Abbreviated Title: E7 Induction Cervical Cancer

NIH Protocol #: 20C0116

Version Date: 11/4/2020

IBC Number: RD-20-III-01

NCT Number: NCT04476251

Title: A Pilot Study of E7 TCR T Cell Induction Immunotherapy for Stage IIB-IVA Cervical Cancer

NCI Principal Investigator: Scott Norberg, DO
Genitourinary Malignancies Branch
National Cancer Institute
10 Center Drive, Room 6B09
Bethesda, MD 20892
Phone: 301-275-9668
Email: scott.norberg@nih.gov

Investigational Agents:

Drug Name:	E7 TCR T cells
IND Number:	19564
Sponsor:	Center for Cancer Research
Manufacturer:	CC DTM

Investigational Device:

Name:	HPV16 Genotyping Assay
Device:	Nonsignificant Risk Device (NSR)
Sponsor:	Center for Cancer Research
Lab:	MolecularMD

Commercial Agents: Fludarabine, Cyclophosphamide, IL-2 (Aldesleukin)

PRÉCIS

Background:

- Cervical cancer is the third most common cause of death among women with gynecologic cancers in the United States. Worldwide, cervical cancer accounts for nearly 300,000 deaths annually.
- Virtually all cases of cervical cancer result from chronic infection with high-risk human papillomavirus (HPV), the most common type being HPV16.
- The treatment of locally advanced cervical cancer consists of chemoradiation +/- extended field radiation therapy. Participants with FIGO (revised 2018) stage III-IVA have the worse prognosis with approximately 50% of the participants dying from their disease within 5 years.
- Induction chemotherapy is an active area of study in this type of cancer. The aim of induction therapy is to reduce the risk of disease recurrence and improve overall survival.
- E7 TCR T cells, administered as a single infusion, have demonstrated safety and clinical activity in advanced, treatment-refractory metastatic HPV+ cancers.

Objectives:

- To determine the feasibility of induction E7 TCR T cell therapy for FIGO (2018) stage IIB-IVA, HPV16+ cervical cancer

Eligibility:

- Participants greater than or equal to 18 years old with FIGO (2018) stage IIB-IVA cervical cancer.
- The cancer must be HPV16+ and participant must be HLA-A*02:01+.
- Participants must be treatment-naïve (i.e., no prior local or systemic treatment, including radiation; prior LEEP procedure or cone biopsy is allowed).

Design:

- This is a single arm, pilot study, testing the feasibility of induction E7 TCR T cell therapy.
- Participants will receive a conditioning regimen of cyclophosphamide and fludarabine, a single infusion of E7 TCR T cells, and systemic aldesleukin.
- Participants will be referred for standard of care definitive therapy (i.e., chemoradiation +/- extended field radiation therapy) within 6 weeks after infusion of E7 TCR T cells.

TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1. INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To determine the feasibility of induction E7 TCR T cell therapy for FIGO (2018) stage IIB-IVA, HPV16+ cervical cancer

1.1.2 Secondary Objective

- To assess relapse-free survival (RFS) at 2 years and 5 years
- To evaluate the safety of induction E7 TCR T cell therapy for FIGO (2018) stage IIB-IVA, HPV16+ cervical cancer
- To determine the percentage of E7 TCR T cells following completion of chemoradiation
- To assess objective response rate following E7 TCR T cell induction therapy

1.1.3 Exploratory Objective(s)

- To perform laboratory research to begin to understand treatment response and to develop biomarkers that predict response
- To determine the percentage of participants able to receive standard chemoradiation without requiring dose-reduction due to hematologic toxicities

1.2 BACKGROUND AND RATIONALE

There are more than 12,000 new cases of cervical cancer diagnosed in the United States each year and the incidence is rising [1, 2]. Virtually all cases (99.7%) of cervical cancer are associated with the human papillomavirus (HPV) [3]. HPV types 16 and 18 account for over

70% of all cervical cancers with more than half of all cases of squamous cell carcinoma caused by HPV type 16 [4]. The primary therapy for FIGO (2018) stages IIB to IVA cervical cancer is chemoradiation +/- extended field radiation therapy [5]. Despite this therapy, 42% of participants with FIGO (2018) stage III cervical cancer will die of their disease within 5 years [6, 7]. These participants are also at a high risk of developing recurrent disease at distant sites [8]. Despite improvements in the treatment of metastatic cervical cancer, more than 90% of participants who develop distant recurrence will die of their disease within five years [9]. While there is no well-established approach to induction chemotherapy in this disease, it is an active area of investigation. The goals of induction chemotherapy are to reduce the risk of disease recurrence and improve overall survival. In a multicenter, phase II trial of 46 women, 68% of participants with locally advanced cervical cancer responded to induction chemotherapy with carboplatin + paclitaxel prior to chemoradiation [10]. The overall survival and progression free survival at 3 years was 66%. This has led to a multicenter, randomized phase III clinical trial (termed the INTERLACE trial) of induction chemotherapy with weekly carboplatin + paclitaxel for 6 cycles followed by standard chemoradiation versus standard chemoradiation alone in participants with locally advanced cervical cancer (NCT01566240). Another clinical trial is testing induction chemotherapy with 3 cycles of carboplatin + paclitaxel every 3 weeks followed by standard therapy in cervical cancer participants with paraaortic lymph node involvement (NCT03534713). In this clinical trial, we are studying induction therapy with E7 TCR T cells followed by standard therapy with chemoradiation +/- extended field radiation therapy in participants with FIGO (2018) stage IIB-IVA cervical cancer.

1.2.1 T Cell Therapy

T cell therapy is a type of treatment in which tumor-targeted T cells are administered for the treatment of cancer. T cells are a part of the adaptive immune system that is specialized for the highly specific cell-mediated killing of other cells, particularly cells that are infected by a virus or other intracellular pathogen. Through mechanisms similar to those by which T cells fight infected cells, T cells can also attack cancer cells. HPV+ cancers are attractive candidates for T cell therapy because they express viral antigens that can be targeted by T cells [11]. The primary antigens for targeting with immunotherapy are the HPV E6 and E7 oncoproteins, which are viral proteins that are constitutively expressed by HPV+ cancers and not expressed by healthy human tissues. E6 and E7 contribute to malignant transformation and to survival of cancer cells, and this functional importance also makes them attractive therapeutic targets.

Previously, our group has conducted clinical trials of T cell therapy for metastatic HPV+ cancers. Our initial clinical trial employed as treatment a single infusion of autologous tumor-infiltrating T cells (TIL), which were preferentially generated from TIL subcultures with E6 and/or E7 reactivity [12, 13]. Participants received a conditioning regimen of cyclophosphamide 60 mg/kg for two days and fludarabine 25 mg/m^2 for five days. Cell infusion was followed by high-dose aldesleukin. In this study, 5/18 participants with cervical cancer experienced tumor responses and 2/11 participants with other HPV+ cancers experienced tumor responses. Within the cervical cancer participants, two participants had complete responses, while the other participants had partial responses. A participant with oropharyngeal cancer experienced complete regression of multiple tumors in his lungs. He had a recurrence of the metastatic cancer in his brain, which was resected, and he is without disease several years after treatment. The magnitude of the HPV-oncoprotein-reactivity of the infused treatment cells correlated with response in participants in this clinical trial. However, both viral and non-viral tumor antigens were targeted by the TIL

administered to two participants with cervical cancer who each had a complete tumor response [12]. Thus, the results of this trial support the ability of T cells to mediate regression of HPV+ cancers, but they do not necessarily validate E6 and E7 as therapeutic targets.

We subsequently conducted a clinical trial in which the E6 antigen was specifically targeted with T cell therapy. To accomplish E6 targeting, autologous peripheral blood T cells were genetically engineered to express an HLA-A*02:01-restricted, HPV16 $E6_{29-38}$-specific T cell receptor (TCR) [14]. The trial was a phase I/II, dose-escalation study. Participants had metastatic HPV16+ cancer that, in most participants, was refractory to multiple systemic agents. Participants received a conditioning regimen of cyclophosphamide 60 mg/kg for two days and fludarabine 25 mg/m^2 for five days. Cell infusion was followed by high-dose aldesleukin. Here, 2/12 participants experienced partial tumor responses; one subsequently had residual disease resected by surgery, and she is without evidence of cancer several years later. Pre-treatment tumor biopsies from three participants were available to study to understand disease response and resistance. One participant who did not respond had loss of HLA-A*02:01 expression by the tumor, which likely made the tumor unable to present the $E6_{29-38}$ epitope to E6 TCR T cells. A second participant who did not respond had a truncating mutation in interferon-gamma receptor 1, a crucial molecule for tumor sensitivity to T-cell-mediated tumor recognition and killing [15, 16]. A participant who responded to treatment did not have mutations, deletions, or loss of heterozygosity in a defined panel of genes related to antigen processing and presentation, and interferon-gamma response. These findings suggest that – in the setting of previously treated, metastatic HPV+ cancer – tumors may acquire mutations that confer resistance to T cell immunotherapy. Tumors appear to acquire mutations that confer resistance to T cell immunity as they progress [17-20]. Awareness of this progressive resistance is driving a movement in oncology toward the earlier application of immunotherapy [21, 22].

1.2.2 E7 TCR T cell preclinical development

We identified an HLA-A*02:01-restricted TCR that targets HPV16 $E7_{11-19}$ from the cervix-infiltrating T cells of a participant with cervical intraepithelial neoplasia who received a therapeutic cancer vaccine targeting HPV-16 E7 [23]. The nucleotide sequence of the TCR was codon optimized for expression in human tissues and the TCR constant regions were swapped for their mouse counterparts, which in other receptors has improved TCR alpha/beta chain pairing. TCR expression was further improved by reversing the order of the alpha and beta genes, and by making cysteine substitutions in the TCR constant regions and hydrophobic substitutions in the transmembrane region of the alpha chain constant region. The TCR sequence insert was cloned into the MSGV1 retroviral vector (**Figure 1**), which was chosen for this clinical trial based on its excellent safety record in treating greater than 200 participants.

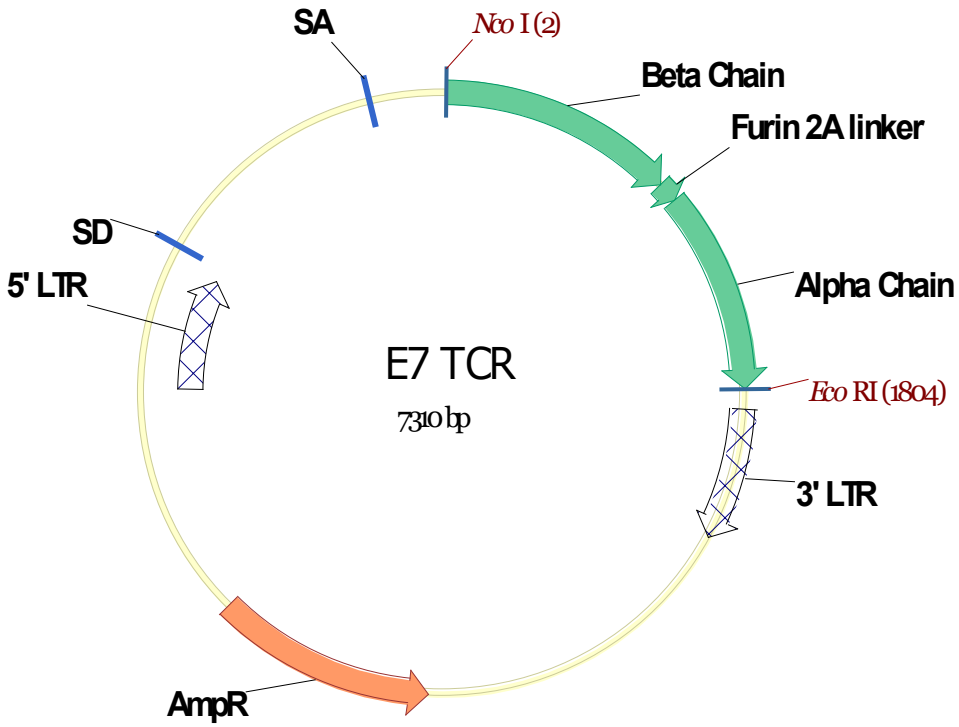


Figure 1: E7 TCR vector map. A TCR targeting $E7_{11-19}$ was isolated from the cervix-infiltrating lymphocytes of a participant with cervical intraepithelial neoplasia. The nucleotide sequence of the alpha and beta chains was determined, codon optimized for expression in human tissues, and the constant regions swapped for their mouse counterparts. A MSGV1 retroviral vector encoding this TCR was constructed. This retroviral vector consists of 7,310 base pairs and includes a 5'LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites (SA). Alpha and beta chains of the E7 TCR are linked by a furin 2A peptide.

Peripheral blood T cells transduced to express the E7 TCR display high avidity for the $E7_{11-19}$ peptide (**Figure 2**) and CD8-independent HLA-A*02:01/$E7_{11-19}$ tetramer binding (**Figure 3**). They specifically recognize a panel of HPV-16+ HLA-A*02:01+ cervical and oropharyngeal cancer cell lines but not cell lines that lack HLA-A*02:01 or HPV-16 (**Figure 4**). Thus, gene engineered T cells expressing the E7 TCR can specifically target HPV-16+ HLA-A*02:01+ cancers. In contrast to TCRs that have had unexpected cross-reactivity against normal human proteins, this TCR was isolated directly from a human T cell. Hence, it was subjected to thymic selection and is unlikely to possess avid reactivity against self-antigens. The complementarity determining regions of the TCR have not been modified; therefore, there is no chance that cross-reactivity has been artificially introduced. The target epitope is derived from a viral protein, and no more than 6 of its 9 amino acids are shared with any human protein (**Table 1**). There is no cross reactivity of this TCR with epitopes of human proteins that share six amino acids or five amino acids plus a conservative amino acid substitution (**Figure 5**). In addition, alanine scanning of $E7_{11-19}$ identified four important residues for recognition (**Figure 6**). Cross reactivity was not detected against epitopes of human proteins that shared these residues (**Table 1**).

