporated CAR-T cells are being used; due to the transient expression of the CAR as compared to stable insertion of the CAR in T cells in other products, the overall effect of our product is completely dependent on the dose of CAR-T cells since there is no expansion of these cells *in vivo*. Therefore, dosing based on Beatty et. Al. will be used [60]. The Beatty et. Al. phase I trial evaluated mRNA-engineered T cells that targeted mesothelin and patients received 3 intravenous infusions or in the case of

patient 2 received 8 intravenous infusions and 2 intratumoral injections, with doses starting at 3×10^8 cells. In the Beatty et. Al. trial, there were no adverse events with weekly dosing at this dose level. Therefore, for this trial the starting dose will be 7.5×10^7 cells/infusion for 3 weekly infusions with a dose escalation to 2×10^8 cells/infusion for 3 weekly infusions with lymphodepletion.

It is understood that cross reactivity due To the expression of the target antigen in normal tissue in addition to the tumor tissue may result in toxicity and can be a major obstacle to the use of CAR-T cells engineered for the treatment of solid malignancies such as ovarian cancer [65]. As a result, in this study mRNA-engineered T cells will be used to evaluate the safety of CAR-T cells targeting antigens on solid tumors in a more controlled and transient manner as previously performed by Beatty et. Al. to demonstrate initial safety of CAR-T cells targeting mesothelin in patients with mesothelin-expressing solid tumors [65]. RNA based CAR-T cells will not persist long term as the RNA is diluted during cellular division and thus it is unlikely to find persistent RNA-expressing cells. Beatty et. Al. showed that maximal levels of transgene introduced in T cells by mRNA were detected within 2 hours of infusion when mRNA-based CAR-T cells were infused directly intravenously [65]. Due to the biodegradable nature of the transgene, the levels then progressively decreased on successive days [65]. This will mitigate the on-target, off-tumor toxicities. It is anticipated that maximal detection of CAR.B7-H3 cells to occur within 7 days following cellular product administration, which is the rationale for the weekly doses.

With regards to our two dosing strategies, dose level will increase by 2.6-fold for each of the three weekly infusions. In other CAR-T trials, the proposed dose increases between cohorts was 3 fold: 3×10^5 cells/kg; 1×10^6 cells/kg; 3×10^6 cells/kg; 1×10^7 cells/kg [60]. Therefore, this dose escalation is consistent with those used in other CAR-T cell trials. While these were not mRNA-engineered trials, this dose increase is consistent and due to the transient nature of the product, this dosing strategy should have less off-tumor on-target toxicity and be safer for the patients.

1.4.2 *Rationale for Lymphodepleting Chemotherapy*

Lymphodepleting chemotherapy has been shown to improve the persistence of modified T cells and has been associated with improved progression free survival.

Brentjens *et al.* studied the safety and persistence of chimeric antigen receptor T cells with antibody against CD19 and found improved clinical benefit in subjects who received prior conditioning chemotherapy and had low tumor burden or minimal residual disease [22]. In addition, subjects that were treated with modified T cells without prior cyclophosphamide had very low frequency of T cells detected in the peripheral blood. In contrast, subjects who received cyclophosphamide conditioning had T cells more readily detected over time in the blood and bone marrow.

Lymphodepleting chemotherapy also reduces the number of the host's suppressive cells, such as regulatory T cells, allowing the genetically modified T cells to expand

and eradicate tumor cells [66]. The conditioning regimen may also stimulate the production of cytokines such as IL-7 or IL-15, which may also favor expansion of the infused cells [23, 67]. Grupp *et al.* showed improved T-cell counts and proliferation in subjects who received CAR-T cells on day 2 after conditioning chemotherapy compared to day 90, supporting the theory that T cell infusion should occur early after conditioning [68].

In this phase 1 dose-finding trial, subjects will receive a lymphodepleting chemotherapy consisting of cyclophosphamide 300 mg/m^2/day and fludarabine 30 mg/m^2/day IV for 3 consecutive days administered 2 – 14 days prior to initial cellular product administration. Cyclophosphamide and fludarabine were chosen given documented cyclophosphamide activity in patients with recurrent epithelial ovarian cancer [69, 70] and the benefits of fludarabine for lymphodepletion prior to CAR T cell infusion [66].

1.5 Correlative Studies

We will perform function and persistence studies, which will include functional assays, such as *in vitro* reactivation of PBMCs and immunophenotyping using flow cytometry. CAR.B7-H3 T cells entering the blood stream will be measured by flow cytometry. Pre- and post-treatment tumor samples and ascites fluid (when available) samples will be obtained and analyzed to evaluate changes after CAR.B7-H3 T cell product administration. Changes in tumor mutation and/or gene expression will be evaluated in the tumor samples or ascites obtained before and after treatment. Gene expression will be measured using either single cell or bulk RNA-sequencing, Nanostring or qPCR. Mutational analysis will be performed using whole genome or whole exome sequencing. T cell repertoire analysis will be evaluated using next generation sequencing of T cell receptor Vβ and/or paired sequencing for Vαβ.

2 STUDY OBJECTIVES

2.1 Primary

2.1.1 ***To determine the safety and tolerability of autologous CAR.B7-H3 T cell product administered intraperitoneally after lymphodepletion with cyclophosphamide and fludarabine to subjects with relapsed or refractory platinum-resistant epithelial ovarian cancer.***

2.2 Secondary

2.2.1 ***To determine the disease control rate (DCR) mediated by autologous CAR.B7-H3 T cell product administered to subjects with relapsed or refractory platinum-resistant epithelial ovarian cancer.***

2.2.2 ***To determine the progression free survival (PFS) after lymphodepletion with cyclophosphamide and fludarabine and administration of the CAR.B7-H3 T cell product in subjects with relapsed or refractory platinum-resistant epithelial ovarian cancer.***

2.2.3 ***To determine the overall survival (OS) in adult subjects with relapsed or refractory platinum-resistant epithelial ovarian cancer following lymphodepletion with cyclophosphamide and fludarabine and infusion with autologous CAR.B7-H3 T cells.***

2.3 Exploratory

3 STUDY ENDPOINTS

3.1 Primary

3.1.1 ***Toxicity will be classified and graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE), version 5.0, CRS Grading Criteria (APPENDIX III – CRS Grading Criteria and Management Guidelines) and ICANS Grading Criteria (APPENDIX IV – Management of Neurotoxicity/Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) from CAR-T Therapy).***

(*Note:* The safety evaluation period for DLT assessment will encompass toxicities related to the cell therapy that are experienced starting on the day of initial CAR.B7-H3 T cell product administration up through 4 weeks after the final (third) CAR.B7-H3 T cell product administration. See Sections 4.1.7 and Section 4.1.8 of the protocol for definitions of DLTs and RP2D, respectively. General safety monitoring will begin at the time of procurement).

3.2 Secondary

3.2.1 ***Disease control rate will be defined as the percentage of subjects with [complete response (CR) + partial response (PR) + stable disease] at 6 months per RECIST 1.1 (APPENDIX II – Tumor Measurement Based on RECIST 1.1) criteria.***

3.2.2 ***PFS will be measured from the time of lymphodepletion prior to infusion with CAR.B7-H3 to progression (as defined per RECIST 1.1 (APPENDIX II – Tumor Measurement Based on RECIST 1.1) or death.***

3.2.3 ***OS will be measured from the date of lymphodepletion prior to CAR.B7-H3 T cell product administration to the date of death.***

3.3 Exploratory

[redacted]

4 STUDY PLAN

This single center, open-label phase 1 study will determine the safety and tolerability of escalating doses of autologous activated T lymphocytes expressing the chimeric antigen receptor specific for B7-H3 in recurrent epithelial ovarian cancer. During dose finding, up to 12 subjects will receive infusions of the cellular product expressing the CAR.B7-H3.

4.1 Study Treatment

4.1.1 *Dose Escalation Rules*

Dose escalation will be performed as described below considering the dose limiting toxicities listed in section 4.1.7. Two doses will be explored with prior lymphodepletion. The starting dose will be 7.5×10^7 cells/infusion (Dose Level (DL) 1). If there are no dose limiting toxicities (DLTs) within 4 weeks of the third cellular product administration in these 3 subjects, then the next cohort will evaluate 2×10^8 cells/infusion (DL2). The maximum tolerated cell dose is defined as a dose with the probability of DLT of 20%. The estimated maximum tolerated cell dose after the 3+3 design is the higher dose with observed DLT rate less than 33%. If enough cells for 3 infusions are not generated, then a subject may be treated at a previously cleared dose level on which they would have enough cells to receive all three infusions. Their DLT data will be incorporated in the dose escalation/de-escalation algorithm and will be used to estimate the maximum tolerated cell dose. A dose level may be expanded to 4-9 subjects to obtain more data at that dose. If more than 3 subjects are enrolled at a dose and ≥0.33 of the total number of subjects on a dose level experiences a DLT, the study would not escalate to the next highest dose level and the MTD would be exceeded. Recommended phase 2 tolerable cell dose (RP2D) will be decided based on the maximum tolerated cell dose and additional factors.

If dose level 1 is not tolerated, subjects may be enrolled on dose level -1 at a dose of 3.75×10^6 cells/infusion. After dose escalation is completed, an expansion cohort will enroll up to 12 subjects to further assess the safety and efficacy of CAR.B7-H3 T cells. Subjects enrolled in the expansion cohort will continue to be monitored for safety using the stopping rules included in Section 11.2.

Table 1 Dose Escalation Rules (3+3)

Number of Subjects with DLT	Action
0 out of 3 subjects	Escalate to next dose level
1 out of 3 subjects	Accrue 3 additional evaluable subjects at current dose level
1 out of 6 subjects	Escalate to the next dose level
2 or more subjects in a dosing cohort	D-escalate to the next lower dose level

The first three subjects evaluated on each dose level must complete a 4-week evaluation period after the initial infusion before additional subjects are dosed in that cohort. Prior to escalating or de-escalating to the next dose level, all subjects in the prior cohort must have cleared the DLT evaluation window.

The doses of cells to be explored are provided in Section 4.1.6.

See Section 4.1.7 for definitions of DLT and Section 4.1.8 for the definition of the RP2D.

4.1.2 *Cell Procurement*

Peripheral blood, up to 300 mL (in up to 3 collections) will be obtained from subjects for cell procurement. In subjects with inadequate lymphocyte count in the peripheral blood, a leukapheresis may be performed to isolate sufficient T cells. The parameters for apheresis will be up to 2 blood volumes.

4.1.3 *Intraperitoneal Catheter Placement*

The intraperitoneal catheter will be placed in patients who are determined to be otherwise eligible for lymphodepletion.

Aspira Drainage system (Meritmedical) will be placed either in the operating room at the time of laparoscopically directed biopsies or, if the patient has ascites, by vascular radiology. This device is cleared by the FDA.

4.1.4 *Lymphodepleting Chemotherapy*

Subjects will require lymphodepletion and will receive a lymphodepleting chemotherapy regimen with cyclophosphamide 300 mg/m^2 and fludarabine 30 mg/m^2 administered IV x 3 days per institutional standard prior to initial cellular product administration. Prophylaxis (e.g., mesna, hydration, antiemetics, etc.) needed prior to cyclophosphamide chemotherapy will be provided per institutional guidelines.

4.1.5 *Premedication for Intraperitoneal Cellular Product Administration*

Subjects may be pre-medicated with diphenhydramine up to 1 mg/kg IV (max 50 mg) and acetaminophen 10 mg/kg PO (max 650 mg) prior to each cellular product administration. We will pre-medicate subjects who have a history of reactions to blood products. Corticosteroids should be avoided given their detrimental effect on the survival of the infused T cells. Anti-emetics in appropriate dosage for each subject will be prescribed as necessary.

4.1.6 *CAR.B7-H3 Cell Administration*

Prior to CAR.B7-H3 administration, in the absence of ascites, 100-200 mL of saline will be infused into subjected intraperitoneally via an IP port/catheter. CAR.B7-H3 T

cell product will be administered by a licensed healthcare individual intraperitoneally over 5-10 minutes via an intraperitoneal port/catheter. The volume of infusion will depend upon the concentration of the cells when frozen and the size of the subject. The expected volume will be up to 50 mL. After the cellular product administration intraperitoneal catheter should be flushed with normal saline solution (up to 2x the volume of the CAR.B7-H3 infusion). The subject will then be rolled per institutional intraperitoneal chemotherapy protocols to distribute the intraperitoneally infused product.