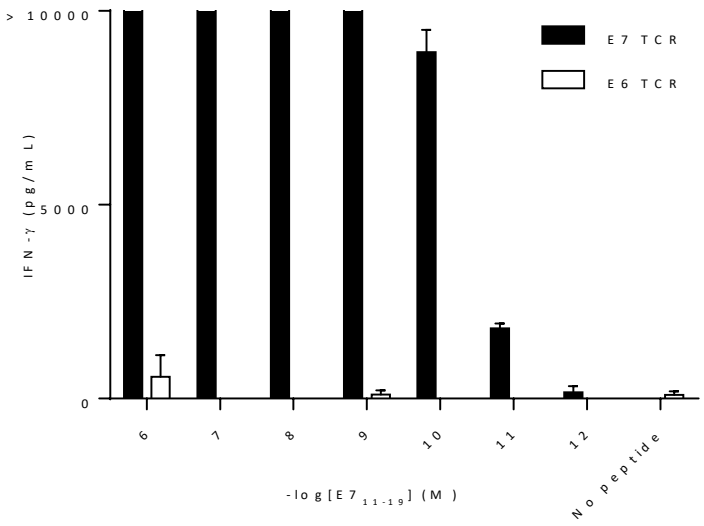


Figure 2: T cells transduced to express the E7 TCR demonstrated high avidity for the E7$_{11-19}$ peptide. T cells from PBMCs were transduced to express the E7 TCR. Functional avidity was tested by co-culture with T2 cells pulsed with titrated concentrations of E7$_{11-19}$ peptide.

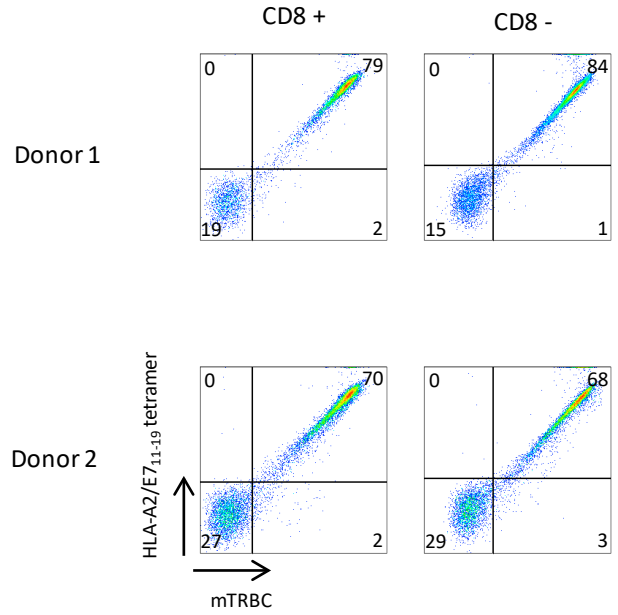


Figure 3: Peripheral blood T cells transduced to express the E7 TCR display CD8-independent HLA-A*02:01/E7$_{11-19}$ tetramer binding. T cells from PBMCs were transduced to express the E7 TCR. Dot plots shown are gated on PI- lymphocytes and either CD8+ or CD8- cells as indicated above the dot plots. The x-axis is mouse T cell receptor beta chain expression. The y-axis is tetramer binding.

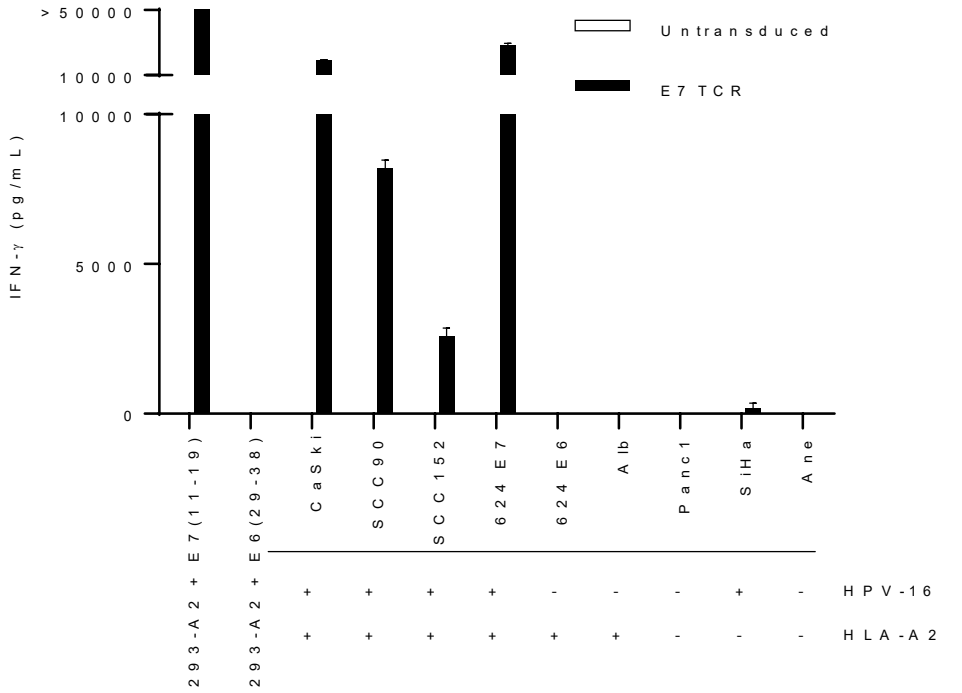
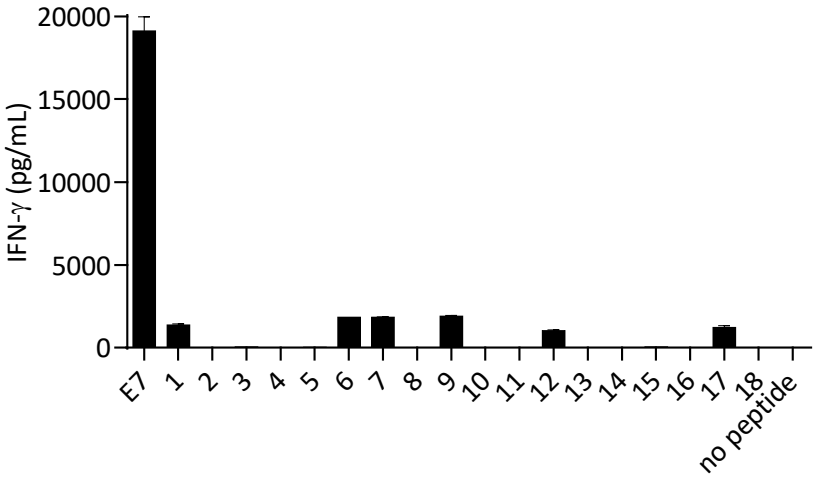


Figure 4: T cells transduced to express the E7 TCR specifically recognized HPV-16+ HLA-A*02:01+ tumor lines T cells transduced with E7 TCR were cocultured with targets expressing HPV-16 and HLA-A2 or with negative controls. Target cell line expression of HPV-16 and HLA-A2 is indicated below each label on the x-axis.



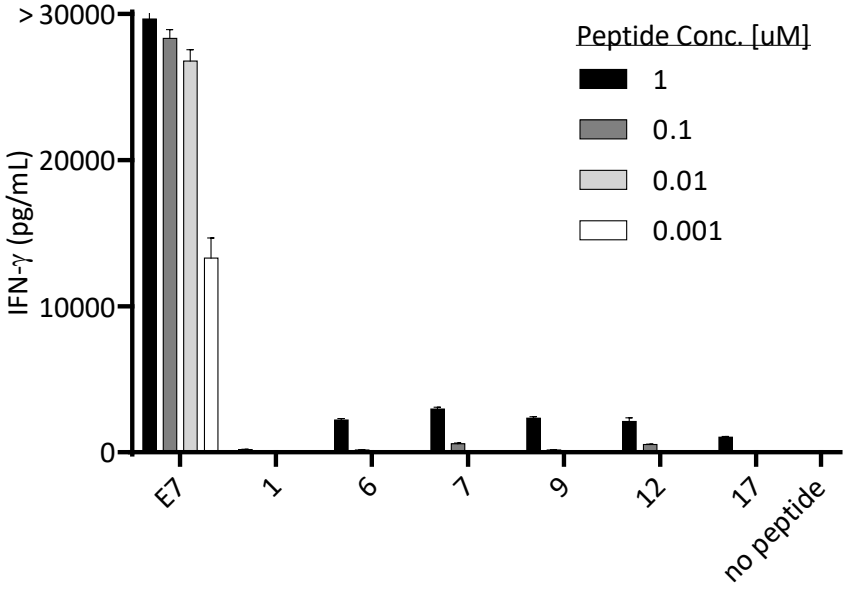


Figure 5: E7 TCR transduced T cells did not show cross reactivity against human peptides. E7 TCR transduced human T cells were tested for recognition of peptides identified by the BLAST search shown in **Table 1**. Target cells were T2 cells loaded with either the E7 peptide (E7) as a positive control, peptides identified by number in **Table 1**, or no peptide (A). Peptides which elicited a weak response by E7TCR were further tested for recognition at titrated concentrations (B).

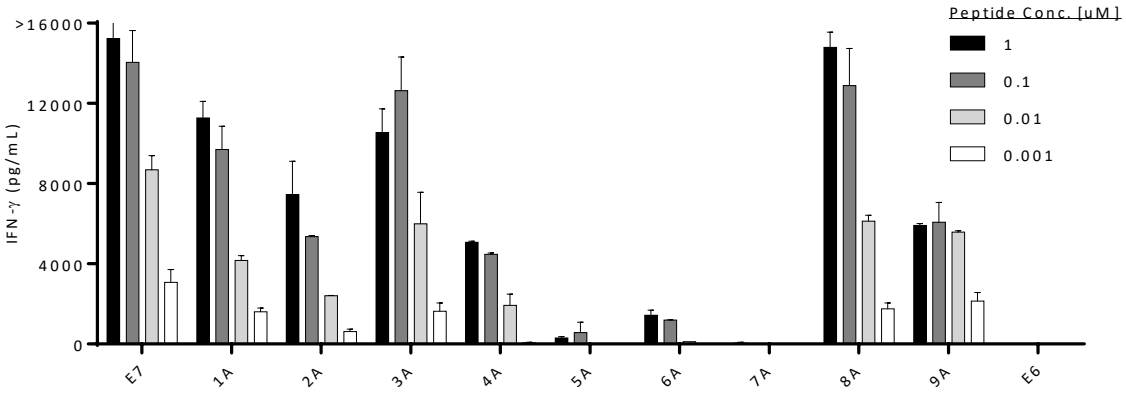


Figure 6: Serial alanine substitutions to the $E7_{11-19}$ target peptide revealed positions 4-7 to be the most crucial for recognition by E7 TCR transduced T cells. Human T cells were transduced to express the E7 TCR. The transduced cells were co-cultured with T2 cells loaded with varying concentrations of $E7_{11-19}$ peptide (E7) or $E7_{11-19}$ with an alanine substitution at the position indicated by the x-axis labels. E7 peptide (E7) is an HLA-A2 restricted negative control peptide.

Peptide No.	Protein	Sequence
	E7 (11-19)	YMLDLQPET
1*	endophilin-B1 isoform 4 [Homo sapiens]	YMLDLQkql
2	uncharacterized serine/threonine-protein kinase SBK3 [Homo sapiens]	gLLDLdPET
3	zinc finger protein 236 [Homo sapiens]	aMLDLEPQh
4	zinc finger protein GLIS1 [Homo sapiens]	sgLgLQPET
5	tensin-1 [Homo sapiens]	lMLDLEPas
6*	clathrin coat assembly protein AP180 isoform c [Homo sapiens]	dLLDLQPDf
7*	translational activator GCN1 [Homo sapiens]	mgLDLQPDl
8	phosphatidate phosphatase LPIN3 isoform X2 [Homo sapiens]	agaDLQPDT
9*	GH3 domain-containing protein isoform 3 precursor [Homo sapiens]	lgLNLQPEq
10	GH3 domain-containing protein isoform 1 precursor [Homo sapiens]	elLNLQPEq
11	protocadherin alpha-9 isoform 2 precursor [Homo sapiens]	lsyELQPET
12*	integrin alpha-IIb preproprotein [Homo sapiens]	YiLDIQPQg
13	tripartite motif-containing protein 66 [Homo sapiens]	pvsDMQPET
14	neural cell adhesion molecule L1 isoform 3 precursor [Homo sapiens]	tqwDLQPDT
15	receptor-type tyrosine-protein phosphatase S isoform X8 [Homo sapiens]	vitNLQPET
16	collagen alpha-1(XII) chain long isoform precursor [Homo sapiens]	meiNLQPET
17*	sacsin isoform 2 [Homo sapiens]	nrLDLQPDl
18	protein AHNAK2 [Homo sapiens]	isgDLQPDT

Peptide No.	Protein	Sequence
	E7 (11-19)	YMLDLQPET
19	Hermansky-Pudlak syndrome 1 protein isoform X8 [Homo sapiens]	pAvDLQPpA
20	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase epsilon-1 isoform 2 [Homo sapiens]	eLiDLQPlI
21	dystrophin isoform X9 [Homo sapiens]	rLsDLQPQI
22	junctophilin-4 [Homo sapiens]	iAqDLQPmL
23	Werner syndrome ATP-dependent helicase [Homo sapiens]	iLqDLQPfL
24	fibronectin isoform 6 preproprotein [Homo sapiens]	tLsDLQPgV

Table 1: BLAST search for peptides with at least 6 (or at least 5 identical + 1 conservative change) amino acids shared with $E7_{11-19}$. Capital/Underlined = Amino Acid Identical to E7 epitope, Capital/Not Underlined = Conservative change. *=Peptides that demonstrates weak cross-reactivity only at supraphysiological concentrations.

1.2.3 E7 TCR T cell clinical trial

We conducted a phase I dose-escalation study of HPV-16 E7 TCR T cells for participants with metastatic HPV16+ cancers (e.g., cervical cancer, anal cancer, head and neck cancer, etc.). Participants received a conditioning regimen of cyclophosphamide 60 mg/kg or 30 mg/kg daily for two days and fludarabine 25 mg/m^2 daily for five days. Cell infusion was followed by high-dose aldesleukin. Objective tumor responses were experienced in 6 of 12 participants. One dose-limiting toxicity occurred in a participant with impaired lung function from rapidly progressing cancer in the lungs. The participant experienced severe lung, cardiovascular, and kidney toxicity that required temporary mechanical ventilation, pressors, and hemodialysis, that resulted in soft tissue injury to the distal lower extremities. No T-cell-mediated off-target toxicity was observed. The maximum administered dose, 1 x 10^{11} TCR+ E7 T cells, was selected as the dose for the phase II portion of the study which is currently ongoing. Six participants have been treated in the phase II portion of the study as of the writing of this protocol, with 3 participants having experienced objective responses.

1.2.4 Safety Considerations

The safety of infusion of large numbers of retrovirally modified tumor reactive T cells has been demonstrated in prior clinical studies. Protocols at the NIH Clinical Center have administered over 1 X 10^{11} tumor infiltrating lymphocytes (TIL) with widely heterogeneous reactivity including CD4, CD8, and NK cells. Further, after treating more than 200 participants with advanced cancers with genetically engineered T cells, NIH studies have not identified a risk of malignant transformation in this setting to date.

The risk of insertional mutagenesis is a known possibility using retroviral vectors. It has been observed in the setting of CD34+ hematopoietic stem cells for the treatment of XSCID, WAS, and X-CGD. It has also been reported with lentiviral transduction in a participant who received CD19 CAR-T cells (it did not cause malignant transformation and may have enhanced the efficacy of the T cells) [24]. With retroviral vector-mediated gene transfer into mature T cells, there has been no evidence of malignancy due to genotoxicity since the first NCI sponsored gene transfer study in 1989. Although continued follow-up of all gene therapy participants will be required, data support the safety of retrovirally transduced mature T cells [25]. While the risk of insertional mutagenesis is low, the proposed protocol follows all current FDA guidelines regarding testing and follow up of participants receiving gene transduced cells [26]. To increase the safety of this protocol, the dose of E7 TCR T cells will be approximately 3.0 x 10^{10}, which is several-fold lower than the maximum dose that was found to be safe in the phase I/II protocol. In addition, the dose of cyclophosphamide will be 30 mg/kg, which is half the dose administered to some participants in the prior phase I/II study, and the maximum doses of aldesleukin will be decreased by 50% to 6.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Participants with histologically or cytologically confirmed carcinoma of the cervix that has not been treated, with clinical staging as follows:

- Lead-in safety cohort: FIGO stage IIIC-IVA (2018 International FIGO Staging System)
- After lead-in safety cohort: FIGO stage IIB-IVA (2018 International FIGO Staging System)

2.1.1.2 HPV16+ tumor and HLA-A*02:01+ HLA type. **Note:** HLA-A*02 is also acceptable for enrollment but not for treatment.

2.1.1.3 Measurable disease by RECIST 1.1 criteria or PERCIST (if not eligible by RECIST 1.1).

2.1.1.4 Age 18 years. Because no dosing or adverse event data are currently available on the use of E7 TCR T cells in participants <18 years of age, children are excluded from this study. **Note:** This age range is consistent with the age of participants with the disease being studied.

2.1.1.5 ECOG performance status 0 or 1 (see **Appendix 1**).

2.1.1.6 Women of child-bearing potential must have a negative pregnancy test because E7 TCR T cells have unknown potential for teratogenic or abortifacient effects. Women of child-bearing potential are defined as all women who are not post-menopausal or who have not had a hysterectomy. **Note:** Postmenopausal will be defined in this study as women over the age of 55 who have not had a menstrual period in at least 1 year.

2.1.1.7 The effects of E7 TCR T cells on the developing human fetus are unknown. For this reason and because the chemotherapy agents used in this trial are known to be teratogenic, women of child-bearing potential must agree to use adequate contraception (e.g., intrauterine device, hormonal or barrier method of birth control; abstinence; tubal ligation or vasectomy) prior to study entry and for four months after treatment. Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.

2.1.1.8 Seronegative for HIV antibody. The experimental treatment being evaluated in this protocol depends on an intact immune system. Participants who are HIV seropositive can have decreased immune-competence and thus be less responsive to the experimental treatment.

2.1.1.9 Seronegative for hepatitis B antigen and hepatitis C antibody. If hepatitis C antibody test is positive, then the participant must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.

2.1.1.10 Must be willing to participate in Gene Therapy Long Term Follow up Protocol (20C0051), which will follow participants for up to 15 years per Food and Drug

Administration (FDA) requirements.

2.1.1.11 Participants must have organ and marrow function as defined below:

• leukocytes	≥3,000/mcL
• absolute neutrophil count	≥1,500/mcL
• platelets	≥100,000/mcL
• hemoglobin	≥9.0 g/dL
• total bilirubin	within normal institutional limits except in participants with Gilbert's Syndrome who must have a total bilirubin < 3.0 mg/dL
• AST(SGOT)/ALT(SGPT)	Serum ALT/AST < 2.5X ULN
• creatinine clearance	Calculated creatinine clearance (CrCl) ≥50 mL/min/1.73 m^2 for participants with creatinine levels above institutional normal (by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation)

2.1.1.12 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Previous treatment for invasive cervical cancer including:

- Chemotherapy or other systemic treatments
- Radiation therapy
- Hysterectomy (prior LEEP procedure or cone biopsy is allowed)

2.1.2.2 Participants who are receiving any other investigational agents.

2.1.2.3 History of severe allergic reactions attributed to compounds of similar chemical or biologic composition to agents used in study.

2.1.2.4 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations at the time of treatment that would limit compliance with study requirements.

2.1.2.5 Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with E7 TCR T cells, breastfeeding should be discontinued if the mother is treated with E7 TCR T cells. These potential risks may also apply to other agents used in this study.

2.1.2.6 Participants with any form of systemic immunodeficiency, including acquired deficiency such as HIV or primary immunodeficiency such as Severe Combined Immunodeficiency Disease, are ineligible. The experimental treatment being evaluated in this protocol depends on an intact immune system. Participants who have decreased

immune competence may be less responsive to the treatment.

2.1.2.7 Participants on immunosuppressive drugs including corticosteroids.

2.1.2.8 Participants with autoimmune diseases such as Crohn's disease, ulcerative colitis, rheumatoid arthritis, autoimmune hepatitis, autoimmune pancreatitis, or systemic lupus erythematosus. Hypothyroidism, vitiligo and other minor autoimmune disorders are not exclusionary.

2.1.2.9 Participants with a second invasive malignancy requiring treatment within the last 2 years are not eligible with the following exceptions:

- Ductal carcinoma in situ (DCIS) of the breast
- Cutaneous skin cancers requiring only local excision

2.1.3 Recruitment Strategies

Participants for this protocol will be recruited via standard CCR mechanisms, including that this protocol may be abstracted into a plain lay language announcement posted on NIH websites and social media platforms, physician and self-referrals as well as various advertising venues. All other specific recruitment advertisements and letters will be submitted to the IRB for approval prior to their implementation.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the consent for this study for screening. Results from outside laboratories are accepted for screening evaluations provided that they were performed in the appropriate timeframe.

2.2.2.1 Any time prior to initiation of study therapy:

- HLA-A*02:01 testing. Tests from CLIA approved laboratories outside the NIH are acceptable. **Note:** HLA-A*02 is also acceptable for determination of eligibility but HLA-A*02:01 is required prior to initiation of treatment.
- HPV16 genotype testing. Tissue from a previous surgery or biopsy may be used or a new biopsy may be obtained. Tests from MolecularMD laboratories outside the NIH are acceptable.
- Confirmation of cervical cancer (pathology report from outside institution is acceptable)

2.2.2.2 Any time within 3 months prior to confirmation of eligibility:

- HBsAg, anti-HCV Antibody, and anti-HIV-1/2 Antibody. Results from outside the NIH are acceptable. (Within 3 months prior to initiation of study therapy or 7 days prior to cell product collection if results are from outside the NIH)
- Anti-HTLV-I/II, Anti-Hbc Antibody, West Nile Virus, HIV-1/HCV/HBV NAT, T.cruzi Antibody.
- Anti CMV antibody titer, HSV serology, and EBV panel (unless known to be positive by a prior test).

2.2.2.3 Any time within 4 weeks prior to confirmation of eligibility:

- History and physical exam, including vital signs, review of performance status and concomitant medications, and a review of the medical record to confirm disease diagnosis and stage.
- Chem 20 equivalent: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (Bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid).
- Thyroid Panel
- CBC with differential and TBNK
- PT/PTT
- Imaging studies including CT scans, MRI scans*, and PET scans may be obtained to confirm staging or if clinically indicated.
- Pregnancy test
- Electrocardiogram (ECG)

***NOTE:** MRIs done in this study may involve the use of contrast agent gadolinium, unless contraindicated.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

2.3.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

2.3.2 Treatment Assignment and Randomization Procedures

Cohorts

Number	Name	Description
1	*Lead-in Cohort*	*Participants with histologically or cytologically confirmed advanced cervical cancer (FIGO (2018) stage IIIC-IVA)*
2	*Post- Lead-in Cohort*	*Participants with histologically or cytologically confirmed advanced cervical cancer (FIGO (2018) stage IIB-IVA)*

Arms

Number	Name	Description
1	*Arm 1*	*E7 TCR T cell Therapy*

Randomization and Arm Assignment

Participants in Cohorts 1 and 2 will be directly assigned to Arm 1.

2.4 BASELINE EVALUATION

Tests performed as part of screening may not need to be repeated if they were performed within the specified window at the discretion of the PI.

2.4.1 Within 30 days prior to apheresis:

- Complete physical examination, including weight and vital signs
- Vein assessment (per apheresis clinic policy)
- Chemistries: Chemistries Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid
- CBC with differential and platelet count
- PT/PTT
- Urinalysis

2.4.2 Any time prior to starting cyclophosphamide:

- Research tumor biopsy (optional)
- Research blood draw

2.4.3 Within 4 weeks prior to starting cyclophosphamide:

- Clinical staging, which may include imaging (e.g., CT scan, MRI scan, PET scan) and/or exam under anesthesia. Scans from referring centers may be used if read by an NIH Clinical Center radiologist.

- Tumor measurements with documentation of the T and N stage and the overall disease stage. Scans from referring centers may be used if the NIH radiologist can obtain reliable tumor measurements.
- Chest x-ray
- ECG
- Cardiac and/or pulmonary testing (e.g., cardiac stress test, echocardiogram, or pulmonary function tests) for participants at elevated risk such as those with COPD, diabetes, hyperlipidemia, hypertension, a history of smoking, or age >60. Participants with cardiac ischemia will not be treated. Participants with a clinically significant arrhythmia or LVEF <45% will be evaluated by a cardiologist. Participants with a FEV1/FVC<70% or FEV1< 80% will be evaluated by a pulmonologist. These tests may be done at screening or at baseline and may be performed at outside institutions. If performed at screening, they do not need to be repeated at baseline as long as they are done within the appropriate timeframe.
- TBNK
- Thyroid panel

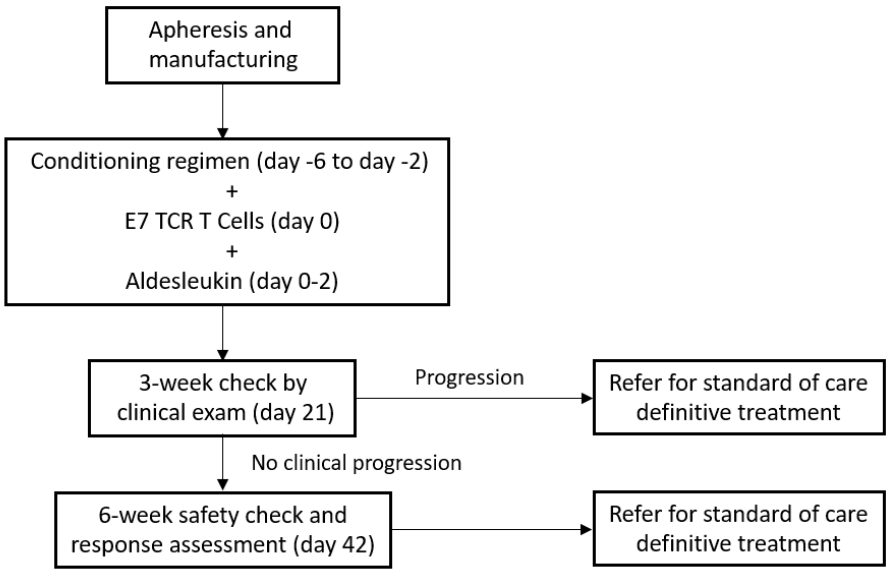
2.4.4 Within 1 week prior to starting cyclophosphamide:

- Complete physical examination, including weight and vital signs
- Chemistries: Chemistries Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid
- CBC with differential and platelet count
- PT/PTT, Fibrinogen and Triglycerides
- Baseline oxygen saturation
- Beta-HCG pregnancy test (serum or urine) for all women of child-bearing potential
- ECOG assessment

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a pilot study testing the feasibility of induction E7 TCR T cell therapy. The study schema is as follows:



3-week (+/- 4 days) check for clinical progression by clinical exam (see below) will be performed at the NIH.

6-week (+/- 4 days) response and safety assessment will be performed at the NIH Clinical Center.

The 3-week clinical check will include a physical exam, adverse event assessment and evaluation by gynecologic oncology to assess for possible hyper-progression.

Tumor measurements at the 6-week response assessment will be performed as described in section **6.4**.

All participants will be referred for standard of care definitive treatment, regardless of response, following the 6-week safety check and response assessment.

3.1.1 Leukapheresis

The participant will undergo a 10-15 liter leukapheresis (generally, 12 liters will be processed to target a yield of 6-10 $x10^9$ lymphocytes) in the Department of Transfusion Medicine (DTM) Dowling Apheresis Clinic according to DTM standard operating procedures. This procedure may occur on this protocol, or protocol 16C0061, if the participant chooses to co-enroll on that protocol. The procedure requires dual venous access and takes approximately 3-4 hours to complete. A central line will be placed if peripheral venous access is not sufficient. Leukapheresis material that is not required for clinical use will be retained and cryopreserved in 10 vials at $100x10^6$ cells per vial with remaining cells stored at $300x10^6$ cells per vial for research and banked on protocol 16C0061 (Tissue Procurement Protocol) if the subject is co-enrolled on that study.