Subjects will receive three weekly CAR.B7-H3 T cell product infusions at the same dose. The cell dose levels that will be evaluated are outlined below. The starting dose will be 7.5×10^7 cells/infusion of transduced cells. If this dose is not tolerated, then a lower dose (DL -1) of 3.75×10^6 cells/infusion will be explored. If there is an insufficient number of cells manufactured to treat a subject with 3 infusions of CAR.B7-H3 T cells on their assigned dose level, then the subject may be treated on a lower dose level. Subjects will only be treated on dose levels for which enough cells have been manufactured to complete all three infusions.

Table 2 Dose levels of CAR.B7-H3 T cells to be tested during dose escalation

Dose Level	Dose (number of cells/infusion)
-1	3.75×10^6
1	7.5×10^7
2	2×10^8

4.1.7 *Definition of Dose Limiting Toxicity*

An event will be considered a DLT per NCI CTCAE criteria, version 5.0, CRS grading criteria (APPENDIX III – CRS Grading Criteria and Management Guidelines) or ICANS grading criteria (APPENDIX IV – Management of Neurotoxicity/Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) from CAR-T Therapy) if it occurs within the DLT reporting period (i.e., evaluation period following the initial cellular product administration and continuing until 4 weeks after the final cellular product administration) as specified below:

- Any treatment-emergent Grade ≥ 3 CRS event that does not decrease to Grade ≤ 2 within 7 days;
- Any treatment-emergent NCI-CTCAE Grade 5 event;
- Any treatment-emergent autoimmune toxicity Grade ≥ 3;
- NCI-CTCAE Grade ≥ 3 allergic reactions related to the cellular product;

- NCI-CTCAE Grades ≥ 3 organ toxicity (cardiac, dermatologic, gastrointestinal, hepatic, pulmonary, renal/genitourinary, or neurologic) not pre-existing or due to the underlying malignancy and occurring within 4 weeks of the final cell infusion;

- Any Grade ≥4 febrile neutropenia or bleeding.

DLTs are defined as at least possibly related to CAR.B7-H3 T cell product. If an apparent DLT is clearly due to underlying epithelial ovarian cancer and is unrelated to the cell infusion, then the investigator will specify that the event is not a DLT.

4.1.8 *Definition of Recommended Phase 2 Dose (RP2D)*

The maximum tolerated cell dose is the higher dose among the two studied with observed DLT rate less than 33%. Recommended phase 2 tolerable cell dose (RP2D) will be decided based on the maximum tolerated cell dose, DLTs observed in the dose-finding portion of the study and additional factors including the ability to manufacture the appropriate quantity of cells and the number of subjects who are removed early from treatment and do not proceed with receiving all 3 infusions.

4.1.9 *Potential Toxicities of the Cellular Product Administration*

Potential toxicities may be categorized as those related to infusion of T cells; cross-reactivity with normal tissues; cytokine release syndrome (CRS), Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS), and macrophage activation syndrome (also refer to Section 8.1.8).

4.1.10 *Clinical Monitoring*

Monitoring will be undertaken according to institutional standards for administration of blood products. Subjects will be monitored for at least 4 hours after each cellular product administration.

Fever will mandate initial hospitalization for subjects discharged after this observation period. Any evidence of ICANS will also mandate hospitalization for all subjects. All phase 1 subjects must reside in local housing (as designated by the Cellular Therapeutics Program Local Housing SOP). Subjects with medical indications for inpatient care or those whose physicians assess that outpatient care will pose an undue risk will remain inpatient for their treatment until indications for inpatient care have resolved. Subjects will be monitored as outlined in the Time & Events Table.

4.1.11 *Dose Delays*

For subjects who receive lymphodepleting chemotherapy, the initial administration of CAR.B7-H3 T cell product may be delayed per Principal Investigator's discretion if a subject has unresolved serious adverse reactions (including pulmonary reactions,

cardiac reactions, or hypotension) from preceding chemotherapies, active uncontrolled infection, or worsening tumor burden following lymphodepleting chemotherapy. This may extend the time between lymphodepletion in those subjects and cellular product administration beyond the 14 days outlined within the protocol.

The44econdd and/or third infusion will be delayed for any treatment emergent non-hematological >grade 2 adverse events determined to be related to the CAR.B7-H3 T cell product. The CAR.B7-H3 T cell administrations may resume when the treatment emergent Aes resolve to baseline. If a subject has treatment delayed >4 weeks, then the subject will be permanently discontinued from treatment.

4.1.12 *Supportive Care*

Subjects will receive supportive care for acute or chronic toxicity, including blood components or antibiotics, and other intervention as appropriate.

4.1.12.1 *Cytokine Release Syndrome*

Please refer to APPENDIX III – CRS Grading Criteria and Management Guidelines for the CRS treatment algorithm.

4.1.12.2 *Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS)*

Please refer to APPENDIX IV – Management of Neurotoxicity/Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) from CAR-T Therapy for the ICANS treatment algorithm.

4.1.12.3 *Macrophage Activation Syndrome*

Please refer to APPENDIX V – Treatment Algorithm for Macrophage Activation Syndrome for the treatment algorithm for macrophage activation syndrome.

4.1.13 *Concomitant Medications/Treatments*

Ideally, subjects should not receive other antineoplastic agents for at least 4 weeks after their final CAR-T cell infusion (for purposes of efficacy evaluation); however, subjects with progressive disease may receive other therapy if needed at the discretion of their attending physician. If subjects receive other therapy for progressive disease, they will come off study

4.2 Time & Events Table

Study Assessments	Screening[1]										Study Treatment Follow-Up[2]							Follow-Up[2, 3]
	Pre-procure-ment	CELL PROCUREMENT || BRIDGING THERAPY[17]	Pre-lymph	LYMPHODEPLETION[13]	W0 D0	W0 D3	W1 D0	W1 D3	W2 D0	W2 D3	W3 D0	W4 D0	W6 D0	M3	M6	M9	M12	
Medical history	×		×		×		×		×		×	×	×	×	×	×	×	×
Physical exam	×		×		×		×		×		×	×	×	×	×	×	×	×
Performance status	×		×		×		×		×		×			×	×	×	×	
Eligibility	×		×		×													
Echocardiography[4]	×																	
Pregnancy test[5]	×		×		×[5]													
Hematology[6]	×		×[6]		×[6]		×		×			×	×	×	×	×	×	
Virus testing[7]	×																	
IP catheter placement			×															
Serum chemistries[6]	×		×[6]		×[6]		×		×			×	×	×	×	×	×	
Serum cortisol			×										×	×	×	×	×	
CA 125	×		×		×								×	×	×	×	×	×[3]
Tumor imaging[8]	×[8]		×[8]										×	×	×	×	×	×[8]
Correlative Blood Sample[9]			×[9]		×[9]	X	×	X	×	X	×	×	×					
HAMA testing[10]			×										×					
Toxicity assessment[11]	×		×		×		×		×		×	×	×					
Certificate of analysis[12]			×															
Infusion of CAR.B7-H3[14]					×		×		×									
Tissue biopsy[15]			×						×			At the Time of Progression						
Ascites collection[16]			×		×	X	×	X	×	x		At the Time of Progression						

Footnotes to Time &Events Table

1. Screening includes tests to confirm eligibility for procurement, lymphodepletion and cell treatment. The presence of two doses of tocilizumab onsite must be confirmed prior to each cell infusion. Some pre-infusion screening tests may not be performed until the day of infusion. A window of 7 days will apply to all procurement labs unless specified below.

2. A window of ±3 days will apply to all study visits for the first 4 weeks unless otherwise noted below. A window of ±1 day will apply to the Week 0 Day 3 visit. A window of +7 days will apply to the 6 week visit. A window of ±10 days will apply to the every 3 months study visits, and a window of ±30 days will apply to visits separated by ≥ 6 months. Yearly follow-up visits during long-term follow-up until disease progression or for a total of 5 years. Follow-up visits on month 3 and beyond can be conducted locally (i.e., local health care provider, local treating oncologist, etc.). The study team would continue to contact the subjects at the long-term follow-up time points and request they visit their local healthcare provider to complete the follow-up test and assessments, where applicable. The study team would collect clinical information, tests and assessments results, other clinical observations from the subject's medical record and their local health care provider.

3. Performed every 3 months (±14 day window) starting at month 3 until disease progression or for up to 2 years post initial infusion and then will be performed every 6 months (±30 day window) thereafter for up to 5 years post initial infusion. Follow-up visits on month 3 and beyond can be conducted locally (i.e., local health care provider, local treating oncologist, etc.).

4. Echocardiography to measure left ventricular ejection fraction (LVEF). A window of 90 days prior to procurement will be allowed for the ECHO. If a subject undergoes cardiac toxic therapy after echocardiography, it must be repeated prior to procurement.

5. Serum pregnancy testing will be done in female subjects of childbearing potential within 72 hours prior to procurement. It should be repeated within 72 hours prior to lymphodepletion. Serum pregnancy test will be done within 7 days of initial cell infusion (does not need to be repeated if pre-lymphodepletion pregnancy test is within this window).

6. Hematology and serum chemistries (CMP) must be collected within 72 hours of lymphodepletion and 24 hours of the initial cellular product administration.

7. HIV, HBV, HCV testing required for confirmation that no active infection exists at the time of procurement. Results of this testing can be pending at the time of cell procurement; only those samples with a confirmed absence of active infection will be used to generate the cellular product. A window of 30 days will apply to virus testing.

8. Tumor imaging for disease assessment will be performed at baseline (within 90 days before procurement) and within 10 days prior to lymphodepletion. Imaging prior to lymphodepletion must occur at least 3 weeks after the subject's most recent therapy to document measurable or assessable disease. Imaging will then occur at 6 weeks ±7-days following the initial infusion of CAR.B7-H3. Imaging will be performed every 3 months (±14 day window) at treating investigator's discretion starting at month 3 until disease progression for up to 2 years post initial infusion and then will be performed every 6 months (±30 day window) thereafter for up to 5 years post initial infusion. The choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., PET, CT scans, MRI, nuclear imaging). If imaging studies are performed at other times after treatment on this study, the data will be collected, and information gained will be used for this study. The tests performed at baseline for assessment of disease should be performed consistently throughout the study. Follow-up

visits on month 3 and beyond can be conducted locally (i.e., local health care provider, local treating oncologist, etc.).

9. The analyses will be used to monitor function and persistence in peripheral blood and will include functional assays such as *in vitro* reactivation of PBMCs in subjects for whom the appropriate reagents are available and for immunophenotyping. Cytokines will also be measured. Blood samples will be collected pre-lymphodepletion and also collected on the day of initial cell infusion at pre-dose (approximately 19 mL of blood), 1 hour (±15 minutes) post infusion, between 3 – 4 hours post infusion and day 3 of each weekly infusion (approximately 16 mL of blood) to monitor function, persistence and cytokine levels. See Section 7.3 and the Laboratory Manual for the amount of blood collected/type of tube used for all of the correlative studies.

10. Serum from blood drawn for functional studies prior to lymphodepletion and week 6 will be stored for measurement of human anti-mouse antibodies (HAMA). These studies will be performed in the event of a suspected immunologic reaction. See Section 7.2.6 for additional details.

11. Data on all adverse experiences/toxicities regardless of seriousness must be collected for documentation purposes only for 4 weeks after the final cellular product administration per NCI-CTCAE criteria, version 5.0, CRS Grading Criteria (APPENDIX III – CRS Grading Criteria and Management Guidelines) and ICANS Grading Criteria (APPENDIX IV – Management of Neurotoxicity/Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) from CAR-T Therapy) from CAR-T Therapy). Adverse event data collection will cease in subjects that receive therapy for relapse of their primary malignancy. Data on disease status will continue to be collected as appropriate.

a. During dose finding the DLT safety assessment period = 4 weeks after the final (third) cellular product administration

b. If CRS signs and symptoms occur, a serum sample (~1 mL) for IL-6 analysis and approximately 20 mL of blood should be collected before instituting treatment for CRS. See Section 7.3.2 for additional details. Sample collection can be repeated daily until signs and symptoms of CRS resolve.

c. Serum (~1 mL) and approximately 20 mL of blood should also be collected for serious toxicities related to cell infusion that develop that require hospitalization. See Section 7.3.2 for additional details.

d. A blood sample (~20 mL) may be collected if any subject develops an AE or event of clinical significance that is thought to be at least possibly related to the CAR T cells.