3.1.2 E7 TCR T cell Preparation

After cells are obtained by apheresis (either on this protocol or protocol 16C0061 if the participant has co-enrolled on that protocol), further cell processing to generate E7 TCR cells will occur in the DTM according to standard operating procedures and the E7 TCR investigational new drug application. If apheresis has been performed on protocol 16C0061 and

the participant consents and is eligible for treatment on this study, cells will be transferred to this study and all cell preparation will occur as part of this protocol. Any unused cells from this protocol can be transferred to 16C0061 and banked for research if a participant is co-enrolled. E7 TCR cells can be produced in approximately 11 to 15 days. Cell products may be cryopreserved during production to accommodate participant treatment schedules. Either freshly-collected cells or cryopreserved cells can be used to initiate the cell-preparation process. Peripheral blood mononuclear cells (PBMC) will be isolated. Sufficient cells for three complete cell productions (2-3 vials at 3-4.5 x 10^9 cells/vial) may be retained in the DTM; the remaining cells may be frozen in 10 vials at 100 x 10^6 cells per vial with excess frozen at 300 x 10^6 cells/vial. Cells will be frozen in the DTM and then transferred to the Blood Processing Core (BPC) where they may be frozen and stored. The contact for the Blood Processing Core (BPC) is pager is 11964, tel # 240 760 6180.

Before infusion, the percentage of T cells expressing the E7 TCR will be determined by flow cytometry. In addition to flow cytometry, further testing of the cells will take place prior to infusion to evaluate for microbial contamination, replication-competent retroviruses, and viability. Details of this testing can be found in the appropriate DTM SOPs. Any remaining cryopreserved pretreatment PBMC collected on this protocol will be transferred from the Department of Transfusion Medicine to the Principal Investigator of this protocol for storage in the Blood Processing Core (BPC) and possible use in research and banked according to protocol 16C0061 (Tissue Procurement Protocol) if subject co-enrolled on that study.

3.1.3 Treatment Phase

PBMC will be obtained by leukapheresis (approximately 2x10^9 to 1x10^{10} cells are obtained). PBMC will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth. Transduction is initiated by exposure of approximately 10x10^6 to 500x10^6 cells to supernatant containing the E7 TCR retroviral vector. These transduced cells will be expanded and tested for their anti-tumor activity. Successful TCR gene transfer will be determined by FACS analysis for the TCR protein. Successful TCR gene transfer for each transduced peripheral blood lymphocyte (PBL) population will be defined as greater than 10% TCR positive cells. Participants will receive up to 3x10^{10} E7 TCR T cells (i.e. TCR+ cells). There is no lower limit of the cell dose as participants will have already received conditioning chemotherapy and it would not be in their interest to withhold a cell product, regardless of dose, as long as it meets release criteria. A central line catheter may be used for the intravenous infusion of E7 TCR T cells.

Prior to receiving the engineered PBL cells, participants will receive a non-myeloablative, but lymphocyte depleting, preparative regimen consisting of cyclophosphamide and fludarabine, on days -6 to -2 before the intravenous infusion of *in vitro* tumor reactive TCR gene-transduced PBL plus IV high dose aldesleukin, as indicated in section **3.2**. Participants will receive one course of treatment. The start date of the course will be the start date of the chemotherapy; the end of treatment date will be at the 3-week check by clinical exam.

3.1.4 Lead-in cohort

The first two participants treated in the lead-in cohort will be staggered by 2 weeks in order to monitor for acute and sub-acute adverse events.

3.2 DRUG ADMINISTRATION

Treatment schedule will be according to the following schedule (see Schedules **3.2.4**). Times are offered as examples and may be changed as long as a similar time relationship between administrations of the drugs is maintained. Study medication start times for drugs given once daily may be within 2 hours of the scheduled time (once it is established at the administration of the first medication). All other medications may be given +/- one (1) hour of the scheduled time; the length of administration may be +/- 15 minutes. Administration of diuretics, electrolyte monitoring and replacement, and hydration should all be performed as clinically indicated – the times noted below are offered only as examples. Chemotherapy infusions maybe slowed or delayed as medically indicated. Intravenous hydration administered during cyclophosphamide will be individualized for participant clinical factors. Participants at risk of adverse clinical consequences from volume overload (e.g., participants with history of pulmonary hypertension or cardiac dysfunction) may be considered for low-dose hydration rates or hemorrhagic cystitis prevention strategies that include mesna alone without intravenous hydration.

3.2.1 Preparative Regimen

The following will comprise a course of therapy for Day -6 through Day -2:

Day -6 and -5:

11 am: Hydrate (if applicable). Begin hydration with 0.9% sodium chloride injection with or without 10 meq/L of potassium chloride at 90 ml/m^2/hour (recommend starting at least 6 hours pre-cyclophosphamide and continue hydration until 24 hours after last cyclophosphamide infusion). Furosemide 10-20 mg IV may be given once daily on cyclophosphamide treatment days to promote diuresis. At any time during the preparative regimen, if the urine output <1.5 mL/kg/hour or if body weight >2 kg over pre-cyclophosphamide value, additional doses of furosemide 10-20 mg IV may be administered. The hydration rate will be capped at 100 mL/hr. The rate of hydration and total time of hydration may be reduced or increased based on urine output and other clinical considerations per the clinical team.

4 pm: Ondansetron (0.15 mg/kg/dose [*rounded to the nearest even mg dose between 8 mg and 16 mg based on participant weight]* IV q 8 hours X 3 days), olanzapine (10 mg PO once daily for 5 days) and aprepitant (125 mg PO on the first day, 80 mg PO daily the following 2 days) will be utilized for prophylaxis for chemotherapy induced nausea and vomiting. Modifications to the antiemetic regimen may be made per clinical team discretion, but corticosteroids should be avoided.

5 pm: Cyclophosphamide 30 mg/kg/day X 2 days IV in 250 mL D5W with mesna 15 mg/kg/day over 1 hour X 2 days. If the participant is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 2.

Begin mesna infusion at 1.5 mg/kg/hour intravenously diluted in a suitable diluent over 23 hours after each cyclophosphamide dose. If the participant is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 2.

Day -6 to Day -2:

Fludarabine 25 mg/m^2/day IVPB daily over 15-30 minutes for 5 days.

If the participant is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Table 2**.** (*The fludarabine will be started approximately 1-2 hours after the cyclophosphamide and mesna on Days -6 and -5*)

3.2.2 Cell Infusion and Other Treatment Administration

The E7 TCR cells will be delivered to the participant care unit by an authorized staff member. Prior to infusion, the cell product identity label is double-checked by two authorized staff (e.g., MD or RN), an identification of the product and documentation of administration are entered in the participant's chart, as is done for blood banking protocols. The dose of E7 TCR T cells will be 3 x 10^{10} TCR+ T cells (unless fewer cells are generated) administered once.

Day 0 (one to three days after the last dose of fludarabine):

- E7 TCR T cells will be administered intravenously over 20 to 30 minutes via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.
- Aldesleukin as described in section **3.2.3** below.

Day 0-4 (Day 0 is the day of cell infusion):

- Fluconazole may be used at the discretion of the treating clinician
- Valacyclovir or Acyclovir; see section **4.2.2**

Day 0-2 (Day 0 is the day of cell infusion):

- Aldesleukin as described in section 3.2.3 below.

Discharge:

PJP prophylaxis may be started at or around the time of discharge. TMP/SMX may be administered at 160mg/800mg every other day or monthly inhaled pentamidine per treating clinician discretion. Refer to section **4.2.1**.

3.2.3 Aldesleukin

Aldesleukin will be administered at a dose of 720,000 IU/kg (based on total body weight) as an intravenous bolus over a 15-minute period beginning within 24 hours after cell infusion and continuing for up to four days (maximum 6 doses). The start of aldesleukin treatment may be delayed up to 3 days after cell infusion if medically necessary. Doses will be preferentially administered every eight hours; however, up to 24 hours may elapse between doses depending on participant tolerance. Aldesleukin dosing will be stopped if toxicities are not sufficiently recovered with supportive measures within 24 hours of the last dose of aldesleukin. Doses will be delayed or stopped if participants reach Grade 3 or 4 toxicity due to aldesleukin except for the reversible Grade 3 toxicities common to aldesleukin such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix 3. Toxicities will be managed as outlined in Appendix 2. In addition, dosing may be held or stopped at the discretion of the treating investigator. Because confusion is a possible side effect of aldesleukin administration, a Durable Power of Attorney may be signed by the participant to identify a surrogate to make decisions if a participant becomes unable to make decisions.

3.2.4 Treatment Schedule Schema/Chart

Therapy	Day										
	-6	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Cyclophosphamide 30 mg/kg IV once daily x 2 days	X	X									
Ondansetron 0.15 mg/kg IV every 8 hours x 3 days	X	X	X								
Olanzapine 10mg PO once daily x 5 days	X	X	X	X	X						
Aprepitant 125 mg PO X1, 80 mg PO daily X2	X	X	X								
Mesna 1.5 mg/kg/hour	X	X									
Fludarabine 25 mg/m IV once daily x 5 days	X	X	X	X	X						
E7 TCR cells							X				
Aldesleukin							X	X	X		

3.3 SAFETY PROTOCOL STOPPING RULES

The study will be halted (immediately stop accrual and treatment) if any of the following occur:

1. Death that occurs within 30 days of E7 TCR T cell injection (other than death related to progressive disease).
2. Grade 3 or 4 toxicity that occurs within 30 days of E7 TCR T cell infusion and that does not resolve to Grade 2 or less within 10 days. **Note:** This applies to any event (s) that is not attributable to the preparative regimen, aldesleukin, underlying disease, or unrelated circumstances including standard of care therapy if administered within 30 days after cell infusion.
3. Feasibility failure in 2 of the first 5 participants or in any 3 participants.

The study will remain on hold until an appropriate evaluation of the cause is determined and a plan of correction, if necessary, is established.

3.4 STUDY CALENDAR

Procedure	**Screening**	**Baseline[4]**	**Before treatment[27]**	**Preparative regimen[7]**	**Day 0**	**During hospitalization[8]**	**Follow-up Period**		
							3-week check by clinical exam[1, 24]	**6-week safety check and response assessment[11]**	**Annual Follow-up[25]**
Medical history	X								X

Procedure	Screening	Baseline[4]	Before treatment[27]	Preparative regimen[7]	Day 0	During hospitalization[8]	Follow-up Period		
							3-week check by clinical exam[1, 24]	6-week safety check and response assessment[11]	Annual Follow-up[25]
Physical exam	X	X			X	X[23]	X	X	
Vital signs	X	X			X[22]	X	X	X	
ECOG Performance Score		X							
NIH Advance Directives Form[2]	X								
Blood chemistries[10]	X	X	X	X		X[23]		X	
Complete blood count (CBC with diff.)	X	X	X	X		X		X	
Thyroid panel (TSH, FT3, FT4)	X	X						X	
HLA typing/HPV genotype testing[18]	X								
TBNK	X	X	X			X[9]		X	
Urinalysis		X		X					
HBsAg, anti-HCV Antibody, and anti-HIV-1/2 Antibody[19]	X								
Anti-HTLV-I/II, Anti-Hbc Antibody, West Nile Virus, HIV-1/HCV/HBV NAT, T.cruzi Antibody [20]	X								
Anti CMV antibody titer, HSV serology, and EBV panel [20]	X								
Pregnancy test in women of childbearing potential[5]	X	X							
Leukapheresis			X[16]						
Biopsy		X						X[26]	
Correlative research studies	X		X		X[14]	X[14]	X	X	
E7 TCR assay[17]						X	X	X	X
Cardiac evaluation[12]		X							
Chest x-ray		X							
ECG	X	X							
Chest CT and MRI or PET [21]	X	X						X	

Procedure	Screening	Baseline[4]	Before treatment[27]	Preparative regimen[7]	Day 0	During hospitalization[8]	Follow-up Period		
							3-week check by clinical exam[1, 24]	6-week safety check and response assessment[11]	Annual Follow-up[25]
Pulmonary Function Tests[3]		X							
Response evaluation								X	
Adverse events			X	→	→	→	→	→	→
Infusion of transduced cells[6]					X				
Additional apheresis or blood draw[13,15]							X		

1. End of treatment visit will occur 3 weeks (21 days +/- 4 days) after the E7 TCR T cell infusion, or when the participant comes off treatment if before 21 days. If the participant cannot return to the Clinical Center for this visit, a request will be made to collect required clinical labs (specify as needed) from a local physician or laboratory. If this is not possible, participants may be assessed by telephone for symptoms.
2. As indicated in section **12.3**, all subjects ≥ age 18 will be offered the opportunity to complete an NIH advance directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended but is not required.
3. For participants with a prolonged history of cigarette smoking, as clinically indicated in section **2.4.3**.
4. Exact timeline is indicated in section **2.4**.
5. For women of child-bearing potential as defined in section **2.1.1.7**.
6. See other treatments in Schedules, section **3.1.3**
7. On days -6 to -2
8. Every 1 to 2 days while hospitalized
9. Once total lymphocyte count is greater than 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (while the participant is hospitalized)
10. Chemistries Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid, PT/PTT, Fibrinogen and Triglycerides. After cell infusion, the following labs will only be collected if clinically indicated: Calcium total, Magnesium total (Mg), Phosphorus, LD, Total Protein, Total CK, Uric Acid, PT/PTT, Fibrinogen and Triglycerides.

[11] 6-week response assessment (42 days+/- 4 days) after cell infusion.

[12] For participants who are greater than or equal to 60 years of age, or who have a history of ischemic heart disease, chest pain or clinically significant atrial and/or ventricular arrhythmias. Participants with a LVEF of less than or equal to 45% will not be eligible, as noted in section **2.4.3**

[13] An approximately 5 liter apheresis may be performed at the 3-week check by clinical exam, if the participant is unable to undergo apheresis, approximately 96 mL of blood may be obtained. Peripheral blood mononuclear cells will be cryopreserved so that immunologic testing may be performed and will be banked under protocol 16C0061 (Tissue Procurement Protocol). PBMC from apheresis may be stored in 10 vials at 100x106 cells per vial with remaining cells stored at 300x106 cells per vial.

[14] See section **5.1.2**.

[15] If the participant is unable to undergo apheresis, approximately 96 mL of blood may be obtained.

[16] This can occur at any time prior to treatment on protocol 16C0061 if the participant is co-enrolled on that protocol. See section **3.1.1** for further details.

[17] Clinical assay performed by the NCI Flow Cytometry Laboratory in the Laboratory of Pathology.

[18] See section **2.2**.

[19] Within 3 months of enrollment or 7 days of cell product collection if results are from outside the NIH

[20] Within 3 months of treatment, results not required for enrollment but required for treatment

[21] To confirm staging or if clinically indicated. (Within 4 weeks of enrollment, results not required for enrollment but required for treatment)

[22] After cell infusion (Day +1 to Day +7): Vital signs will be monitored hourly (+/- 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated

[23] As clinically indicated

[24] 21 days (+/- 4 days) after cell infusion.

[25] Participants will be contacted (either by phone or visit to NIH) every 1 year (+/- 1 months) following completion of standard of care therapy for a total of 5 years to determine the dates and types of additional therapies (if any) that participants received after definitive treatment, the status of their disease and recent imaging studies. If participants have not had any imaging within 3 months of the annual follow-up, participants can have imaging performed at the NIH. Participants will also be contacted after completion of chemoradiation to have a tube of blood drawn and tested for persistence of E7 TCR T cells.

[26] Refer to section **5.1.3**.

[27] Within 4 weeks prior to treatment.

3.5 On-Study Evaluations

Please see the study calendar in section **3.4** for details regarding on-study evaluations.

3.5.1 Long-term Follow-Up

Long-term follow-up of participants receiving gene transfer is required by the FDA and must continue even after the participant comes off the study. Long-term follow-up will be done under a different protocol (20C0051) for which the participant will be co-enrolled. Refer to section **5.3**.

3.6 Cost and Compensation

3.6.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.6.2 Compensation

No compensation is provided for participation in this study.

3.6.3 Reimbursement

This study offers subject reimbursement or payment for travel, lodging and/or meals while participating in the research. The amount, if any, is guided by NIH policies and guidelines.

The NCI generally does not cover expenses during screening. If a subject is scheduled for and begins treatment, the NCI will cover the cost for some of the expenses. Some of these costs may be paid directly by the NIH and some may be reimbursed to the subject. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.7 Criteria for Removal from Protocol Therapy and Off Study Criteria

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 21 days following the last dose of study therapy.

3.7.1 Criteria for removal from protocol therapy

- Completion of protocol therapy
- Initiation of standard of care definitive therapy
- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Positive pregnancy test

3.7.2 Off-Study Criteria

- Completed study follow-up period

- Participant requests to be withdrawn from study
- Death
- Screen failure
- Lost to follow-up
- Inability to generate a cell product. A second attempt may be made to generate a cell product from the participant. If the second attempt fails, that participant will be removed from the study and replaced with another participant.
- The investigators decide to end the study
- The investigators decide it is in the participant's best interest
- Substantial participant non-compliance that prevents compliance with the study requirements.
- Permanent loss of capacity to give consent

3.7.3 Lost to Follow-Up

A participant will be considered lost to follow-up if he or she fails to return for three (3) scheduled visits and is unable to be contacted by the study site staff.

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

- The site will attempt to contact the participant and reschedule the missed visit (i.e., ideally within 2-3 days of the missed visit) and counsel the participant 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 participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 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 participant's medical record or study file.
- Should the participant 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.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 PROHIBITED MEDICATIONS

Current use of immunosuppressive medication, EXCEPT for the following:

- Intranasal, inhaled, topical steroids, or local steroid injection (e.g., intra-articular injection);
- Systemic corticosteroids at physiologic doses ≤ 10 mg/day of prednisone or equivalent; or,
- Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication)

4.2 INFECTION PROPHYLAXIS

Note: Other medications may be substituted or medications may be held at the discretion of the treating investigator. Below are guidelines, and suggested medications and schedule to be used; however, they can be altered by the treating physician as clinically indicated.

4.2.1 Pneumocystis Jirovecii Pneumonia

Participants may receive the fixed combination of trimethoprim and sulfamethoxazole [SMX] as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) P.O. daily three times a week on non-consecutive days, beginning at or around the time of discharge from the hospital.

Pentamidine may be substituted for TMP/SMX-DS in participants with sulfa allergies or per investigator discretion. It will be administered aerosolized at 300 mg per nebulizer within one week of chemotherapy start date.

4.2.2 Herpes Virus Prophylaxis

Participants will be given either acyclovir 800mg PO twice daily (preferred) or valacyclovir 500mg PO twice daily (alternate) or, if unable to tolerate PO: acyclovir 250mg/m2 IV q 12 hr. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made, or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g., ganciclovir. In renal disease, the dose is adjusted as per product labeling.

Prophylaxis for pneumocystis and herpes will continue for 6 months. If the CD4 count is less than 200 at six months post-chemotherapy, prophylaxis will continue for at least six months and until the CD4 count is greater than 200 for two consecutive measures. If a participant misses less than 20% of their doses for either medication a protocol deviation does not need to be filed.

4.2.3 Fungal Prophylaxis (Fluconazole)

This will be decided by the treating clinician.

4.2.4 Empiric Antibiotics

Participants will start on broad-spectrum antibiotics as per current institutional guidelines for fever of 38.3°C once or two temperatures of 38.0°C or above at least one hour apart, AND an ANC < 500/mm^3. Infectious disease consultation may be obtained for participants with unexplained fever or infectious complications.

4.3 BLOOD PRODUCT SUPPORT

Using daily CBC's as a guide, the participant will receive platelets and packed red blood cells (PRBC's) as needed. As a general guideline, participants may be transfused for:

- Hemoglobin < 8 gm/dL
- Platelets < 10,000/mm^3

Note: Participants may be transfused at a higher platelet count as clinically indicated, such as:

- Increased risk for bleeding such as undergoing an invasive procedure or presence of metastatic lesion likely to bleed
- fever greater than 38.5°C
- sepsis

All blood products will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection.

4.4 NEUTROPHIL RECOVERY

Participants may receive Filgrastim for count recovery when clinically indicated.

4.5 OTHER CONCOMITANT MEDICATIONS TO CONTROL SIDE EFFECTS

Concomitant medications to control side effects of therapy may be given. Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg q4h), indomethacin (50-75 mg q8h) and ranitidine (150 mg g12h). If participants require steroid therapy they will be taken off treatment. Participants who require transfusions will receive irradiated blood products. Ondansetron 0.15 mg/kg/dose IV every 8 hours may be administered for nausea and vomiting. Additional anti-emetics may be administered as needed for nausea and vomiting uncontrolled by ondansetron. Antibiotic coverage for central venous catheters may be provided at the discretion of the investigator.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

The amount of blood that may be drawn from adult participants for research purposes shall not exceed 10.5 mL/kg or 550mL; whichever is smaller, over any 8-week period.

5.1.1 Pre-cell infusion evaluations

- At baseline/screening 12 CPT tube and 2 SST tubes may be collected. One or more CPT tube(s) may be used to collect 4mL of plasma, which could then be frozen in 4mL vials. PBMC from the remainder of the CPT tubes may be frozen in aliquots of 10 x 10^6 cells/vial. Serum from SST tubes may be aliquoted into four vials of 0.5-1mL each. All samples will be processed in the Blood Processing Core (BPC). Additional research blood may be collected and studied under protocol 16C0061 (Tissue Procurement Protocol).
- At day -6 prior to cell infusion 6 CPT tubes and 1 SST tube may be collected. One or more CPT tube(s) may be used to collect 4mL of plasma, which could then be frozen in 4mL vials. PBMC from the remainder of the CPT tubes may be frozen in aliquots of 10 x 10^6 cells/vial. Serum from SST tubes may be aliquoted into four vials of 0.5-1mL each. All samples will be processed in the Blood Processing Core (BPC).

5.1.2 Post cell infusion evaluations

- 2 SST tube (4mL each) may be collected daily from serum starting on the day of

chemotherapy and continuing through the end of hospitalization. Serum will be processed in the Blood Processing Core (BPC) and may be aliquoted into four vials of 0.5-1 mL each.

- Once total lymphocyte count is greater than 200/mm^3, the following samples may be drawn and sent to the Blood Processing Core (BPC) on Monday, Wednesday, and Friday x 5 days, then weekly (while the participant is hospitalized). Send to the Blood Processing Core (BPC). Attention: NCIBloodcore@mail.nih.gov. Building 10, room 5A08 pager is 11964, tel # 240 760 6180.
    - 6 CPT tubes (8mL each). One CPT tube daily may be used to collect 4mL of plasma, which could then be frozen in 4mL vials. PBMC from the remainder of the CPT tubes may be frozen in aliquots of 10 x 10^6 cells/vial
- Following discharge, at each scheduled follow-up visit 6 CPT tubes and 1 SST tube may be collected. One or more CPT tube(s) may be used to collect 4mL of plasma, which could then be frozen in 4mL vials. PBMC from the remainder of the CPT tubes may be frozen in aliquots of 10 x 10^6 cells/vial. Serum from SST tubes may be aliquoted into four vials of 0.5-1 mL each. All samples will be processed in the Blood Processing Core (BPC).

5.1.3 Tumor Biopsies (optional)

- Biopsy of tumors are optional and will be performed at baseline and approximately 6 weeks following T cell infusion. Biopsies may be performed in the clinic using either direct visualization or indirect visualization based on the preference of the physician performing the biopsy. In cases where the tumor is easily visualized, multiple biopsies may be collected but should not exceed 5 per tumor site. Local anesthetic with lidocaine may be used prior to each biopsy. In cases where the tumor is not accessible using either direct or indirect visualization in the clinic, the biopsy performed in the operating room under general anesthesia. Biopsies will only be performed if it does not interfere with the response assessment and the participant provides consent.
- Specimens may be transported by the assigned research nurse to Dr. Christian Hinrichs' lab for sample labeling. Contact: Nisha Nagarsheth, Bldg 10, room 4B-04, phone 772-349-3229.
- Following labeling, samples will be transported by an assigned lab member to the Blood Processing Core (BPC) where they will be frozen in optimal cutting temperature compound. Contact: Blood Processing Core (BPC) Attention: NCIBloodcore@mail.nih.gov, Building 10, room 5A08 pager is 11964, tel # 240 760 6180.
- Some of these samples will be archived and analyzed under another protocol 16C0061 (Tissue Procurement Protocol) if the subject is also enrolled on that study.

5.1.4 Immunological Testing

- Apheresis may be performed prior to and approximately 3 weeks after the treatment. Apheresis product will be transferred to the Blood Processing Core (BPC) Attention: NCIBloodcore@mail.nih.gov. Building 10, room 5A08 pager is 11964, tel # 240 760

6180. Cell product may be frozen in 10 vials at concentration 100 x 10^6 cells/mL and additional vials at 300 x 10^6 cells/mL.

- At other time points, peripheral blood lymphocytes (PBL) and plasma may be obtained from whole blood by purification using centrifugation. These samples may be transferred directly to the Blood Processing Core (BPC) lab for processing. Plasma may be frozen in 4mL vials. PBL may be frozen in aliquots of 10 x 10^6 cells/vial
- Possible laboratory research studies on tumor biopsies are as follows: Expression of p16, CD3, CD4, CD8, MHC I, and MHC II by immunohistochemistry; flow cytometry to determine the frequency of E7 TCR T cells in the samples; generation and characterization of TIL cells; generation and characterization of tumor cell lines. IHC quantification may be performed and include scoring of the intensity and frequency of staining. Flow cytometry data may be analyzed with FlowJo software.
- Possible laboratory research studies on PBMC and PBL are as follows: Specific cytolysis determined by impedance-based assay, frequency of effector cells as determined by ELISPOT, quantity of cytokine production as determined by coculture assay with cytokine quantification, cytokine production by intracellular flow cytometry, phenotypic analysis by flow cytometry. Immunological assays may be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the T cells cryopreserved at the time of infusion.
- Possible laboratory research on serum or plasma are as follows: HPV DNA quantification, cytokine quantification
- The planned methods for performing the laboratory studies above are as described in Stevanovic, et al, *Journal of Clinical Oncology*, 2015 and Draper, et al, Clinical Cancer Research, 2015. [12, 14]
- The laboratory studies are considered exploratory. Statistical analysis may be performed in consultation with a biostatistician.
- Specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study and protocol 16C0061 (Tissue Procurement Protocol).
- Genomic studies will not be pre-planned and will be conducted under protocol 16C0061 (Tissue Procurement Protocol).

5.2 SUMMARY OF SAMPLE COLLECTION

Test/ assay	**Volume blood (approx)**	**Type of tube**	**Collection point (+/- 48hrs)**	**Location of specimen analysis**
Plasma/ PBMC	48-96 mL	CPT	<4 weeks prior to cell infusion, day-6	Clinical Pharmacology Blood Processing Core (BPC), Bldg 10 room 5A08.

Test/ assay	Volume blood (approx)	Type of tube	Collection point (+/- 48hrs)	Location of specimen analysis
Serum	8-16 mL	SST	<4 weeks prior to cell infusion, day-6	Clinical Pharmacology Blood Processing Core (BPC), Bldg 10 room 5A08.
Plasma/ PBMC	48 mL	CPT	Post cell infusion day 1, 3, every Mon/Wed/Fri x 5 days, weekly until discharge and follow-up visits	Clinical Pharmacology Blood Processing Core (BPC), Bldg 10 room 5A08.
Serum	Variable, based on length of hospitalization and duration of follow-up	SST	Daily at the start of chemo until discharge from hospital, follow-up visits	Clinical Pharmacology Blood Processing Core (BPC), Bldg 10 room 5A08.
Biopsy (optional)	N/A	N/A	Prior to starting chemotherapy and approximately 6 weeks following T cell infusion	Processed in Hinrichs Lab, then transported to Clinical Pharmacology Blood Processing Core (BPC), Bldg 10 room 5A08.

5.3 GENE THERAPY-SPECIFIC FOLLOW-UP

Persistence of TCR transduced cells will be assessed by quantitative PCR and/or flow cytometry at follow-up visits 1, 3, 6 and 12 months after cell infusion, or until TCR-expressing cells are no longer detectable. If any participant shows an increasing population of TCR gene transduced T cells at month six or later (by FACS staining or qPCR), the previously archived samples will be subjected to techniques to identify predominant clonal populations of transduced cells that would suggest transformation. These cells will be obtained from the CPTs drawn for research at follow up visits or under the long-term gene therapy follow up protocol if the participant is off study.