12. Certificate of Analysis generated at completion of required studies for production/QA of cells. The CofA must be available prior to lymphodepletion.

13. Subjects will receive lymphodepleting chemotherapy consisting of cyclophosphamide of 300 mg/m^2/day administered IV and fludarabine 30 mg/m^2/day administered IV over 3 consecutive days.

14. CAR.B7-H3 T cells will be administered 2 – 14 days after lymphodepletion. Additional dose delays beyond 14 days may be allowed as detailed in Section 4.1.11. Research personnel will keep track of any subjects who undergo procurement but do not undergo treatment with cellular product, and the reason for withholding treatment.

15. CT-guided, ultrasound-guided or operative research biopsy at the time of intraperitoneal catheter placement. CT-guided or ultrasound-guided research biopsy at the time of intraperitoneal catheter removal. A biopsy at the time of disease progression may be performed at the investigator's discretion

for subjects who have initial response at their week 6 imaging. Subjects with progression at their week 6 tumor imaging will not require a biopsy.

16. Collection of ascites fluid at the time of intraperitoneal catheter placement, at the time of each cellular product administration and at day 3 of each weekly infusion (if ascites fluid is present).

17. Peripheral blood, up to 300 mL (in up to 3 collections) will be obtained from subjects for cell procurement. In subjects with inadequate lymphocyte count in the peripheral blood, a leukapheresis may be performed to isolate sufficient T cells. The parameters for apheresis will be up to 2 blood volumes.

5 STUDY POPULATION

During the period after cell procurement and during CAR-T cell production, subjects are allowed to receive additional standard of care therapy e.g., chemotherapy or radiation therapy to stabilize their disease if the treating physician feels it is in the subject's best interests.

5.1 Inclusion Criteria Prior to Cell Procurement

5.1.1 Written informed consent and HIPAA authorization for release of personal health information. Subjects or their Legally Authorized Representative must sign a consent to undergo cell procurement. Subject will be given a copy of the informed consent form.

5.1.2 Age ≥ 18 years at the time of consent.

5.1.3 Subject has adequate performance status as defined by ECOG score of ≤ 2 (see APPENDIX VI – ECOG Performance Status [75]).

5.1.4 Subjects must have histologically or cytologically confirmed epithelial ovarian, peritoneal or fallopian tube cancer and must have a histological diagnosis of a high-grade serous histology based on local histopathological findings.

5.1.5 Subjects must have recurrent platinum-resistant or platinum-refractory disease defined as:

5.1.5.1 Disease that has progressed by imaging while receiving platinum OR

5.1.5.2 Disease that has recurred within 6 months of the last receipt of platinum-based chemotherapy. Rising CA-125 only is not considered as platinum-resistant or refractory disease.

5.1.5.3 Having received at least 2 prior regimens.

5.1.5.4 Have failed prior therapy with a PARP inhibitor if the subject has a germline or somatic BRCA mutation.

5.1.6 Subjects must have evaluable disease – defined as:

5.1.6.1 Measurable disease per RECIST 1.1 (see APPENDIX II – Tumor Measurement Based on RECIST 1.1) OR

5.1.6.2 Non-measurable disease (defined as solid and/or cystic abnormalities on radiographic imaging that do not meet RECIST 1.1 definitions for target lesions) OR

5.1.6.3 Ascites and/or pleural effusion that has been pathologically demonstrated to be disease-related in the setting of a CA-125 > 2 × ULN.

5.1.7 Subjects must be able to have an intraperitoneal port placed either by vascular interventional radiology or surgically in the operating room. (Note: The intraperitoneal port will not be placed until the subject is determined to be otherwise eligible to receive the CAR.B7-H3 infusion and until the subject is determined to be otherwise eligible to receive lymphodepletion).

5.1.8 Female subjects of childbearing potential must be willing to abstain from heterosexual activity or to use 2 forms of highly effective methods of contraception from the time of informed consent until 180 days after study treatment discontinuation. The two contraception methods can be comprised of two barrier methods, or a barrier method plus a hormonal method or an intrauterine device that meets < 1% failure rate for protection from pregnancy in the product label.

5.1.9 Subject is willing and able to comply with study procedures based on the judgement of the investigator.

5.1.10 Subject is willing to undergo a biopsy prior to treatment, at the time of intraperitoneal catheter removal, and at the time of disease progression (if not at their first scan), and the tumor site is determined to be safe by the treating investigator for biopsy collection.

5.1.11 Subjects must not be pregnant or lactating (Note: Breast milk cannot be stored for future use while the mother is being treated on study).

5.1.12 Subjects must not be deemed unlikely to be a candidate for successful intraperitoneal catheter placement by radiographic assessment.

5.1.13 Subject must not have intraparenchymal lung metastases (note that pleural effusions are not exclusionary and that subjects with intraparenchymal liver disease and subjects with retroperitoneal disease are allowed on the study).

5.1.14 Subject must not have brain metastases. A subject with prior brain metastasis may be considered if they have completed their treatment for brain metastasis at least 4 weeks prior to being screened for eligibility, have been off of corticosteroids for ≥ 2 weeks, and are asymptomatic.

5.1.15 Subject must not have current signs and/or symptoms of bowel obstruction or signs and/or symptoms of a bowel obstruction within 3 months prior to starting treatment.

5.1.16 Subject must not have a history of intra-abdominal abscess within the past 3 months.

5.1.17 Subject must not have a history of gastrointestinal perforation.

5.1.18 Subject must not have a history of symptomatic diverticular disease, confirmed by CT or colonoscopy.

5.1.19 Subjects must not be dependent on intravenous hydration or total parenteral nutrition.

5.1.20 Subject must not have a known additional malignancy that is active and/or progressive requiring treatment; exceptions include basal cell or squamous cell skin cancer, in situ cervical or bladder cancer, or other cancer for which the subject has been disease-free for at least five years.

5.1.21 Subject must not be currently using systemic corticosteroids at doses ≥10 mg prednisone daily or its equivalent; those receiving <10 mg daily may be enrolled at discretion of investigator. Inhaled steroids are allowed. Physiologic replacement hydrocortisone at doses 6-12 mg/m²/day is allowed. Equivalently dosed alternative steroids are allowed at discretion of investigator, though not to exceed 10 mg prednisone per day.

5.1.22 Subject must not have an active infection with HIV, HBV, HCV (tests can be pending at the time of cell procurement; only those samples confirming lack of active infection will be used to generate transduced cells). Note: To meet eligibility subjects are required to be negative for HIV antibody or HIV viral load, negative for Hepatitis B surface antigen, negative for HCV antibody or HCV viral load.

5.1.23 Subjects must not have a history of intolerance to fludarabine. .

5.1.24 Subject has life expectancy ≥ 3 months.

5.1.25 Subject has evidence of adequate organ function as defined by:

5.1.25.1 Total bilirubin ≤ 1.5 × ULN, unless attributed to Gilbert's Syndrome

5.1.25.2 AST / ALT ≤ 3 × ULN (Note: if intrahepatic liver metastases are present, AST and ALT must be ≤ 5 × ULN)

5.1.25.3 Creatinine ≤ 2 × ULN

5.1.25.4 Left ventricular ejection fraction (LVEF) ≥ 40%, as measured by ECHO, with no additional evidence of decompensated heart failure.

5.1.25.5 Pulse oximetry ≥ 90% on room air

5.1.26 Subjects must have active disease by imaging assessment within 90 days prior to procurement.

5.1.27 Female subjects of childbearing potential must have a negative serum pregnancy test within 72 hours prior to cell procurement. Note: Females are considered of childbearing potential unless they are surgically sterile (have undergone a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or they are naturally postmenopausal for at least 12 consecutive months. Documentation of postmenopausal status must be provided.

5.2 Eligibility Criteria Prior to Lymphodepletion

5.2.1 Written informed consent explained to, understood by and signed by the subject prior to lymphodepletion; subject given a copy of informed consent form.

5.2.2 Subject has an intraperitoneal catheter/port in place. (Note: The intraperitoneal port will not be placed until the subject is determined to be otherwise eligible to receive lymphodepletion prior to the CAR.B7-H3 infusion).

5.2.3 Subject had no major surgery within 28 days prior to lymphodepletion.

5.2.4 Subject has not received any investigational agents or any tumor vaccines within 21 days prior to lymphodepletion.

5.2.5 Subjects must have stopped systemic chemotherapy or radiation therapy for at least 21 days prior to lymphodepletion.

5.2.6 Subject must have stopped bevacizumab for at least 6 weeks prior to lymphodepletion.

5.2.7 Subject must have stopped hormonal therapy (tamoxifen, letrozole, etc.) for at least 21 days prior to lymphodepletion.

5.2.8 Subject is not receiving a prohibited medication at the time of starting lymphodepletion up through 72 hours after the last dose of cyclophosphamide listed in Section 8.2.1.7.

5.2.9 No current use of systemic corticosteroids at doses ≥10 mg prednisone daily or its equivalent; those receiving <10 mg daily may be enrolled at discretion of investigator. Inhaled steroids are allowed. Physiologic replacement hydrocortisone at doses 6-12 mg/m²/day is allowed. Equivalently dosed alternative steroids are allowed at discretion of investigator, though not to exceed 10 mg prednisone per day.

5.2.10 Imaging results from within 10 days prior to lymphodepletion. Imaging must occur at least 3 weeks after most recent therapy (used as baseline measure for documentation of progression before the lymphodepletion) to document measurable or assessable disease. Imaging does not need to be repeated if it is within 10 days prior to lymphodepletion. Subjects must have evaluable disease defined as:

5.2.10.1 Measurable disease per RECIST 1.1 (see APPENDIX II – Tumor Measurement Based on RECIST 1.1) OR

5.2.10.2 Non-measurable disease (defined as solid and/or cystic abnormalities on radiographic imaging that do not meet RECIST 1.1 definitions for target lesions) OR

5.2.10.3 Ascites and/or pleural effusion that has been pathologically demonstrated to be disease-related in the setting of a CA-125 > 2 × ULN.

5.2.11 Subject must demonstrate adequate organ function prior to lymphodepletion as defined below. All tests must be obtained within 48 hours prior to lymphodepletion:

5.2.11.1 Hemoglobin ≥ 8 g/dL

5.2.11.2 Absolute neutrophil count ≥ 1.0×10^9/L

5.2.11.3 Platelet count ≥ 100×10^9/L

5.2.11.4 Total bilirubin ≤ 1.5 × ULN, unless attributed to Gilbert's Syndrome

5.2.11.5 AST / ALT ≤ 3 × ULN (Note: if intrahepatic liver metastases are present, AST and ALT must be ≤ 5 × ULN)

5.2.11.6 Creatinine ≤ 2 × ULN

5.2.12 Subject does not have intraparenchymal lung metastases (Note: Pleural effusions are not exclusionary and that subjects with intraparenchymal liver disease and subjects with retroperitoneal disease are allowed on the study.)

5.2.13 Subject does not have a known brain metastasis. A subject with prior brain metastasis may be considered if they have completed their treatment for brain metastasis at least 4 weeks prior to lymphodepletion, have been off corticosteroids for ≥ 2 weeks, and are asymptomatic.

5.2.14 ***Subject must have available autologous transduced activated T cells product that meets the Certificate of Analysis acceptance.***

5.2.15 Subject does not have current signs and/or symptoms of bowel obstruction or signs and/or symptoms of a bowel obstruction within the last 3 months prior to lymphodepletion.

5.2.16 Subject does not have a history of intra-abdominal abscess within 3 months of lymphodepletion.

5.2.17 Subject does not have a history of gastrointestinal perforation.

5.2.18 Subject does not have a history of symptomatic diverticular disease, confirmed by CT or colonoscopy.

5.2.19 Subject is not dependent on intravenous hydration or total parenteral nutrition.

5.2.20 Negative serum pregnancy test within 72 hours prior to lymphodepleting therapy for female participants of childbearing potential. Note: Females are considered of childbearing potential unless they are surgically sterile (have undergone a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or they are naturally postmenopausal for at least 12 consecutive months.

5.2.21 Subject is not lactating (Note: Breast milk cannot be stored for future use while the mother is being treated on study).