Participants' blood samples will be obtained and undergo analysis for detection of replication competent retroviruses (RCR) by PCR prior to cell infusion and at 3, 6, and 12 months post cell administration. Blood samples will be archived annually thereafter if all previous testing has been negative with a brief clinical history. These cells will be obtained from the CPTs drawn for research at follow up visits or under the separate long-term gene therapy follow up protocol (20C0051) if the participant is off study.

5.4 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management System. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

5.4.1 Samples Managed by Dr. Figg's Blood Processing Core (BPC)

5.4.1.1 Blood Collection

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov

The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.

5.4.1.2 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to participants without Labmatrix access. The data recorded for each sample includes the participant ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Participant demographics associated with the clinical center participant number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.4.2 Hinrichs Laboratory

Samples transferred to the Hinrichs laboratory will be barcoded and tracked with Labmatrix.

Laboratory research data will be stored on the NCI secure server in the Hinrichs laboratory folder with secure access by laboratory personnel only. Access to personally identifiable information (PII) is limited to the PI and study personnel who interact directly with the participant and their samples.

5.4.3 Protocol Completion/Sample Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LabMatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a participant withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the participant, if so requested).. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

Sample barcodes are linked to participant demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to participant information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.5 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

Genomic studies will be conducted under protocol 16C0061 (Tissue Procurement Protocol) if participant is co-enrolled.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system, C3D and Labmatrix, and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

The key for assignment of participant code identification numbers with the personal identifiers will be stored in a secure database. This key will not be shared with other investigators. Investigators conducting the individual sample testing will only have access to coded identification numbers and coded participant information (i.e., treatment regimens, treatment responses, diagnoses, pathology information).

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per

requirements in section **7.2.1**.

6.1.1 Adverse Event (AE) Recording

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day -6, through Day 21. Beyond 21 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

6.1.2 Reporting of Laboratory Events

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the participant's outcome.
- Grade 1 AEs will not be recorded

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded and linked data in an NIH-funded or approved public repository.
- Coded and linked data in BTRIS (automatic for activities in the Clinical Center)
- Identified or coded and linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- An NIH-funded or approved public repository: Clinicaltrials.gov
- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

6.3 GENOMIC DATA SHARING PLAN

The GDS Policy does not apply to this protocol because there are no genomic tests done on this study (i.e., any genomic studies would be done on a separate protocol for participants who are co-enrolled).

6.4 RESPONSE CRITERIA

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) for participants with solid tumors [27]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria. Imaging modalities used in this study will be consistent with current standard of care according to NCCN guidelines. Scans will be obtained at 4-week intervals as outlines in section **3.1.1**

6.4.1 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as:

- By chest x-ray: ≥20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under: as ≥10 mm
    - Scan slice thickness >5 mm: double the slice thickness
- With calipers on clinical exam: ≥10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. 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, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

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

6.4.2 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment 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 is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g., for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the

image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published.[28-30] In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer.[31]

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If

the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

6.4.3 PERCIST Criteria Summary

Note: Participants that do not have measurable disease by RECIST v1.1 may have their disease measured using PERCIST [32-34]. If participants have disease measurable by both criteria, response will be monitored using RECIST v1.1.

Response to Treatment

The PERCIST categories for response to treatment

The PERCIST categories for response to treatment	
Complete metabolic response (CMR)	Complete resolution of FDG uptake in all lesions
Partial metabolic response (PMR)	≥ 30% Reduction of the SULpeak and an absolute drop of 0.8 SULpeak units
Progressive metabolic disease (PMD)	≥ 30% Increase in the SULpeak of the FDG uptake and an absolute increase of 0.8 SULpeak, or appearance of FDG-avid new lesions
Stable metabolic response (SMD)	Does not qualify for CMR, PMR, or PMD

1. **Complete Metabolic Response:** a target lesion must show complete resolution of FDGuptake, with FDG uptake less than the mean SUL of the liver and indistinguishable from that of the surrounding background.
    a. This requires data for all other lesions to return to background levels and that no new FDG-avid lesions in a pattern typical of cancer appear.

b. When no FDG-avid tumor is visible, the mean SUL_{peak} of the anatomic location situated as close as possible to the site of the original tumor should be measured.

c. A normal structure with high FDG uptake, such as a bowel loop that occupies the site of the original target lesion, should be avoided in the measurement of results of the follow-up study.

d. A complete metabolic response according to PERCIST 1.0 does not require the SUL_{peak} to decrease to zero.

2. **Partial metabolic response**: a decrease of greater than or equal to 30% and of at least 0.8 SUL units must be shown between the most intense evaluable lesion at baseline and the most intense lesion at follow-up (not necessarily the same lesion).

    a. Also requires the following:

        i. a decrease in SUL_{peak} of greater than or equal to 0.8 SUL units in the target lesion

        ii. no new FDG-avid lesions in a pattern typical of cancer

        iii. no identifiable increase in size greater than 30% in the target lesion

        iv. no SUL_{peak} or identifiable increase in size greater than 30% in a nontarget lesion.

3. **Stable metabolic disease**: an increase or decrease in SUL_{peak} of less than 30% is required.

4. **Progressive Metabolic Disease**: Lesions must show an increase of greater than or equal to 30% and an increase of at least 0.8 SUL units in a target lesion or development of a new lesion or more than one new lesions.

    a. This can include new FDG-avid lesions in a pattern typical of cancer, an increase in SUL_{peak} or an identifiable anatomic increase in size greater than or equal to 30% in target lesions, or unequivocal progression in nontarget lesions.

*The choice between partial metabolic response (PMR) and stable metabolic disease (SMD) depends on the difference between the percentage of change in SUL and absolute SUL. If the decrease from baseline to follow-up is sufficient, the response is PMR. If the decrease is less than 30% or 0.8 SUL units, the response is SMD

Required Baseline Parameters for PET

- Measurement of the "hottest" single tumor and background area on images, usually of the liver, is required at baseline. (if diseased liver, can use descending thoracic aorta)
- A single target lesion at each time point is selected as the primary parameter on the basis of the concept that the most metabolically active tumor focus corresponds to the most aggressive portion of the tumor, which is the most clinically important area.
- With PERCIST, standardized uptake value corrected for lean body mass (SUL) are used and the maximum over 1cc volumes of interest (SUL_{peak}) is chosen instead of the widely used single-pixel maximum standardized uptake value (SUV_{max}). This fixed-volume approach is used to minimize the effect of differences in voxel sizes, reducing variability

and bias.

Target Lesion at Baseline

- The SUL_{peak} is measured in the single hottest tumor.
- For a tumor to be measurable at baseline, the SUL_{peak} must be greater than or equal to one and a half times the mean SUL in the 3-cm diameter spherical VOI plus two times its standard deviation to have a minimum threshold for evaluation.
- A minimal level of tumor uptake at baseline is proposed to ensure that a decline in FDG uptake with therapy can be measured in the dynamic range of the imager (ie, that it remains higher than background uptake), to decrease the likelihood that a change is due to chance, and to minimize overestimation of response or progression.
- When the SUL_{peak} of the tumor at baseline is lower than this threshold, the tumor is considered not measurable with PERCIST 1.0

Follow-up imaging

- Known areas of iatrogenic or benign FDG uptake should not be selected as the target lesion, even when such a focus has the highest SUL_{peak} value.
- PERCIST requires the reader to select the hottest tumor at each time point, and the target lesion selected at baseline may not the hottest tumor at follow-up.
- To be assessable, differences between baseline and follow-up SUL in the liver must be *(a)* less than or equal to 20% of the larger of the two liver measurements and *(b)* less than or equal to 0.3 SUL units.
- The imaging conditions that allow for comparison are as follows: *(a)* The difference between the injection-to-imaging time of the baseline study and to that of the follow-up study should, though not required, to be less than or equal to 15 minutes. *(b)* The injection- to-imaging start time for both baseline and follow-up imaging should, though not required, to be greater than or equal to 50 minutes and less than or equal to 70 minutes.

Objective Response

- PERCIST encourages recording of the percentage of change in tumor metabolism as a continuous variable with notation of the number of weeks since treatment was begun. The percentage of change in target lesion(s) is computed as:

  $$\mathbf{100 \cdot (FTL_{SULpeak} - BTL_{SULpeak})/BTL_{SULpeak}}$$

  - $FTL_{SULpeak}$ is the SUL_{peak} of the follow-up target lesion
  - $BTL_{SULpeak}$ is the SUL_{peak} of the baseline target lesion.

6.4.4 RECIST Criteria Summary

6.4.4.1 Evaluation of Target Lesions

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

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

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

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

6.4.4.2 Evaluation of Non-Target Lesions

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

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

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

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

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.4.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The participant's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participants with Measurable Disease (i.e., Target Disease)

Target Lesions	**Non-Target Lesions**	**New Lesions**	**Overall Response**	**Best Overall Response when Confirmation is Required***
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	

PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

6.4.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time cell infusion until the first date that progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.5 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each

participant while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

6.6 FEASIBILITY ASSESSMENT

Feasibility will be assessed by three criteria. Failure by any one criterion will be scored as a feasibility failure.

1. Delivery of the E7 TCR T cell induction therapy without an increase in tumor T or N stage (for example an increase from T1 to T2) between baseline and the last response assessment before referral for definitive treatment (with an absolute increase in tumor size of at least 5 mm). This criterion will be assessed by determining the T and N stage at baseline and at the 6-week response assessment. An increase in either T stage or N stage will be scored as a feasibility failure. Participants that develop hyper-progression at the 3-week safety check will also be considered a feasibility failure.
2. Initiation of definitive therapy without a delay in treatment related to toxicity of the induction therapy. This criterion will be assessed by review of the medical records from the treating center and by communication with the clinical team delivering the definitive therapy. If there is a delay, the toxicity type and grade that was the cause will be recorded. The duration of the delay that resulted from the toxicity will be recorded. Any delay related to the toxicity of the induction therapy will be scored as a feasibility failure. Furthermore, participants that require dose reduction in chemoradiation that is definitely due to the induction therapy will be scored as a feasibility failure.
3. If any participant starts the conditioning regimen chemotherapy but does not receive cells for any protocol-related reason (e.g., toxicity of the conditioning regimen, manufacturing failure, tumor hyper-progression), it will be considered a feasibility failure.

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical

Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention **s**hould be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of E7 TCR T cells as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the E7 TCR T cells but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, the IRB continuing review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.4.2.1 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- the title and purpose of the trial
- clinical site
- the Principal Investigator
- clinical protocol identifiers;
- participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed,
- if the trial has been completed, a brief description of any study results.

7.4.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.5 NIH Required Data and Safety Monitoring Plan

7.5.1 Principal Investigator/Research Team

The clinical research team will meet weekly on a regular basis when participants are being actively treated on the trial to discuss each participant. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior participants.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section 7.2.1 will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each participant to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Safety Monitoring Committee (SMC)

This protocol will be periodically reviewed by an intramural Safety Monitoring Committee. Initial review will occur as soon as possible after the annual NIH Intramural IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC based on the risks presented in the study. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period.

The SMC review will focus on unexpected protocol-specific safety issues that are identified during the conduct of the clinical trial.

Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a participant or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (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 (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see section 8.1.3)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for participant convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the participant or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the participant or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in section **8.4**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **8.4**.

All SAE reporting must include the elements described in section 8.2.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 WAIVER OF EXPEDITED REPORTING TO CCR

As death due to disease progression is part of the study objectives (relapse free survival), and captured as an endpoint in this study, it will not be reported in expedited manner to the sponsor. However, if there is evidence suggesting a causal relationship between the study drug and the event, report the event in an expedited manner according to section **8.3**.

8.4.1 Specific Toxicities

Hematological toxicities as outlined below will not be included in expedited reporting to CCR because these are expected toxicities from the conditioning regimen (commercial product):

CTCAE System Organ Class	Adverse Event	Grade	Prolongation of Hospitalization	Expected Frequency	Attribution
Investigations	Neutrophil count decreased	1-3	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	Neutrophil count decreased	4, if < 14 days	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Infection	Febrile Neutropenia	3-4	Expected	60%	Commercial Product (Cyclophosphamide and fludarabine in combination with aldesleukin)
Blood and lymphatic system disorders	Anemia	1-3	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	Platelet count decreased	1-3	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	Platelet count decreased	4, if < 14 days	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	White blood cell decreased	1-4	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)

CTCAE System Organ Class	Adverse Event	Grade	Prolongation of Hospitalization	Expected Frequency	Attribution
Investigations	Lymphocyte count decreased	1-4	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	CD4 lymphocytes decreased	1-4	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)

For participants that start standard of care treatment prior to 21 days from cell infusion, we will only record and report adverse events and serious adverse events that are possibly related to E7 TCR T cells.

The PI will submit a summary table of all grade 3-5 events, whether or not considered related to the product, every 6 months. The report shall include the number of participants treated in the timeframe, the number of events per AE term per grade which occurred in the 6-month timeframe and in total since the start of the study, attribution, and type/category of serious.

Reports will be submitted to the Center for Cancer Research (CCR) at OSROSafety@mail.nih.gov.

The Sponsor might request case summaries for those events if, upon review, the Sponsor determines that an aggregate safety report is required (21CFR312.32(c)(1)(iv)).

8.5 Safety Reporting Criteria to the Pharmaceutical Collaborators

Reporting will be per the collaborative agreement with Kite Pharma (CRADA #03022).

8.6 Reporting Pregnancy

8.6.1 Maternal exposure

If a participant becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section 8.1.2) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.7 Regulatory Reporting for Studies Conducted Under CCR-Sponsored IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected

adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug 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, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 SPONSOR PROTOCOL NON-ADHERENCE REPORTING

Protocol non-adherence is defined as any noncompliance with the clinical trial protocol, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol non-adherence identified by the Staff or the site Monitor on the OSRO Site Protocol Non-Adherence Log. The protocol-specific, cumulative non-adherence log should be maintained in the site essential documents file and submitted to OSRO via OSROMonitoring@mail.NIH.gov on the **first business day of each month over the duration of the study**. In addition, any non-adherence to the protocol should be documented in the participant's source records and reported to the local IRB per their guidelines. OSRO required protocol non-adherence reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights of the participants are protected, that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Monitoring based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. The intensity and frequency of monitoring will be based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. OSRO Monitoring visits and related activities will be conducted throughout the life cycle of each protocol, with the first activity being before study start to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will take place at the study site(s). Monitoring visit reports will describe visit activities, observations, findings of protocol non-adherence and associated action items or follow-up required for resolution of findings. Monitoring reports will be distributed to the study PI, NCI CCR QA, coordinating center (if applicable) and the OSRO regulatory file.

If protocol non-adherence is identified by the Monitor (i.e., any noncompliance with the clinical trial protocol, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the site Staff) the Monitor will note the observation, review with site Staff and if unresolved, request that the Staff document the non-adherence on the protocol-specific OSRO Site Protocol Non-Adherence Log (see Section **8.8**).

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESES

10.1.1 Primary efficacy endpoints:

The primary objective of this protocol is to determine the feasibility of induction E7 TCR T cell therapy for FIGO (2018) stage IIB-IVA, HPV16+ cervical cancer.

The endpoint to be measured is the fraction of subjects for whom E7 TCR induction therapy is feasible as defined in section **6.6**.

10.1.2 Secondary efficacy endpoints:

The secondary endpoints will be to assess the relapse-free survival at 2 years and 5 years following definitive standard of care therapy, to evaluate the safety of E7 induction therapy, to determine the percentage of E7 TCR T cells following completion of chemoradiation and to assess objective response rate following E7 induction therapy.

10.2 SAMPLE SIZE DETERMINATION

This study evaluates the fraction of participants who can receive induction therapy with E7 TCR cells without failing the feasibility criteria in section **6.6**. For a given participant, this will be considered a success. It would be desirable if the fraction of participants who are able to achieve success were consistent with 80-85%. The trial will first enroll 5 participants who are FIGO (2018) stage IIIC-IVA to assess feasibility in a small number of high-risk participants. If after these first 5 participants have been treated, the number of participants with a success is not at least 3 (3/5=60%), the trial will stop since the one sided 90% upper confidence bound on 2/5 is 75.3%, indicating inconsistency with a desirable 80% success rate or greater. If at least 3 have success, these 5 participants will be included in this pilot trial of 15 evaluable participants who undergo the E7 TCR cell administration. If in these 15 there are 12 or more who can be considered a success, the probability of this occurring is 9.1% if the true probability of success is 60%, 17.0% if the true probability of success is 65%, 30% if the true probability of success is 70%, 65% if the true probability of success is 80%, and 82% if the true probability of success is 85%. Thus, if 12/15 (80%) or more of participants who undergo the E7 TCR T cell administration achieve a success, this would be more consistent with 80-85% or more probability of this being the case than with 65% or less probability of this being the case, and thus would be considered an acceptable fraction.

It is anticipated that up to one participant per month may enroll onto this trial. Thus, accrual is expected to be completed in approximately 1.5 years. To allow for a small number of inevaluable participants, up to 18 participants may be treated. A maximum of 5 participants evaluable for feasibility will be treated in the lead-in cohort (cohort 1). A maximum of 10 participants evaluable for feasibility will be treated in the post-lead-in cohort (cohort 2). The accrual ceiling will be set at 180 participants to allow for up to 90% rate of screen failure.

10.3 POPULATIONS FOR ANALYSIS

All subjects who start chemotherapy conditioning regimen under this protocol will be included in the analyses.

10.3.1 Feasibility Analysis

All participants who start conditioning chemotherapy and who can be evaluated for the three feasibility criteria in section **6.6** will be evaluable for feasibility.

10.3.2 Toxicity Analysis

All participants will be evaluable for toxicity from the time of their treatment with E7 TCR T cells.

10.3.3 Response Analysis

Participants who receive E7 TCR T cell therapy and either experience clinical progression at the 3-week clinical check or are evaluated at the 6-week response assessment will be evaluable for response. Response will be classified according to the definitions below.

10.4 STATISTICAL ANALYSES

10.4.1 General approach

The fraction who achieve a success will be determined and reported.

10.4.2 Analysis of the primary efficacy endpoints

The fraction who achieve a success among those who start conditioning chemotherapy and who can be evaluated for the three feasibility criteria in section **6.6,** with 95% confidence intervals on the fraction reported as well.

10.4.3 Analysis of the secondary efficacy endpoints

The 2 year and 5 year RFS will be estimated from a Kaplan-Meier curve of RFS.

To evaluate the toxicity of induction E7 TCR T cell therapy for FIGO (2018) stage IIB-IVA, HPV16+ cervical cancer, the types and grades of toxicity obtained will be report and findings described.

The objective response rate (PR+CR) following E7 TCR T cell induction therapy will be estimated and reported along with a 95% confidence interval.

10.4.4 Safety analysis

Adverse events will be recorded and reported as defined in Section **6.1.1**.

10.4.5 Baseline Descriptive Statistics

Demographic and clinical characteristics of all participants will be reported.

10.4.6 Planned interim analyses

An early interim look at the fraction with success after 5 participants with FIGO (2018) stage IIIC-IVA have been treated will be conducted.

10.4.7 Subgroup analyses

None will be performed.

10.4.8 Tabulation of individual participant data

None will be provided

10.4.9 Exploratory analyses

The exploratory objectives are:

- To perform laboratory research to begin to understand treatment response and to develop biomarkers that predict response.
- To determine the percentage of participants able to receive standard chemoradiation without requiring dose-escalation due to hematologic toxicities.

Statistical tests performed will be considered exploratory with results presented without formal adjustment but interpreted in the context of the number of tests performed.

11 COLLABORATIVE AGREEMENTS

11.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

Coded, linked samples may be provided to Kite Pharma for assistance in performing the research studies described in section **5.1**. The code key will not be provided to collaborators. Kite Pharma is collaborating in the development of the E7 TCR. A CRADA between NCI and Kite Pharma is in place (CRADA # 03022). Kite Pharma will be providing funding for this study.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

The participants to be entered in this protocol have advanced HPV-associated cervical cancer and are more likely to have persistent or recurrent disease after standard therapy.

Only female participants are affected by this condition and, therefore, eligible for the study. Subjects from all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between participant safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments, on the one hand, and the need to explore sex and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded, or a follow-up study may be written to investigate those differences more fully.

12.2 PARTICIPATION OF CHILDREN

The use of the non-myeloablative regimen in this protocol entails serious discomforts and hazards for the participant, such that fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life-threatening metastatic cancer. Since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit. Should results of this study indicate efficacy in treating metastatic cancer, which is not responsive to other

standard forms of therapy, future research can be conducted in the pediatric population to evaluate potential benefit in that participant population.

12.3 RISK/BENEFIT ASSESSMENT

12.3.1 Known Potential Risks

Over 400 participants have been treated in the Surgery Branch, NCI with Tumor-Infiltrating Lymphocytes (TIL). Early toxicities related specifically to the infusion of the cells (those which are seen immediately following cell infusion and prior to aldesleukin administration) are generally mild and include fevers, chills, headache, and malaise. Toxicities which occur following administration of aldesleukin but are thought to be related to the cells include immune mediated events such as vitiligo, transient uveitis, hearing loss and vestibular dysfunction. The use of the non-myeloablative regimen prior to cell administration increases the toxicity of this treatment as profound myelosuppression occurs in all participants. In 93 participants treated with TIL using the non-myeloablative chemotherapy regimen with or without total body irradiation, there was one treatment related death (NMA + 200 cGy TBI) due to an unexpected but preexisting diverticular abscess. In the 101 participants treated in subsequent randomized trial 2 treatment related deaths occurred, both due to the TBI component of the treatment regimen.

The major discomforts of the research are those of nausea and vomiting, mucositis, anorexia, diarrhea, fever and malaise. Side effects of common drugs used in this regimen include:

- Cyclophosphamide: Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting, SIADH.
- Fludarabine: Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, neurologic toxicity, and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL participants treated with fludarabine.
- Antimicrobials in general: Allergic reactions, renal impairment, nausea, vomiting, hepatic damage, marrow suppression, photosensitivity.
- High-dose aldesleukin administration: A listing of these side effects in 525 participants who received 1,039 treatment courses are listed in Appendix 3.

12.3.1.1 Biopsy

The risks associated with biopsies are pain and bleeding at the biopsy site. To minimize pain, local anesthesia will be used. Rarely, there is a risk of infection at the sampling site.

12.3.1.2 Blood Sampling

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

12.3.1.3 Leukapheresis

There may be some tingling in the face, mouth and fingers due to the medicine used to keep the blood from clotting during the procedure. The nurses may give a calcium-containing antacid to take away this tingling. Rarely, people may experience lightheadedness or dizziness. Rare complications of this procedure are lowered blood pressure, lightheadedness, dizziness, nausea, possible problems with the cell separator machine which would not allow the red cells and plasma to be returned and bleeding or bruising where the needles are put in your arms.

12.3.1.4 Intravenous Catheter

The risks associated with placing some catheters include pain, bleeding, infection and collapsed lung. The long-term risks of the catheter include infection and clotting of your veins. It may be necessary to remove the catheter.

12.3.1.5 CT scan, PET, MRI and Ultrasound

Although rare, the intravenous (IV) contrast material involved in some CT, PET and MRI scans causes medical problems or allergic reactions in some people. Most reactions are mild and result in hives or itchiness. In rare instances, an allergic reaction can be serious and potentially life threatening. Participants will be asked if they have had prior reaction to contrast material during medical tests. There are no anticipated risks for the ultrasound procedure.

12.3.1.6 Risks related to Radiation Exposure

This research study involves exposure to radiation from chest x-ray, CT scans, PET scans, and CT-guided biopsies. The amount of radiation exposure that may be received from these procedures is equal to approximately 9.3 rem. A rem is a unit of absorbed radiation.

Every day, people are exposed to low levels of radiation that come from the sun and the environment around them. The average person in the United States receives a radiation exposure of 0.3 rem per year from these sources. This type of radiation is called “background radiation.” This study will expose participants to more radiation than they get from everyday background radiation. No one knows for sure whether exposure to these low amounts of radiation is harmful to your body.

The chest X-ray, PET and CT scans in this study may expose participants to the roughly the same amount of radiation as 31 years worth of background radiation. Being exposed to too much radiation can cause harmful side effects such as an increase in the risk of cancer. The risk depends on how much radiation they are exposed to. Please be aware that about 40 out of 100 people (40%) will get cancer during their lifetime, and 20 out of 100 (20%) will die from cancer. The risk of getting cancer from the radiation exposure in this study is 0.9 out of 100 (0.9%) and of getting a fatal cancer is 0.4 out of 100 (0.4%).

12.3.2 Known Potential Benefits

The experimental treatment has a chance to provide potential clinical benefit by shrinking of the tumor or lessening of symptoms, such as pain, that are caused by the cancer. With no known additional risks related to the previously produced vector for participants who have received cells with vectors made the potential benefit outweighs the potential risks.

12.3.3 Assessment of Potential Risks and Benefits

The standard approach to the administration of high-dose aldesleukin in all studies is to continue dosing until Grade 3 or 4 events occur. The most commonly seen Grade 4 events are pulmonary and renal impairment, and mental status changes. These toxicities may sometimes require intubation for protection of the participant’s airway. It is important to note that although these participants require significant supportive measures during this period, all toxicities are reversible, and the overwhelming majority of participants have suffered no long-term sequelae following this treatment regimen. However, fatal complications are possible, and it is therefore only appropriate to carry out this experimental treatment in the context of life-threatening cancer.

Toxicities seen on protocols using this non-myeloablative regimen and aldesleukin that occur during the follow up period are rare but have included EBV lymphoma following prolonged lymphopenia, herpes zoster infection, and sensory neuropathy likely related to fludarabine.

12.4 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided to the participant for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms) per discretion of the designated study investigator and with the agreement of the participant. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

*Please note that consent for treatment (consent labeled Treatment - Affected Patient) must be obtained by a designated appropriately licensed study investigator (e.g., MD, NP, PA, DO). However, study investigators not falling into this category (e.g. RNs) who are designated as able to obtain consent, may do so for non-treatment procedures such as screening.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigators, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met

- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical

records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the National Cancer Institute (NCI). This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NCI.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 ALDESLEUKIN

14.1.1 Source/Acquisition and Accountability

Aldesleukin (interleukin-2) will be provided by the NIH Clinical Pharmacy Department from commercial sources.

14.1.2 Formulation/Reconstitution

Aldesleukin, NSC #373364, is provided as single-use vials containing 22 million IU (~1.3mg) IL-2 as a sterile, white to off-white lyophilized cake plus 50mg mannitol and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/mL or 1.1 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. Since vials contain no preservative, reconstituted solution should be used within 24 hours.

14.1.3 Storage

Intact vials are stored in the refrigerator (2 to 8°C) protected from light. Each vial bears an expiration date.

14.1.4 Dilution/Stability

Reconstituted aldesleukin should be further diluted with 50 mL of 5% Human Serum Albumin (HSA). The HSA should be added to the diluent prior to the addition of IL-2. Dilutions of the reconstituted solution over a 1000-fold range (i.e., 1 mg/mL to 1 mcg/mL) are acceptable in either glass bottles or polyvinyl chloride bags. Aldesleukin is chemically stable for 48 hours at refrigerated and room temperatures, 2 to 30°C.

14.1.5 Administration

The dosage will be calculated based on total body weight. The final dilution of aldesleukin will be infused over 15 minutes. Aldesleukin will be administered as an inpatient.

14.1.6 Toxicities

Expected toxicities of aldesleukin are listed in the product label and in **Appendix 2**. Grade 3 toxicities common to aldesleukin include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix 1. Additional Grade 3 and 4 toxicities seen with aldesleukin are detailed in **Appendix 2**.

14.2 FLUDARABINE

(Please refer to package insert for complete product information.)

14.2.1 Description

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

14.2.2 Source/Acquisition and Accountability

Fludarabine will be purchased by the NIH Clinical Pharmacy Department from commercial sources. Fludarabine is supplied in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.