5.2.22 Subject does not have rapidly progressive disease, per treating oncologist's discretion.

5.2.23 Subject is a good candidate for CAR.B7-H3 T cell therapy, per treating oncologist's discretion.

5.3 Eligibility Criteria Prior to Cellular Product Administration After Lymphodepletion

5.3.1 Subject has no evidence of uncontrolled infection or sepsis.

5.3.2 Negative serum pregnancy within 7 days of the initial cellular product administration. If the pre-lymphodepletion pregnancy test is within the 7 day window, then the pregnancy test does not need to be repeated.

5.3.3 Evidence of adequate organ function as defined by:

5.3.3.1 Total bilirubin $\leq 2 \times$ ULN, unless attributed to Gilbert's syndrome

5.3.3.2 AST / ALT $\leq 5 \times$ ULN, unless attributed to intrahepatic liver metastases

5.3.3.3 Creatinine $\leq 3 \times$ ULN

5.3.4 Subject has no clinical indication of rapidly progressing disease in the opinion of the clinical investigator.

5.3.5 Subject is a good candidate for treatment with CAR.B7-H3 cell product per the clinical investigator's discretion.

5.4 Single Subject Exceptions

Eligibility single subject exceptions are not permitted for Lineberger Comprehensive Cancer Center Investigator Initiated Trials under any circumstances. Other types of single subject exceptions may be allowed if proper regulatory review has been completed in accordance with Lineberger Comprehensive Cancer Center's Single Subject Exceptions Policy.

6 STUDY CONDUCT

6.1 Duration of Therapy

Therapy in the LCCC1818-ATL study involves three sequential intraperitoneal administrations of CAR.B7-H3 T cell product. Study treatment will be administered unless the subject meets criteria delineated in Section 6.3 including:

- Subject decides to withdraw from study treatment,
- The second and/or third infusion is delayed due to treatment emergent non-hematological >grade 2 adverse events determined to be related to the CAR.B7-H3 T cell product that do not resolve to baseline for >4 weeks or
- General or specific changes in the subject's condition render the subject unacceptable for further treatment in the judgment of the investigator.

6.2 Duration of Follow-Up

Subjects will be followed for up to 5 years or until death, whichever occurs first. Subjects removed from the study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Subjects who experience unequivocal disease progression after a cellular product administration will come off study.

6.3 Removal of Subjects from Protocol Therapy

Subjects will be removed from protocol therapy and the Principal Investigator notified when any of the criteria listed below apply.

- Subject experiences a DLT
- Progressive disease is noted during disease re-evaluation
- Subject develops Grade 4 non-hematologic toxicity after cell treatment
- Subject experiences Grade 3 – 4 allergic reaction to investigative agents
- The second and/or third infusion is delayed due to treatment emergent non-hematological >grade 2 adverse events determined to be related to the CAR.B7-H3 T cell product that do not resolve to baseline for >4 weeks
- Positive pregnancy test
- Subject decides to withdraw from therapy
- Physician decision

The reason for discontinuation of protocol therapy will be documented on the eCRF. In the case where a subject decides to prematurely discontinue protocol therapy ("refuses treatment"), the subject should be asked if she or he may still be contacted for further scheduled study assessments. The outcome of that discussion should be documented in both the medical records and in the eCRF.

6.4 Off-Study Criteria

Subjects will be removed from the study if they meet any of the below criteria:

- After completion of 5 years of follow-up OR the subject has been determined to have progressive disease (whichever is earliest)
- Death
- Lost to follow up
- Withdrawal of consent for any further data submission

6.5 Subject Withdrawal

If a subject decides to withdraw from the study (and not just from protocol therapy) an effort should be made to complete and report study assessments as thoroughly as possible. At the time of withdrawal, the investigator should attempt to establish as completely as possible the reason for the study withdrawal.

- The subject should be asked if they are willing to allow for the abstraction of relevant information from their medical record in order to meet the long term follow up (e.g., survival) objectives outlined in the protocol.
- A complete final evaluation at the time of the subject's study withdrawal should be obtained with an explanation of why the subject is withdrawing from the study.
- If the subject is noncompliant and does not return for an end of study follow up assessment, this should be documented in the eCRF.
- If the reason for removal of a subject from the study is an adverse event, the principal specific event will be recorded on the eCRF.

Excessive subject withdrawals from protocol therapy or from the study can render the study un-interpretable; therefore, unnecessary withdrawal of subjects should be avoided.

6.6 Subjects Lost to Follow-Up

Subject will be considered lost to follow-up if he or she fails to return for three scheduled visits and is unable to be contacted by the study site staff.

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

- The site will attempt to contact the subject and reschedule the missed visit and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.

- Before a subject is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the subject (where possible, three telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record or study file.

- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

7 DESCRIPTION OF STUDY PROCEDURES

7.1 Clinical Assessments

Clinical assessments will be performed as outlined in the Time & Events Table.

Procurement happens after pre-procurement screening and prior to lymphodepletion and initial cellular product administration. Any therapy received after procurement and prior to cellular product administration must be documented. Additionally, research personnel must keep track of any subjects who undergo procurement but do not undergo treatment with cellular products, and the reason for withholding treatment.

7.1.1 *Demographics*

Demographic information (date of birth, gender, race, ethnicity) will be recorded at the pre-procurement screening visit.

7.1.2 *Medical History*

Relevant medical history, including history of current disease, other pertinent history, and information regarding underlying diseases will be recorded at procurement, pre-lymphodepletion (if applicable) and pre-infusion screening visits and a focused medical history on symptoms/toxicity will be performed thereafter. BRCA mutation status should be captured, if known. In the event that a subject experiences an SAE or AE on study that leads to hospitalization, the number of days that the subjects spends in the hospital should be captured.

7.1.3 *Physical Examination*

A complete physical examination including height (at initial screening only), weight, vital signs and performance status.

Baseline information collected prior to procurement or cellular product administration will be compared to changes that occur during study treatment. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

Vital signs (temperature, heart rate, respiratory rate, pulse oximetry, and blood pressure) will be performed by either the investigator or other qualified staff (MD, NP, RN, or PA).

7.1.4 *Performance Status*

Performance status will be assessed by the ECOG scale provided in APPENDIX IV – Management of Neurotoxicity/Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) from CAR-T Therapy.

7.1.5 *Assessment of Safety*

All subjects consented for the study will be included in the safety analysis. The safety analysis will be divided into the following categories: events occurring between cell procurement and lymphodepletion, events occurring between lymphodepletion and CAR.B7-H3 product administration, and events occurring after CAR.B7-H3 cell product administration. Each subject will be assessed periodically for the development of any toxicity according to the Time & Events Table. Toxicity will be assessed according to the NCI CTCAE, version 5.0, CRS Grading Criteria provided in APPENDIX III – CRS Grading Criteria and Management Guidelines and Neurotoxicity/ICANS Grading Criteria provided in APPENDIX IV – Management of Neurotoxicity/Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) from CAR-T Therapy.

7.1.6 *Assessment of Efficacy*

All subjects who receive three intraperitoneal infusions of CAR.B7-H3 T cell product will be included in the efficacy analysis. To assess disease response, imaging will be performed within 10 days of lymphodepletion and at 6 weeks (±7-day window) following the initial intraperitoneal administration of CAR.B7-H3 T cell product. If imaging is consistent with progressive disease, the subject will come off of study. Imaging will be performed every 3 months (±14 day window) starting at month 3 until disease progression for up 2 years post initial infusion and then will be performed every 6 months (±30 day window) thereafter for up to 5 years post initial infusion. The Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1 criteria will be used for measurement of tumor response (see APPENDIX II – Tumor Measurement Based on RECIST 1.1). If imaging studies are performed at other times after treatment on this study, the data will be collected, and information gained will be used for this study. The choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., CT scans, MRI, PET scans). The tests performed at baseline for assessment of disease should be performed consistently throughout the study. Additionally, CA 125 tumor marker level will be measured according to the Time & Events Table.

7.2 Laboratory Assessments

7.2.1 *Hematology*

Blood will be obtained during the study as outlined in the Time & Events Table and sent to the clinical site hematology laboratory for assessment of complete blood count with white blood cell differential.

7.2.2 *Serum Chemistries*

Blood will be obtained during the study as outlined in the Time & Events Table and sent to the clinical site chemistry laboratory for determination of a comprehensive

metabolic panel (sodium, potassium, chloride, CO2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase).

7.2.3 *Cancer Antigen-125 Level*

CA-125 will be assessed as outlined in the Time & Events Table.

7.2.4 *Serum Cortisol Level*

Serum cortisol will be assessed as outlined in the Time & Events Table.

7.2.5 *Virus Testing*

HIV, HBV, HCV testing required for confirmation that no active infection exists; results can be pending at the time of cell procurement; only those samples confirming lack of active infection will be used to generate transduced cells.

7.2.6 *HAMA Testing*

Serum from blood drawn for functional studies at baseline and at week 6 will be stored for measurement of human anti-mouse antibodies (HAMA). These studies will be performed in the event of a suspected immunologic reaction.

7.2.7 *Pregnancy Testing*

A serum pregnancy test will be obtained from female subjects who are of childbearing potential as outlined in the Time & Events Table.

- Serum pregnancy test done within 72 hours prior to procurement
- Serum pregnancy test done within 72 hours prior to lymphodepletion
- Serum pregnancy test done within 7 days prior to cellular product administration (does not need to be repeated if the pre-lymphodepletion pregnancy is within window)

7.3 Correlative Studies

The following investigations will be used to monitor function and persistence in peripheral blood and safety of transduced T cells at time points indicated in the Time and Events Tables. Please consult the study Laboratory Manual for details on correlative blood sample collection. If a subject's hemoglobin is less than 8.0 g/dL at any of the evaluation times, the amount of blood drawn for the evaluation will be reduced and may be obtained over more than one venipuncture, if necessary.

Cytokines: Our standard array for cytokine determination includes multiple cytokines (UNC Immune Monitoring Core) which among others include: IL-2, IFNγ, TNFα, IL-6, IL-10, IL-13, IL-1Rα, IL-8, IL-12, IL-15, and GM-CSF.

7.3.1 *Function and Persistence Studies*

Immunophenotyping for the presence of the CAR construct will also be conducted when applicable. Additionally, we will use surface immunophenotyping and/or intracellular cytokine by flow cytometry to evaluate both CAR-expressing and other T cells post-infusion. Blood sampling for characterizing the pharmacokinetics of the CAR.B7-H3 T cell product will follow the correlative blood sample collections defined in the Time & Events Table. As this is an RNA based product and CAR T cells will be infused intraperitoneally, we anticipate the maximum detection of CAR.B7-H3 T cells in the peripheral blood to occur within the first 4 hours upon administration if CAR-T cells enter the bloodstream from the peritoneal space. Beatty et. al. showed that maximal levels of transgene introduced in T-cells by mRNA were detected within 2 hours of infusion when mRNA-based were infused directly intravenously [60]. Due to the biodegradable nature of the transgene the levels then progressively decreased on successive days. Quantitative RT-polymerase chain reaction (RT-PCR) will be used to quantify the persistence of the CAR.B7-H3 T cells. mRNA will be extracted from total PBMC and reverse transcribed. CAR expression will then be quantified by real time PCR using primers and probes spanning a specific sequence of the CAR transgene. Function and persistence studies may also include functional assays such as *in vitro* reactivation of PBMCs in subjects for whom the appropriate reagents are available.

7.3.2 *Sample Collection Requirement if CRS or Severe Toxicity Occurs*

A serum sample (~1 mL) for IL-6 analysis and approximately 20 mL of blood should be collected in any subject who develops signs and symptoms of CRS regardless of whether their symptoms meet severity requirements for DLT. This sample should be collected before treatments are administered to alleviate CRS symptoms. Subsequent samples may be collected at the discretion of the investigator (e.g., a single sample collection may be obtained each day until the event resolves) and will depend on the condition of the subject.

In addition, a serum sample (~1 mL) for IL-6 analysis and approximately 20 mL of blood should be collected in any subject who develops a SAE (i.e., requiring hospitalization) related to the cell infusion. Subsequent samples may be collected at the discretion of the investigator.

An additional blood sample of ~20 mL may be collected if any subject develops an AE or event of clinical significance that is thought to be at least possibly related to the CAR T cells.

7.3.3 *Fresh Tissue Studies*

Subjects with measurable disease per RECIST 1.1 criteria will have their tissue specimens collected prior to or at the time of intraperitoneal catheter installation via CT-guided, ultrasound-guided or operative biopsy; at the time of intraperitoneal catheter removal via CT-guided or ultrasound-guided biopsy. Subjects who have initial response at their week 6 imaging may have a biopsy performed at the time of progression (if not at their first scan). This will be at the investigator's discretion. Biopsy material will be used to analyze molecular signature of the tumor before and after treatment and at the time of progression, to study immunogenomics. This will be performed using either single cell or bulk RNA-sequencing, NanoString analysis or quantitative PCR. Tumor mutation analysis will be performed by whole genome or whole exome analysis.

B7H3 expression will be measured by performing staining on fixed samples using methodologies that have been previously published for glioblastoma in Neham *et. al.* [71].

7.3.4 *Ascites Fluid Analysis*

If present, ascites fluid will be collected by large volume paracentesis at the time of intraperitoneal catheter installation; at the time of each cellular product administration; and at day 3 after each weekly infusion; and at the time of progression. Ascites fluid will be used to isolate cells by Ficoll-Paque processing, which will then be sub-divided and 1 fraction will be cryopreserved and the other will be pelleted to be used fresh. Cells will be used to analyze molecular signature of the tumor before and after treatment, to study immunogenomics. This will be performed using either single cell or bulk RNA-sequencing, NanoString analysis or quantitative PCR. Tumor mutation analysis will be performed by whole genome or whole exome analysis.

7.4 Biobank Repository

Subjects participating in this trial may consent to allow researchers to store their biological specimens. Participants in this trial will also be required to sign a separate HIPAA authorization form to allow investigators to review their medical records.

CAR.B7-H3 T cells will be stored at the GMP facility according to standard procedures. Other specimens collected during this study as described in Sections 7.3.3 and 7.3.4 of this protocol will be stored at the University of North Carolina at Chapel Hill in the immunotherapy laboratory in locked liquid nitrogen tanks with controlled access. The samples will be labeled with the study ID number and date and time of sample collection.

The purpose of this repository or biobank is to store samples for immediate or future analyses to answer study-related questions, not to increase general knowledge outside the parameters of the study. These goals will be accomplished using several different kinds of specimens collected during the study as described in section 7.3.

These specimens include:

- Tissue samples from biopsies or ascites
- Unused CAR.B7-H3 T cells
- Unused additional material collected during procurement (peripheral blood mononuclear cells)
- Leftover tissue obtained for any standard of care procedure that may provide additional information about CAR.B7-H3 T cell therapy

For example, analyses will be performed to assist in determining if the CAR.B7-H3 T cells are associated with the tumor and if the CAR.B7-H3 T cells are present.

Information about the subject's disease will be linked to the specimens stored in the repository database. Immunotherapy laboratory-associated research staff, LCCC Bioinformatics staff who support the database and the LCCC Data Warehouse, and researchers with IRB-approval for access to personal health information for each subject in this study will be able to link specimens to relevant medical information. Some results from laboratory analyses that occurred during the subject's participation in the clinical study may also be included. This information may be important for understanding how the subject's cancer developed and responded to treatment.

Storage Time:

- CAR.B7-H3 T cells and material from procurement are to be stored for up to 15 years for subject treatment and safety purposes. If storage of the CAR-T cells and/or extra material from procurement is no longer deemed necessary as determined by the Principal Investigator with appropriate consult, as needed (e.g., attending physician, treating physicians, etc.), the CAR-T cells and/or the extra material from procurement may be released for archiving or destruction earlier than 15 years. At the time of release determination by the Principal Investigator, if the subject did not consent for their specimens to be used for further research or quality assurance/quality control (QA/QC) activities, the specimens will be destroyed at 5 years. The subject may withdraw their consent at any time to prevent the use of their specimens for further research or QA/QC activities. Withdrawing of consent will result in the destruction of the samples at 5 years or at the time consent is withdrawn if this occurs after the initial 5 years in storage.
- Archived samples will be stored in the GMP lab (the ACT facility) and will be designated in the inventory system for use in laboratory research and/or QA/QC activities. Samples will be de-identified when they are used for laboratory research or QA/QC activities. Total storage time (initial storage and archiving) may be up to 15 years, and then the samples will be destroyed.

- Tissue obtained from tumor biopsies or ascites, or any leftover tissue obtained through standard of care procedure that may provide additional information about CAR.B7-H3 T cell therapy will be stored for up to 15 years.

7.5 Protocol Deviations

A protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs;
- Has no substantive effect on the risks to research participants;
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected;
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance meets any of the following criteria:

- Has harmed or increased the risk of harm to one or more research participants;
- Has damaged the scientific integrity of the data collected for the study;
- Results from willful or knowing misconduct on the part of the investigator(s);
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

If a deviation or violation occurs, please follow the guidelines below:

Protocol Deviations: In the event a deviation from protocol procedures is identified, record the deviation in OnCore®.

Protocol Violations: Violations should be reported to the IRB of record by UNC personnel within one (1) week of the investigator becoming aware of the event.

Unanticipated Problems: Any events that meet the criteria for “Unanticipated Problems” must be reported by the study personnel to the IRB of record.

8 STUDY DRUG MANAGEMENT

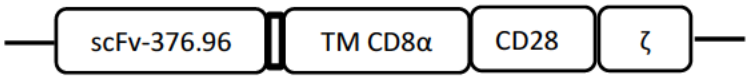
8.1 CAR.B7-H3 Cell Product

Activated T lymphocytes will be generated using UNC Lineberger Advanced Cellular Therapeutics Facility previously validated SOPs. Briefly, PBMC will be activated with anti-CD3 and anti-CD28 antibodies, and then expanded using IL-7 and IL-15. Anti-CD3 and anti-CD28 are now available in GMP grade and our validation studies show improved cell transduction when both anti-CD3 and anti-CD28 antibodies are combined.

8.1.1 *Construction of the mRNA*

The single chain antibody (scFv) targeting the B7-H3 molecule is cloned in frame with the hinge and transmembrane domain of CD8a, 4-1BB and ζ endodomains. A schematic representation of the constructs is shown in Figure 5.

Figure 5 Schematic Representation of the Construct



8.1.2 *Generation of Genetically Modified T-cells*

All manufacturing procedures will be performed in our GMP facility located at the address below:

UNC Lineberger Advanced Cellular Therapeutics Facility

6101 Quadrangle Drive, Suite 150

Chapel Hill, NC 27517

The cells will be manufactured as dictated by Standard Operating Protocols (SOPS).

8.1.3 *Source Material*

See Section 4.1.2 (procurement).

8.1.4 *Cell Activation and ex vivo Expansion*

PBMC are activated during *in vitro* culture with monoclonal antibodies (GMP grade) against CD3 and CD28. After initial culture period, the activated T cells will be further expanded in culture to reach sufficient numbers by feeding them with IL-7 and IL-15 for an estimated culture period of 7 – 12 days.

8.1.5 *RNA Electroporation of T cells*

Following 7 – 12 days of expansion, T cells are washed, resuspended and transferred to electroporation cuvettes, where they are electroporated with RNA coding for the CAR.B7-H3 using a closed system electroporation device. Immediately after electroporation, the cells are washed and returned to culture for recovery.

8.1.6 *Cryopreservation of T cells*

After recovery (typically overnight), cells will be harvested from culture and cryopreserved following previously validated SOPs. Cells will also be tested for expression of the gene construct and potency using an assay as discussed with FDA. All lines will be analyzed for phenotype and sterility. The results will be reviewed by Quality Assurance (QA) prior to issuing a Certificate of Analysis.

8.1.7 *Testing*

Products that meet study specific release criteria, as detailed on the Certificate of Analysis that accompany each infusion product, will be infused as per section 4.1.6. Complete sterility test results are currently required for product release prior to infusion. However, if a positive sterility testing result is reported after the product was infused, the FDA and other relevant parties would be notified as per our manufacturing standard operating procedure (SOP) and our clinical research SOP. Subjects would be managed per established practice.

8.1.8 *Potential Toxicities*

Potential toxicities may be categorized as those related to infusion of T cells: cross-reactivity with normal tissues, cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), and macrophage activation syndrome (MAS).

8.1.8.1 *Cross-Reactivity*

B7-H3 has limited expression on normal tissues. In addition, the intraperitoneal infusion of the CAR.B7-H3 T cells should further mitigate the potential systemic toxicity.

8.1.8.2 *Cytokine Release Syndrome*

CRS is the most common side effect of CAR-T cell therapy and is potentially life threatening [72]. Symptoms can vary from mild flu-like symptoms to more severe toxicities such as vascular leak, hypotension, coagulopathy, pulmonary edema and multi-organ failure. CRS-associated toxicities, when severe, require intensive medical management including vasoactive pressors, mechanical ventilation, anti-epileptics, and antipyretics [53]. Quite recently, tocilizumab, a monoclonal

antibody specific to the IL-6 receptor, has been approved by the FDA for the treatment of patients with CRS. Cytokine elevations are detectable in most patients; however, the degree of elevation does not correlate with the severity of CRS or response to therapy. The most significantly elevated cytokines associated with CRS are IL-10, IL-6, and IFN-γ. IL-6 is an inflammatory cytokine involved in a large number of immune processes, including neutrophil trafficking, acute phase response, angiogenesis, B cell differentiation, and auto-antibody production. In severe CRS with T-cell engaging therapies, IL-6 levels peak during maximal T cell proliferation. High levels of IL-6, present in the context of CRS likely initiates a pro-inflammatory IL-6-mediated signaling cascade.

8.1.8.3 *Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS)*

The underlying pathophysiology for immune effector cell-associated neurotoxicity syndrome (ICANS) is not fully understood [54, 72]. CAR-T cells in the CNS may play a role. However, the heightened systemic inflammatory and cytokine state resulting from CAR-T therapy may also be a factor, as other therapies associated with increased cytokine levels have also been associated with neurologic toxicities, such as high-dose interleukin-2 (IL-2) and blinatumomab.

The symptoms and manifestations of ICANS are broad and range from confusion/altered mental status to seizures. Routine monitoring is critical in subjects receiving CAR-T therapy to identify neurologic symptoms early and neurology should be consulted for any subjects who exhibit early signs/symptoms of neurotoxicity. Early interventions should be employed to prevent worsening, especially if therapies are already indicated such as corticosteroids.

8.1.8.4 *Macrophage Activation Syndrome*

Macrophage activation syndrome (MAS) has been found previously in patients with acute lymphoblastic leukemia receiving CAR-T cell therapy [73]. Macrophage activation syndrome is characterized by pancytopenia, liver insufficiency, coagulopathy and neurological symptoms, and is thought to be mediated by uncontrolled proliferation and activation of T cells leading to macrophage activation and differentiation and cytokine production with hemophagocytosis. The pathophysiology of MAS is complex but appears to have some similarities to hemophagocytic lymphohistiocytosis (HLH). Correlative evaluations have shown that hepatic biopsy samples in patients with hemophagocytic lymphohistiocytosis including a subgroup with macrophage activation syndrome demonstrated the presence of CD8+ T cells generating IFNγ with macrophages generating IL-6 and TNF. There was overproduction of IL-18, which may play a role in the polarization of T cells.

Diagnosis of macrophage activation syndrome: pancytopenia, fever, elevated AST, ALT, triglycerides and LDH, increased PT and PTT associated with increased number of fibrin split products and decreased fibrinogen.

Additionally, splenomegaly, hepatomegaly and CNS dysfunction characterized by lethargy, irritability, disorientation, headache, seizures and coma can be found.

8.2 Lymphodepleting Chemotherapy

Subjects will receive lymphodepleting chemotherapy with cyclophosphamide 300 mg/m^2/day and fludarabine 30 mg/m^2/day IV for three consecutive days administered 2-14 days prior to initial cellular product administration.

8.2.1 *Cyclophosphamide*

Cyclophosphamide is an alkylating agent that has anticancer activity. It is converted in the liver to active alkylating metabolites such as phosphoramide mustard by cytochrome-P450 enzymes. These alkylating metabolites interfere with the growth of susceptible rapidly proliferating malignant cells. Alkyl radicals intercalate into DNA strands and interfere with DNA replication.

Cyclophosphamide is commercially available and will not be supplied free of charge to the patient. Cyclophosphamide should be handled and stored per institutional guidelines in accordance with the manufacturer's prescribing information.

8.2.1.1 *How Supplied*

Cyclophosphamide is supplied as 500 mg, 1 gm and 2 gm vials containing white powder for IV administration.

8.2.1.2 *Storage Requirements/Stability*

Cyclophosphamide vials should be stored at or below 25°C (77°F). Cyclophosphamide does not contain any antimicrobial preservative and care must be taken to assure the sterility of prepared solutions.

8.2.1.3 *Handling and Dispensing*

Cyclophosphamide must be dispensed by authorized personnel according to local regulations. Cyclophosphamide should be stored in a secure area according to local regulations.

8.2.1.4 *Preparation*

Cyclophosphamide should be reconstituted in 25 mL (500 mg), 50 mL (1 gm) or 100 mL (2 gm) normal saline or sterile water for injection, USP, for IV infusion (i.e., per institutional guidelines). Shake vigorously to dissolve the drug. To minimize risk of dermal exposure, always wear gloves when handling vials containing cyclophosphamide sterile powder for injection.

8.2.1.5 *Dosage and Administration*

Cyclophosphamide 300 mg/m^2/day will be administered intravenously for 3 consecutive days 2 – 14 days prior to initial cellular product administration.

8.2.1.6 *Adverse Events Associated with Cyclophosphamide*

Please refer to the prescribing information for cyclophosphamide for complete information.

Possible side effects:

- Nausea, vomiting, diarrhea
- Urinary bladder toxicity
- Bone marrow suppression
- Gonadal suppression
- Myelodysplasia
- Alopecia
- Immunosuppression
- Hyperpigmentation of the skin

Full prescribing information on cyclophosphamide is available at:

http://medlibrary.org/lib/rx/meds/cyclophosphamide-1/

8.2.1.7 *Prohibited Medications*

Drugs that should not be administered concomitantly with cyclophosphamide:

- Carbamazepine
- Idarubicin
- Natalizumab
- Etanercept
- Palifermin

Consumption of alcohol should be avoided while the subject is receiving cyclophosphamide. In addition, subjects should avoid the consumption of

grapefruit or grapefruit juice during treatment with cyclophosphamide. Vaccination with live vaccines is prohibited (see product insert for details). In addition, there are 46 medications known to have major interactions with cyclophosphamide provided at this link: https://www.drugs.com/drug-interactions/cyclophosphamide-index.html?filter=3&generic_only=.

8.2.2 *Fludarabine*

Fludarabine phosphate for injection is a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-β-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination by adenosine deaminase. Fludarabine phosphate is indicated for the treatment of adult patients with B-cell chronic lymphocytic leukemia who have not responded to or whose disease has progressed during treatment with at least one standard alkylating agent containing regimen. Details on this product may be found in the Prescribing Information http://medlibrary.org/lib/rx/meds/fludarabine-phosphate-10/

8.2.2.1 *Formulation*

Fludarabine is supplied in clear glass vials. Each vial of sterile lyophilized solid cake contains 50 mg of the active ingredient fludarabine phosphate, USP, 50 mg of mannitol, USP, and sodium hydroxide to adjust to pH to 7.7. The pH range for the final product is 7.2 to 8.2. Reconstitution with 2 mL of sterile water for injection, USP results in a solution containing 25 mg/mL of fludarabine phosphate, USP, intended for intravenous administration.

8.2.2.2 *Storage*

Fludarabine should be stored in a secure area according to local regulations at 20° to 25°C (68° to 77°F). Excursions permitted between 15° to 30° C (59° to 86°F). Fludarabine should be retained in original package until time of use.

8.2.2.3 *Handling and Dispensing*

Fludarabine is commercially available and will not be supplied free of charge to the subject. Fludarabine should be handled and stored per institutional guidelines in accordance with the manufacturer's prescribing information. Local requirements for disposal of hazardous drugs should be followed per institutional policy.

8.2.2.4 *Preparation*

Refer to the package insert for instructions on preparation for IV administration (see http://medlibrary.org/lib/rx/meds/fludarabine-phosphate-10/).

8.2.2.5 *Dose and Administration*

Fludarabine (30 mg/m^2/day × 3 days) will be administered by IV infusion for lymphodepletion prior to cellular product infusion.

8.2.2.6 *Adverse Events Associated with Fludarabine*

Please refer to the prescribing information for fludarabine for complete information. The information below summarizes the warnings and precautions from the prescribing information.

8.2.2.6.1 Dose Dependent Neurologic Toxicities

There are clear dose dependent toxic effects seen with fludarabine for injection, USP. Dose levels approximately 4 times greater (96 mg/m^2/day for 5 to 7 days) than that recommended for CLL (25 mg/m^2/day for 5 days) were associated with a syndrome characterized by delayed blindness, coma, and death.

8.2.2.6.2 Bone Marrow Suppression

Severe bone marrow suppression, notably anemia, thrombocytopenia, and neutropenia, has been reported in patients treated with fludarabine phosphate for injection. Cumulative myelosuppression may be seen. While chemotherapy-induced myelosuppression is often reversible, administration of fludarabine phosphate for injection requires careful hematologic monitoring.

8.2.2.6.3 Autoimmune Reactions

Instances of life-threatening and sometimes fatal autoimmune phenomena such as hemolytic anemia, autoimmune thrombocytopenia purpura, Evans syndrome, and acquired hemophilia have been reported to occur after one or more cycles of treatment with fludarabine.

8.2.2.6.4 Transfusion Associated Graft vs. Host Disease (GVHD)

Transfusion-associated GVHD has been observed after transfusion of non-irradiated blood in fludarabine-treated patients.

8.2.2.6.5 Pulmonary Toxicity

A high incidence of fatal pulmonary toxicity has been observed when fludarabine is administered in combination with pentostatin for treatment of CLL.

8.2.2.6.6 Pregnancy Category D

Fludarabine can cause fetal harm when administered to a pregnant woman. Women of childbearing potential should be advised to avoid becoming pregnant if they are to receive therapy with fludarabine.

8.2.2.6.7 Male Fertility and Reproductive Outcomes

Males with female partners of childbearing potential should use contraception during and after cessation of fludarabine. Possible sperm DNA damage raises concerns about loss of fertility and genetic abnormalities in fetuses.

8.2.2.6.8 Renal Impairment

Subjects with creatinine clearance 30 to 79 mL/min should have their fludarabine dose reduced and monitored for excessive toxicity. Fludarabine should not be administered to patients with creatinine clearance < 30 mL/min.

8.2.2.6.9 Prohibited Concomitant Therapy

Medications known to have major interactions with fludarabine are provided at this link: https://www.drugs.com/drug-interactions/fludarabine-index.html?filter=3&generic_only=. These medications are prohibited while the subject is receiving fludarabine.

Ensure that subjects are not receiving any of the prohibited medications listed at the links provided while they are undergoing lymphodepletion with bendamustine and fludarabine.

9 ADVERSE EVENTS

9.1 Definitions

9.1.1 *Adverse Event (AE)*

An adverse event (AE) is any untoward medical occurrence (e.g., an abnormal laboratory finding, symptom, or disease temporally associated with the use of cellular product in a subject or clinical investigation subject administered a cellular product and which does not necessarily have a causal relationship with this treatment). An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of a central line) need not be considered an AE and should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the investigator to the study therapy.

9.1.2 *Suspected Adverse Reaction (SAR)*

A suspected adverse reaction (SAR) is any AE for which there is a reasonable possibility that the cellular product is the cause. Reasonable possibility means that there is evidence to suggest a causal relationship between the cell infusion and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by cellular product.

Causality assessment to a cellular product is a medical judgment made in consideration of the following factors: temporal relationship of the AE to cellular product exposure, known mechanism of action or side effect profile of study treatment, other recent or concomitant cellular product exposure, normal clinical course of the disease under investigation, and any other underlying or concurrent medical conditions. Other factors to take into account when considering whether cellular product is the cause of the AE:

- Single occurrence of an uncommon event known to be strongly associated with cellular product exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome).

- One or more occurrences of an event not commonly associated with cellular product exposure, but otherwise uncommon in the population (e.g., tendon rupture); often more than one occurrence from one or multiple studies would be needed before the sponsor could determine that there is reasonable possibility that the cellular product caused the event.

- An aggregate analysis of specific events observed in a clinical trial that indicates the events occur more frequently in the cellular product treatment group than in a concurrent or historical control group.

9.1.3 *Unexpected AE or SAR*

An AE or SAR is considered unexpected if the specificity or severity of it is not consistent with prior experience in CAR-T therapy trials or the applicable product information.

9.2 Documentation of Non-Serious Adverse Events or SARs

For non-serious AEs or SARs, documentation must begin at the time of procurement when the consent form is signed by the subject prior to procurement and continues through the 3 week follow-up period after cellular treatment is discontinued. Any AEs or SARs experienced by the subject related to these procedures (procurement, lymphodepletion, and cellular product administration) must be documented. Adverse event data collection will cease in subjects that receive therapy for relapse of their primary malignancy. The DLT assessment period will start at the time of the initial cellular product administration through the 3 week DLT follow up period after the final (third) cellular product administration. Subjects will be followed for safety and documentation of AEs for 4 weeks after the final cell infusion.

Collected information should be recorded in the electronic case report forms (eCRF) for that subject. A description of the event, its severity or toxicity grade, onset and resolved dates (if applicable), and the relationship to the study drug should be included.

10 SERIOUS ADVERSE EVENT

10.1 Definition of Serious Adverse Event

An AE or SAR is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Requires inpatient hospitalization (>24 hours) or prolongation of existing hospitalization*;
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse study treatment related experience when, based upon appropriate medical judgment, they may jeopardize the subject or subjects and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. For reporting purposes, also consider the occurrences of pregnancy as an event which must be reported as an important medical event.

*Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.

Pregnancy that occurs during the study must also be reported as an SAE.

10.2 Documentation of SAEs or Serious SARs

10.2.1 *Timing*

After informed consent but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected (e.g. SAEs related to invasive procedures such as biopsies, medication washout).

For any other experience or condition that meets the definition of an SAE or a serious SAR, recording of the event must begin at procurement and continue through the 3 week follow-up period after treatment with the final cell infusion is initiated.

10.2.2 *Documentation and Notification*

SAEs or Serious SARs must be recorded in the SAE console within OnCore® for that subject within 24 hours of learning of its occurrence. The Regulatory Associate and Medical Monitor must be notified via email of all SAEs within 24 hours of learning of its occurrence.

10.3 Adverse Event Reporting

10.3.1 *IRB Reporting Requirements*

The IRB will be notified of all SAEs that qualify as an Unanticipated Problem as per the IRB's Policies within 7 days of the Investigator becoming aware of the problem. These events must be reported to the sponsor within 24 hours of learning of the occurrence.

10.3.1.1 *Pregnancy*

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on study, or within 4 weeks of the subject's last dose of study treatment should be recorded as SAEs. The subject is to be discontinued immediately from the study.

The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

The Investigator will follow the female subject until completion of the pregnancy and must document the outcome of the pregnancy (either normal or abnormal outcome) and report the condition of the fetus or newborn to the UNC Study Coordinator. If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE.

10.3.2 *FDA Expedited Reporting Requirements for Studies Conducted Under an IND*

A sponsor must report any suspected adverse reaction that is both serious and unexpected and related to the cellular product to the FDA. The sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the cellular product and the adverse event. For the definition of SAR refer to Section 9.1.2.

The sponsor must submit each IND safety report on the MedWatch Form 3500A.

10.3.2.1 *Timing*

FDA must be notified of potential serious risks within 15 calendar days after the sponsor determines the event requires reporting. FDA must be notified of unexpected fatal or life-threatening suspected adverse reactions as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. As a result, the sponsor must be notified of the SAE by the investigator within 24 hours of the event. If the results of a sponsor's investigation show that an adverse event not initially determined to be reportable is reportable, the sponsor must report such suspected adverse reaction in an IND safety report as soon as possible, but in no case later than 15 calendar days after the determination is made.

10.3.2.2 *Follow-Up*

The sponsor must promptly investigate all safety information it receives. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and as such the sponsor should be updated within 24 hours of the information being available via a follow-up MedWatch Form 3500A.

10.3.2.3 *Notification of Investigators*

The sponsor must notify all participating investigators (i.e., all investigators to whom the sponsor is providing cell infusion under its INDs or under any investigator's IND) in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

10.3.2.4 *Process*

If the sponsor deems that an event is both a serious adverse reaction (SAR) AND unexpected, it must also (in addition to OnCore®) be recorded on the MedWatch Form 3500A. Unexpected adverse events or adverse reaction refers to an event or reaction that is not listed in the Investigator's Brochure/IND or is not listed at the specificity or severity that has been observed; or if an Investigator's Brochure is not required or available, is not consistent with the risk information described in the general investigation plan or elsewhere in the current IND application.

10.3.2.5 *Additional Reporting Requirements*

The following additional items must be reported via IND safety report:

- Findings from other studies. The sponsor must report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND, and whether or not conducted by the sponsor, that suggest a significant risk to humans exposed to the cell infusion.

- Findings from animal or *in vitro* testing. The sponsor must report any findings from animal or in vitro testing, whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the cell infusion, such as reports of mutagenicity, teratogenicity, or carcinogenicity, or reports of significant organ toxicity at or near the expected human exposure.

- Increased rate of occurrence of serious suspected adverse reactions.

- Grade 4 or 5 cellular production infusion reactions, CRS or ICANS.

10.3.2.6 *Additional Guidance*

Please refer to 21CFR312.32 and "Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE Studies" for additional information and reporting requirements. All IND Safety Reports will be submitted in accordance with these regulations/guidances.

10.3.3 *Institutional Biosafety Committee (IBC) Reporting Requirements*

In addition to the local IRB, any qualifying serious adverse events (SAEs) must be reported to the Institutional Biosafety Committee (IBC). The IBC is responsible for reviewing recombinant DNA research conducted at or sponsored by the institution for compliance with NIH Guidelines. Experiments covered by the NIH Guidelines and approving those research projects that are found to conform with the NIH Guidelines. As such, the IBC is charged with ensuring compliance with all surveillance, data reporting and adverse event reporting requirements set forth in the NIH Guidelines. The UNC study coordinator will be responsible for notifying the Regulatory Associate within 48 hours should any reportable event occur.

11 STATISTICS

11.1 Study Design

This single center, open-label phase I dose-finding trial seeks to determine if CAR-T cells targeting the B7-H3 antigen can be safely administered with lymphodepletion to women with recurrent platinum-resistant epithelial ovarian cancer. Up to 12 subjects will be assigned in the dose-escalation portion of the study. The modified 3+3 design will be used. See Section 4.1.1 for the description of the dose-finding algorithm.

The maximum tolerated cell dose is defined as a dose with the probability of DLT of 20%. The estimated maximum tolerated cell dose after the 3+3 design is the higher dose among the two studied with observed DLT rate less than 33%. RP2D will be decided based on the maximum tolerated cell dose, DLTs observed in the dose-finding portion of the study and additional factors including the ability to manufacture the appropriate quantity of cells and the number of subjects who are removed early from treatment and do not proceed with receiving all 3 infusions.

11.2 Stopping Rules

The trial will be stopped if a death is observed. Sequential boundaries will be used to monitor Grade ≥ 4 non-hematologic toxicity at least possibly related to CAR-T cells. The accrual will be halted if excessive numbers of these toxicities are seen, that is, if the number of these toxicities is equal to or exceeds b_n out of n subjects with full follow-up (Table 3). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 0.1 when the rate of Grade ≥ 4 non-hematologic toxicity at least possibly related to CAR-T cells is equal to the acceptable rate of 0.2.

Table 3 Stopping Boundaries

Number of Patients, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Boundary, b_n	-	-	3	3	4	4	4	5	5	5	6	6	6	7	7	7	7	8	8	8	9

11.3 Sample Size, Accrual and Duration of Accrual

The number of subjects required for this study will depend on DLTs we observe. Our expectation is that up to 9 adult subjects will be needed for the dose-escalation portion of the study. Subjects who do not receive all three infusions will be replaced.

After dose escalation is completed, an expansion cohort will enroll additional subjects to further assess the safety and efficacy of CAR.B7-H3 T cells, for the total of up to 21 subjects in the trial.

Retrospective analysis of B7H3 expression will be performed. We expect to enroll 4 B7H3 negative subjects and 17 B7H3 positive subjects. Though the numbers are small, enrolling B7H3 negative subjects might provide valuable data on relative treatment effects in B7H3

negative subjects and B7H3 positive subjects. For example, if none of the four B7H3 negative subjects have disease control and 11 or more of the B7H3 positive subjects have disease control, we will conclude that the disease control rates are significantly different in the two subgroups and further investigation of the treatment in the B7H3 negative group is not warranted.

The duration of accrual is expected to be 48 months.

11.4 Data Analysis Plans

Descriptive statistics will be utilized to summarize toxicity overall and by dose.

All subjects who receive an infusion of CAR.B7-H3 T cells will be included in the safety analysis. Adverse event data and corresponding toxicity grades through 4 weeks after the last CAR-T cell infusion and during long-term follow up will be summarized. Tables will be generated to summarize incidence of subjects experiencing at least one episode of each adverse event, incidence of adverse events causing withdrawal, and incidence of serious adverse events. The total number of episodes for each event reported, the severity, and attribution to study therapy of each episode reported will also be displayed.

Listings of adverse events by subjects will include the time to onset, the duration of each event, the severity of each event, the relationship of the event to study therapy, whether it was a serious event, and whether it caused withdrawal. Safety data will be summarized by dose level.

For laboratory data, descriptive statistics (means, standard deviations, medians and ranges) at each evaluation point through 6 weeks after initial cellular product administration will be calculated. Laboratory data collected intervals dictated by the Time & Events Table for the first year will also be summarized.

Disease control rate will be estimated as a proportion of subjects with complete response (CR) + partial response (PR) + stable disease and 95% confidence interval computed. PFS applies to all subjects and will be measured from the time of lymphodepletion prior to infusion with CAR.B7-H3 to progression or death from any cause; subjects not known to have any of these events are censored on the date they were last examined. OS will be measured from the date of lymphodepletion prior to CAR.B7-H3 T cell product administration to the date of death. PFS and OS will be estimated using the Kaplan-Meier method.

Toxicity and durability of response to therapy will be modeled as a function of changes in the expression of genes and/or proteins from before to after treatment to find the genes and/or proteins with the strongest association with toxicity and durability of response.

12 STUDY MANAGEMENT

12.1 Required Documentation

Before the study can be initiated at any site, the following documentation must be provided to the Clinical Protocol Office (CPO) at the University of North Carolina.

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list.
- CVs and medical licensure for the principal investigator and any sub-investigators who will be involved in the study.
- Form FDA 1572 appropriately filled out and signed with appropriate documentation.
- Financial Disclosures.
- CAP and CLIA Laboratory certification numbers and institution lab normal values.
- Executed clinical research contract.

12.2 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s) and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the subject will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the subject and the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing an IRB approved consent form.

Prior to a subject's participation in the trial, the written informed consent form should be signed and personally dated by the subject and by the person who conducted the informed consent discussion.

12.3 Institutional Biosafety Committee Approval

In accordance with the NIH Guidelines, any experiments involving the deliberate transfer of recombinant or synthetic nucleic acid molecules, or DNA or RNA derived from recombinant or synthetic nucleic acid molecules, into human research participants (human gene transfer), no human gene transfer experiment shall be initiated until Institutional Biosafety Committee (IBC) approval (from the clinical trial site) has been obtained and all applicable regulatory authorization(s) and approvals have been obtained.

12.4 Registration Procedures

All subjects must be registered (prior to procurement) by the Cellular Immunotherapy Study Coordinator at UNC before enrollment to the study. Prior to registration at the time of cell procurement, eligibility criteria must be confirmed with the Cellular Immunotherapy Study Coordinator.

12.5 Adherence to Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study subject requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

12.6 Emergency Modifications

UNC investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB/IEC approval/favorable opinion.

For any such emergency modification implemented, the IRB must be notified within five (5) business days of making the change.

12.7 Study Files and Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor correspondence to Investigators, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed subject consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a cell infusion seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing

application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

12.8 Source Documentation

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study subjects. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator or site will be responsible for assuring that all the required data will be collected and entered into the eCRFs. Periodically, monitoring visits will be conducted, and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all eCRFs will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

12.9 Data and Safety Monitoring Plan

The Principal Investigator will provide continuous monitoring of subject safety in this trial with periodic reporting to the Data and Safety Monitoring Committee (DSMC).

Meetings/teleconferences will be held at a frequency dependent on study accrual. These meetings will include the investigators as well as study coordinators, data coordinators, regulatory associates, clinical data management associates and any other relevant personnel the principal investigator may deem appropriate. At these meetings, the research team will discuss all issues relevant to study progress, including enrollment, safety, regulatory, data collection, etc. Prior to dose escalation, dose escalation meetings will be held to determine whether enrollment should continue on the next dose level. These meetings will include investigators, the Medical Monitor, the people responsible for review and evaluation of information relevant to the safety of the drug, the biostatistician, study coordinators and clinical data management associates.

The team will produce summaries or minutes of these meetings. These summaries will be available for inspection when requested by any of the regulatory bodies charged with the safety of human subjects and the integrity of data including, but not limited to, the oversight of the IRB, the Oncology Protocol Review Committee (PRC) or the North Carolina TraCS Institute Data and Safety Monitoring Board (DSMB).

The UNC LCCC Data and Safety Monitoring Committee (DSMC) will review the study on a regular (quarterly to annually) basis, with the frequency of review based on risk and complexity as determined by the UNC Protocol Review Committee. The UNC PI will be

responsible for submitting the following information for review: 1) safety and accrual data including the number of subjects treated; 2) significant developments reported in the literature that may affect the safety of participants or the ethics of the study; 3) preliminary response data; and 4) summaries of team meetings that have occurred since the last report. Findings of the DSMC review will be disseminated by memo to the UNC PI, PRC, the UNC IRB and DSMB.

13 AUDITING AND MONITORING

The UNC LCCC will serve as the coordinating center for this trial. Data will be collected through a web based electronic data capture system, Advarra EDC. All data will be collected and entered into Advarra by research coordinators from UNC LCCC.

The sponsor will provide direct access to source data/documents for trial-related monitoring, audits, IRB/IEC review, and regulatory inspection. As an investigator initiated study, this trial will also be audited and reviewed by the LCCC compliance committee every six or twelve months. It will also be monitored according to LCCC SOPs, within 8 weeks of the first patient enrolled, and subsequently every 4 months while there are subjects in the treatment period. Monitoring will occur annually once the study is closed to accrual and all subjects are in the follow-up period.

14 AMENDMENTS

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the subject, a revised consent form might be required.

15 STUDY DISCONTINUATION

Both Lineberger Comprehensive Cancer Center and the Principal Investigator reserve the right to terminate the study at the investigator's site at any time. Should this be necessary, Lineberger Comprehensive Cancer Center or a specified designee will inform the appropriate regulatory authorities of the termination of the study and the reasons for its termination, and the Principal Investigator will inform the IRB/IEC of the same. In terminating the study, Lineberger Comprehensive Cancer Center and the Principal Investigator will assure that adequate consideration is given to the protection of the subjects' interests.

16 CONFIDENTIALITY

All information generated in this study is considered highly confidential and must not be disclosed to any person or entity not directly involved with the study unless prior written consent is gained from Lineberger Comprehensive Cancer Center. However, authorized regulatory officials, IRB/IEC personnel, Lineberger Comprehensive Cancer Center and its authorized representatives are allowed full access to the records.

Identification of subjects and eCRFs shall be by initials, screening and treatment numbers only. If required, the subject's full name may be made known to an authorized regulatory agency or other authorized official.

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18 APPENDICES

18.1 APPENDIX I – Names of Study Personnel

Sponsor:	Lineberger Comprehensive Cancer Center
Principal Investigator:	Linda Van Le, MD [redacted]
Medical Monitor:	Natalie Grover, MD [redacted]
Clinical Protocol Office:	Lineberger Comprehensive Cancer Center The University of North Carolina at Chapel Hill 450 West Drive, 3rd Floor, Chapel Hill, NC 27599-7295
Biostatistician:	Anastasia Ivanova, PhD [redacted]

18.2 APPENDIX II – Tumor Measurement Based on RECIST 1.1

See the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1 [74] for additional details on RECIST 1.1.

Measurable disease will be defined as the presence of at least one measurable lesion that can be accurately measured in at least one dimension with the longest diameter a minimum size of:

- >10 mm by CT scan (CT scan slice thickness no greater than 5 mm)
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20 mm by chest X-ray.

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

All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with ≥10 to <15 mm short axis) as well as truly non-measurable lesions, will be considered non-measurable. Lesions considered truly non-measurable include: leptomeningeal disease; ascites; pleural/pericardial effusion; inflammatory breast disease; lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment. 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 should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. Clinical lesions will only be considered measurable when they are superficial and ≥10 mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesions is recommended.

18.2.1 *Baseline Documentation of Target and Non-Target Lesions*

All measurable lesions up to a maximum of 5 lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longer diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as "present" or "absent", or in rare cases "unequivocal progression".

18.2.2 *Evaluation of Target Lesions using RECIST 1.1 Criteria*

NOTE: In addition to the information below, also see section 4.3.2 in the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1 [74] for special notes on the assessment of target lesions.

Complete response (CR) − Disappearance of all target lesions. Any pathological lymph node (LN) (whether target or non-target) must have decreased in short axis to <10mm.

Partial response (PR) − At least a 30% decrease in the sum of the LD of the target lesions taking as reference the baseline sum LD.

Progressive Disease (PD) − At least a 20% increase in the sum of the LD of the target lesions taking as reference the smallest sum LD recorded since the treatment started including baseline 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 5mm. The appearance of one or more new lesions also constitutes PD.

Stable disease (SD) − Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.

18.2.3 *Evaluation of Non-Target Lesions using RECIST 1.1 Criteria*

Complete response (CR) − Disappearance of all non-target lesions and normalization of tumor marker levels. All LN must be non-pathological in size (<10mm short axis).

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

Progressive disease (PD) − Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

18.2.4 *Evaluation of Best Overall Response using RECIST 1.1 Criteria*

The best overall response is the best response recorded from the start of the study treatment until the end of treatment provided the confirmation criteria are met. To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed > 4 weeks after the criteria for response are first met. If a CR/PR cannot be confirmed the original "response" should be considered stable disease. The best overall response will be defined according to the following table (Table 4).

Table 4 Best Overall Response

Overall Response First Time Point	Overall Response Subsequent Time Point	BEST Overall Response
CR	CR	CR
CR	PR	SD, PD, or PR[1]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE[2]	SD provided minimum criteria for SD duration met, otherwise, NE[2]
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE[2]	SD provided minimum criteria for SD duration met, otherwise, NE[2]
NE	NE[2]	NE[2]

[1] If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the subject had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

[2] NE=unevaluable

18.3 APPENDIX III – CRS Grading Criteria and Management Guidelines

BACKGROUND

Immunotherapies in cancer care are becoming more widely available. As these therapies are being used more commonly, clinicians must be aware of their unique toxicities and the optimal strategies that are recommended for the management of these toxicities.

Cytokine release syndrome (CRS) has been observed with several different immunotherapies, including monoclonal antibodies, bi-specific antibodies, T-cell checkpoint inhibitors, and novel T-cell therapies. It is characterized by widespread activation and proliferation of lymphocytes leading to an abundant release of inflammatory cytokines well above physiologic levels. This cytokine storm can manifest in many ways from constitutional symptoms to cardiovascular and neurologic compromise. Management of this cytokine release storm involves both supportive care, and if clinically warranted, immunosuppression that blunts the aggressive cytokine response. However, administration of immunosuppressive therapies may also counter the desired immune response against targeted tumor cells. Thus, it is important that clinicians be prudent and reserve certain immunosuppressive strategies for the most appropriate clinical scenario.

SIGNS/SYMPTOMS & CLINICAL GRADING

Severity of cytokine release syndrome is variable and may be influenced by tumor burden at the time of treatment with the immune-directed therapy or other pre-existing comorbidities. Clinical grading is important for appropriate management. Organ systems affected by CRS and their corresponding signs and symptoms are listed below in Table 5 and criteria for clinical grading are outlined below in Table 6.

Table 5 Signs and Symptoms of CRS

Organ system	Signs/Symptoms
Constitutional	Fever, rigors, malaise, fatigue, anorexia, headache, myalgias/arthralgias, nausea/ vomiting
Dermatologic	Rash
Gastrointestinal	Nausea/vomiting/diarrhea
Respiratory	Tachypnea, hypoxemia (potentially requiring supplemental oxygen/ventilation)
Cardiovascular	Tachycardia, hypotension
Coagulation	Disseminated intravascular coagulation (DIC) characterized by elevated D-dimer, hypofibrinogenemia, bleeding
Renal	Azotemia

Organ system	Signs/Symptoms
Hepatic	Transaminitis, hyperbilirubinemia
Neurologic	Altered mental status, confusion, delirium, aphasia, hallucinations, tremor, seizures, ataxia

CRS Grading/Severity

The outlined clinical grading criteria is designed to guide clinicians in the management of CRS. Many of the signs and symptoms associated with CRS can also be attributable to other common complications of cancer therapy such as neutropenic fever, other infectious complications, and tumor lysis syndrome. Thus, in applying the criteria below, clinicians should exercise appropriate clinical judgement in each subject-specific scenario in an effort to distinguish true CRS from other cancer treatment-related toxicities. This will ensure appropriate delivery of care and avoidance of therapies that may otherwise not be indicated.

Table 6 CRS Grading

Grade 1
Fever ≥38°C No hypotension No hypoxia
Grade 2
Fever ≥38ºC Hypotension not requiring vasopressors Hypoxia requiring low-flow nasal cannula (≤6 L/minute) or blow-by
Grade 3
Fever ≥38ºC Hypotension requiring a vasopressor with or without vasopressin Hypoxia requiring high-flow nasal cannula (>6 L/minute), facemask, nonrebreather mask, or Venturi mask
Grade 4
Fever ≥38ºC Hypotension requiring multiple vasopressors (excluding vasopressin) Hypoxia requiring positive pressure (e.g., CPAP, BiPAP, intubation, mechanical ventilation)

Grade 5 – Death
Death

Management of CRS

CRS will be managed per institutional guidelines provided at the following link:

https://unchcs.sharepoint.com/sites/MCBMSCT/Shared%20Documents/Forms/AllItems.aspx?id=%2Fsites%2FMCBMSCT%2FShared%20Documents%2FAdult%20CRS%20Management%20Guidelines%20V%204%20042522%2Epdf&parent=%2Fsites%2FMCBMSCT%2FShared%20Documents

18.4 APPENDIX IV – Management of Neurotoxicity/Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) from CAR-T Therapy

Neurotoxicity from CAR-T therapy can occur as part of cytokine release syndrome (CRS) or as an independent process. The underlying pathophysiology for neurologic toxicity from CAR-T therapy is not fully understood. CAR-T cells in the CNS may play a role. However, the heightened systemic inflammatory and cytokine state resulting from CAR-T therapy may also be a factor.

The symptoms and manifestations of neurotoxicity are broad and range from confusion/altered mental status to seizures. Routine monitoring is critical in patients receiving CAR-T therapy to identify neurologic symptoms early and neurology should be consulted for any patients that exhibit early signs/symptoms of neurotoxicity. Early interventions should be employed to prevent worsening, especially if therapies are already indicated such as corticosteroids.

Immune Effector Cell-Associated Encephalopathy (ICE) Score

The ICE score is a neurological assessment score that quantifies the severity of neurologic impairment. Each item in the assessment is associated with the point value indicated (Table 7). The higher the score the better, with an ICE score of 10 indicating a normal neurological assessment. The score should then be considered with other assessments to accurately grade the patients ICANS as is outlined in management of ICANS below.

Table 7 ICE

Assessment	Score (10 = Normal)
Orientation	
Year	**1**
Month	**1**
City	**1**
Hospital	**1**
Name 3 Objects (example: point to clock, pen, and button)	**3**
Ability to follow simple commands, e.g., "Show me 2 fingers" or "Close your eyes and stick out your tongue"	**1**

Assessment	Score (10 = Normal)
Ability to write a standard sentence	**1**
Ability to count backwards from 100 by 10	**1**
Total	**10**

Neurotoxicity/ICANS Grading

Neurotoxicity/ICANS will be graded as described below in Table 8.

Table 8 ICANS Grading

Signs/Symptoms
Grade 1
ICE Score: 7-9 (mild impairment) **Level of Consciousness:** Awakens spontaneously **Seizure:** None **Motor Findings:** None **Elevated ICP/Cerebral edema:** None
Grade 2
ICE Score: 3-6 (moderate impairment) **Level of consciousness:** Awakens to voice **Seizure:** None **Motor Findings:** None **Elevated ICP/Cerebral edema:** None
Grade 3
ICE Score: 0-2 (severe impairment) **Level of consciousness:** Awakens only to tactile stimulus **Seizure:** Any clinical seizure (focal or generalized) that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention **Motor findings:** None **ICP/Cerebral Edema:** Elevated ICP; Focal/local edema on neuroimaging

Grade 4
ICE Score: Patient in critical condition, and/or obtunded and cannot perform assessment of tasks **Level of consciousness:** Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma **Seizure:** Life threatening prolonged seizure (> 5 min) or repetitive clinical or electrical seizures without return to baseline in between **Motor findings:** Deep focal motor weakness such as hemiparesis or paraparesis **Elevated ICP/Cerebral edema:** Diffuse cerebral edema on neuroimaging; Decerebrate or decorticate posturing; Cranial nerve VI palsy; Papilledema; Cushing's triad
Grade 5
Death

Neurotoxicity/ICANS Treatment Algorithm

The Neurotoxicity/ICANS treatment algorithm is available at the link provided below:

https://unchcs.sharepoint.com/sites/MCBMSCT/Shared%20Documents/Forms/AllItems.aspx?id=%2Fsites%2FMCBMSCT%2FShared%20Documents%2FAdult%20CRS%20Management%20Guidelines%20V%204%20042522%2Epdf&parent=%2Fsites%2FMCBMSCT%2FShared%20Documents

References:

1. Neelapu SS, et al. Chimeric antigen receptor T-cell therapy – assessment and management of toxicities. Nature Reviews Clinical Oncology 2018; 15(1):47-62.

2. Frisen L. Swelling of the optic nerve head: a staging scheme. J Neurol Neurosurg Psychiatry 1982;45(1):13-8.

3. Lee DW et al. ASBMT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. Biology of Blood and Marrow Transplantation 2018.

18.5 APPENDIX V – Treatment Algorithm for Macrophage Activation Syndrome

MAS can be diagnosed with any of the following criteria:

- Laboratory (need 4 or more of these for diagnosis plus 2 or more clinical criteria)
  - Decreased platelet count < 150×10^9/L
  - Increased AST
  - Decreased WBC < 4×10^9/L.
  - Hypofibrinogenemia (< 250 mg/dL)
  - Increase in triglycerides
  - Increase in ferritin
- Clinical Criteria:
  - CNS dysfunction (irritability, disorientation, lethargy, headache, seizures or coma)
  - Hemorrhage or easy bleeding
  - Hepatomegaly and/or splenomegaly (> 3 cm below the costal margin)
  - Fever (temperature > 38ºC)
- Additional criteria include evaluating bone marrow for the presence of macrophages phagocytosing hematopoietic cells.

Initial evaluation should include the following:

- CBC with differential and reticulocyte count
- AST, ALT, alkaline phosphatase, LDH, total bilirubin
- Basic metabolic panel including sodium, creatinine, BUN
- Ferritin level
- Triglyceride level

- CT scan with contrast or MRI scan with contrast (if renal function permits) in the setting of CNS symptoms

- Spinal tap with evaluation for infection and the presence of CAR-T cells + cytokines such as IL-6 if this can be done without significant concern for bleeding.

Research evaluations which should be drawn and can assist in the diagnosis include:

- Soluble IL-2 receptor

- Soluble CD163

Treatment will be either high dose corticosteroids at 2 mg/kg of prednisone or an equivalent +/- etoposide similar to institutional therapy for HLH. Other therapies that should be considered in refractory cases include anakinra, cyclosporine A or tacrolimus, and therapies targeted to TNF such as etanercept, infliximab or adalimumab.

18.6 APPENDIX VI – ECOG Performance Status [75]

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.