14.2.3 Formulation/Reconstitution

Fludarabine Phosphate Injection, USP is supplied both as a 50 mg vial in the form of a white, lyophilized solid cake and as a 50 mg per 2 mL vial containing a clear, sterile solution (25 mg/mL). Both formulations contain 25 mg of mannitol and sodium hydroxide for pH adjustment. Following reconstitution of the lyophilized vial with 2 mL of sterile water for injection to a concentration of 25 mg/mL, the solution has a pH of 7.7. Fludarabine Phosphate Injection available as a sterile solution has a pH of 6.8.

14.2.4 Stability

Following reconstitution, fludarabine is stable for at least 16 days at room temperature. Since no preservative is present, the manufacturer recommends that fludarabine be used within 8 hours of reconstitution of the lyophilized product or within 8 hours of vial entry for the fludarabine solution. Expiration dating of prepared fludarabine doses will follow institutional guidelines. Specialized references should be consulted for specific compatibility information.

14.2.5 Storage

Intact vials should be stored refrigerated (2 to 8°C).

14.2.6 Administration

Fludarabine is administered as an IV infusion in 100 mL 0.9% sodium chloride, USP or 5% Dextrose in Water, USP over approximately 30 minutes. The doses will be based on body surface area (BSA). If participant is obese (BMI >35), drug dosage will be calculated using practical weight as described in **Table 2**.

Table 2. Modification of Dose Calculations* in participants whose BMI is greater than 35

1. **BMI Determination:**

   BMI = weight (kg) / [height (m)]^2

2. **Calculation of ideal body weight**

   Male = 50kg + 2.3 (number of inches over 60 inches)

   Example: ideal body weight of 5'10" male

   50 + 2.3 (10) = 73 kg

   Female = 45.5kg + 2.3 (number of inches over 60 inches)

   Example: ideal body weight of a 5'3" female

   45.5 + 2.3 (3) = 57kg

3. **Calculation of "practical weight"**

   Calculate the average of the actual and the ideal body weights. This is the practical weight to be used in calculating the doses of chemotherapy and associated agents designated in the protocol.

14.2.7 Toxicities

At doses of 25 mg/m^2/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in CLL participants treated with fludarabine. Hemolytic anemia has been reported after 1 or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include, cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL. Treatment on previous adoptive cell therapy protocols in the Surgery Branch have caused persistently low (below 200) CD4 counts, and 1 participant developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

14.3 CYCLOPHOSPHAMIDE

(Refer to FDA-approved package insert for complete product information.)

14.3.1 Description:

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

14.3.2 Source/Acquisition and Accountability

Cyclophosphamide will be obtained from commercially available sources by the Clinical Center Pharmacy Department.

14.3.3 Stability

Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2 to 8°C.

14.3.4 Administration

It will be diluted in 250 mL D5W and infused over 1 hour. The dose will be based on the participant's body weight. If participant is obese (BMI>35) drug dosage will be calculated using practical weight as described in **Table 2**.

14.3.5 Toxicities

Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of participants; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as an uroprotective agent in participants receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all participants. Participants who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in participants with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, participants should be watched closely for cyclophosphamide toxicity (due to allopurinol induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophosphamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of

cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

14.4 CELL PREPARATION (E7 TCR TRANSDUCED PBL) (IND # 19564)

The procedure for the expanding the human PBL and the Certificate of Analysis (CoA) are similar to those approved by the Food and Drug Administration and used at the NCI in ongoing protocols. The PBL will be transduced with retroviral supernatant containing the E7 TCR. The risks of retroviral transduction of human PBL are discussed in section **1.2.4**.

14.4.1 Retroviral Vector Containing the E7 TCR gene

The retroviral vector supernatant (PG13-MSGV1-E7-TCR) encoding a T cell receptor directed against HPV16 $E7_{11-19}$) was prepared and preserved following cGMP conditions in the Surgery Branch Vector Production Facility (SBVPF). The E7 TCR vector was produced by the Surgery Branch Vector Production Facility. The backbone is the MSGV1 retrovirus that has been used in prior gene therapy clinical trials. It was produced using a PG13-based packaging line.

The retroviral vector E7 TCR consists of 7,310 bps including the 5'LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites, alpha and beta chain genes of the E7 TCR. The alpha and beta chains are linked by a P2A peptide. The vector was codon optimized for expression by human cells with constant region exchanged for murine counterparts with an added disulfide bond and hydrophobic substitutions in the alpha chain constant region transmembrane domain.

The physical titer will be determined by transduction of PBL with serial dilutions of the vector. TCR expression on the cell surface will be measured using FACS following staining with an anti-mouse constant region antibody. The titer will be measured as transducing units per milliliter. Portions of the supernatant will be stored at -80°C at Surgery Branch, NCI, American Type Culture Collection (ATCC), Rockville, MD, and the NIH Clinical Center Department of Transfusion Medicine. These storage facilities are equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in *ex vivo* transduction of participant PBL. There will be no re-use of the same unit of supernatant for different participants. Retroviral titer has been shown to be stable after immediate thawing and immediate administration (coating the tissue culture wells previously coated with Retronectin). Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2). The specific guidelines for Biosafety Level-2 (BSL-2) can be viewed at http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_sect_IV.pdf.

14.5 HPV16 GENOTYPING ASSAY (NSR DEVICE)

In order to be eligible for the study, participants are required to have HPV16-associated, high-grade cervical intraepithelial neoplasia. HPV16 Genotyping assay testing is not FDA approved for this purpose; however, it is being used in this study as a diagnostic device. Validation assays to support the use of the assay have been submitted to the IND. All documentation is in the IND files.

According to 21 CFR 812.3(m), a significant risk device presents a potential for serious risk to the health, safety and welfare of a subject and meets the significant risk criteria listed in the table below along with the sponsor's conclusions with regard to the applicability of these criteria to

the current study. The device has been assessed by the sponsor as non-significant risk per the below.

	Applicable to current study	**Justification**
Is an implant	No	The HPV16 genotyping assay test is not introduced into the subject
Is used in supporting or sustaining human life	No	The device is diagnostic
Is of substantial importance in diagnosing, mitigating or treating disease or preventing impairment of human health	No	While the device is diagnostic, we do not believe it presents a potential for serious risk to the health and welfare of the subject. The assessment of HPV16 positivity is only used to determine eligibility for the study and is assessed to help to increase the possibility that all persons enrolling on the study might derive benefit from therapy. Persons that are deemed ineligible to enroll on the basis of this test are eligible for studies within GMB that are not reliant on this test.
Otherwise poses a risk	No	Testing will be performed on archival samples or on fresh tissue that is collected at screening for confirmation of diagnosis.

15 REFERENCES

1. Viens LJ, Henley SJ, Watson M, Markowitz LE, Thomas CC, Thompson TD, Razzaghi H, Saraiya M: **Human Papillomavirus-Associated Cancers - United States, 2008-2012**. *MMWR Morb Mortal Wkly Rep* 2016, **65**(26):661-666.
2. Berman TA, Schiller JT: **Human papillomavirus in cervical cancer and oropharyngeal cancer: One cause, two diseases**. *Cancer* 2017, **123**(12):2219-2229.
3. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N: **Human papillomavirus is a necessary cause of invasive cervical cancer worldwide**. *J Pathol* 1999, **189**(1):12-19.
4. Li N, Franceschi S, Howell-Jones R, Snijders PJ, Clifford GM: **Human papillomavirus type distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological type and year of publication**. *Int J Cancer* 2011, **128**(4):927-935.
5. Koh WJ, Abu-Rustum NR, Bean S, Bradley K, Campos SM, Cho KR, Chon HS, Chu C, Clark R, Cohn D *et al*: **Cervical Cancer, Version 3.2019, NCCN Clinical Practice Guidelines in Oncology**. *J Natl Compr Canc Netw* 2019, **17**(1):64-84.
6. Macdonald OK, Chen J, Dodson M, Lee CM, Gaffney DK: **Prognostic significance of histology and positive lymph node involvement following radical hysterectomy in carcinoma of the cervix**. *Am J Clin Oncol* 2009, **32**(4):411-416.
7. Matsuo K, Machida H, Mandelbaum RS, Konishi I, Mikami M: **Validation of the 2018 FIGO cervical cancer staging system**. *Gynecol Oncol* 2019, **152**(1):87-93.
8. Fagundes H, Perez CA, Grigsby PW, Lockett MA: **Distant metastases after irradiation alone in carcinoma of the uterine cervix**. *Int J Radiat Oncol Biol Phys* 1992, **24**(2):197-204.
9. Hong JH, Tsai CS, Lai CH, Chang TC, Wang CC, Chou HH, Lee SP, Hsueh S: **Recurrent squamous cell carcinoma of cervix after definitive radiotherapy**. *Int J Radiat Oncol Biol Phys* 2004, **60**(1):249-257.
10. McCormack M, Kadalayil L, Hackshaw A, Hall-Craggs MA, Symonds RP, Warwick V, Simonds H, Fernando I, Hammond M, James L *et al*: **A phase II study of weekly neoadjuvant chemotherapy followed by radical chemoradiation for locally advanced cervical cancer**. *Br J Cancer* 2013, **108**(12):2464-2469.
11. Hinrichs CS: **Molecular Pathways: Breaking the Epithelial Cancer Barrier for Chimeric Antigen Receptor and T-cell Receptor Gene Therapy**. *Clin Cancer Res* 2016, **22**(7):1559-1564.
12. Stevanovic S, Draper LM, Langhan MM, Campbell TE, Kwong ML, Wunderlich JR, Dudley ME, Yang JC, Sherry RM, Kammula US *et al*: **Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells**. *J Clin Oncol* 2015, **33**(14):1543-1550.
13. Stevanovic S, Pasetto A, Helman SR, Gartner JJ, Prickett TD, Howie B, Robins HS, Robbins PF, Klebanoff CA, Rosenberg SA *et al*: **Landscape of immunogenic tumor**

antigens in successful immunotherapy of virally induced epithelial cancer. *Science* 2017, **356**(6334):200-205.

14. Draper LM, Kwong ML, Gros A, Stevanovic S, Tran E, Kerkar S, Raffeld M, Rosenberg SA, Hinrichs CS: **Targeting of HPV-16+ Epithelial Cancer Cells by TCR Gene Engineered T Cells Directed against E6**. *Clin Cancer Res* 2015, **21**(19):4431-4439.

15. Newport MJ, Huxley CM, Huston S, Hawrylowicz CM, Oostra BA, Williamson R, Levin M: **A mutation in the interferon-gamma-receptor gene and susceptibility to mycobacterial infection**. *N Engl J Med* 1996, **335**(26):1941-1949.

16. Shin DS, Zaretsky JM, Escuin-Ordinas H, Garcia-Diaz A, Hu-Lieskovan S, Kalbasi A, Grasso CS, Hugo W, Sandoval S, Torrejon DY *et al*: **Primary Resistance to PD-1 Blockade Mediated by JAK1/2 Mutations**. *Cancer Discov* 2017, **7**(2):188-201.

17. del Campo AB, Kyte JA, Carretero J, Zinchencko S, Mendez R, Gonzalez-Aseguinolaza G, Ruiz-Cabello F, Aamdal S, Gaudernack G, Garrido F *et al*: **Immune escape of cancer cells with beta2-microglobulin loss over the course of metastatic melanoma**. *Int J Cancer* 2014, **134**(1):102-113.

18. Khong HT, Restifo NP: **Natural selection of tumor variants in the generation of "tumor escape" phenotypes**. *Nat Immunol* 2002, **3**(11):999-1005.

19. Reiman JM, Kmieciak M, Manjili MH, Knutson KL: **Tumor immunoediting and immunosculpting pathways to cancer progression**. *Semin Cancer Biol* 2007, **17**(4):275-287.

20. McGranahan N, Rosenthal R, Hiley CT, Rowan AJ, Watkins TBK, Wilson GA, Birkbak NJ, Veeriah S, Van Loo P, Herrero J *et al*: **Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution**. *Cell* 2017, **171**(6):1259-1271 e1211.

21. Ferris RL, Goncalves A, Baxi SS, Martens UM, Gauthier H, Langenberg M, Spanos WC, Leidner RS, Kang H, Russell J *et al*: **An open-label, multicohort, phase 1/2 study in patients with virus-associated cancers (CheckMate 358): Safety and efficacy of neoadjuvant nivolumab in squamous cell carcinoma of the head and neck (SCCHN)**. *Ann Oncol* 2017, **28**.

22. Forde PM, Chaft JE, Smith KN, Anagnostou V, Cottrell TR, Hellmann MD, Zahurak M, Yang SC, Jones DR, Broderick S *et al*: **Neoadjuvant PD-1 Blockade in Resectable Lung Cancer**. *N Engl J Med* 2018, **378**(21):1976-1986.

23. Jin BY, Campbell TE, Draper LM, Stevanovic S, Weissbrich B, Yu Z, Restifo NP, Rosenberg SA, Trimble CL, Hinrichs CS: **Engineered T cells targeting E7 mediate regression of human papillomavirus cancers in a murine model**. *JCI Insight* 2018, **3**(8).

24. Fraietta JA, Nobles CL, Sammons MA, Lundh S, Carty SA, Reich TJ, Cogdill AP, Morrissette JJD, DeNizio JE, Reddy S *et al*: **Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells**. *Nature* 2018, **558**(7709):307-312.

25. Scholler J, Brady TL, Binder-Scholl G, Hwang WT, Plesa G, Hege KM, Vogel AN, Kalos M, Riley JL, Deeks SG *et al*: **Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells**. *Sci Transl Med* 2012, **4**(132):132ra153.

26. Bear AS, Morgan RA, Cornetta K, June CH, Binder-Scholl G, Dudley ME, Feldman SA, Rosenberg SA, Shurtleff SA, Rooney CM *et al*: **Replication-competent retroviruses in gene-modified T cells used in clinical trials: is it time to revise the testing requirements?** *Mol Ther* 2012, **20**(2):246-249.

27. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M *et al*: **New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1)**. *Eur J Cancer* 2009, **45**(2):228-247.

28. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, Eisenberger MA, Higano C, Bubley GJ, Dreicer R *et al*: **Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group**. *J Clin Oncol* 2008, **26**(7):1148-1159.

29. Rustin GJ, Quinn M, Thigpen T, du Bois A, Pujade-Lauraine E, Jakobsen A, Eisenhauer E, Sagae S, Greven K, Vergote I *et al*: **Re: New guidelines to evaluate the response to treatment in solid tumors (ovarian cancer)**. *J Natl Cancer Inst* 2004, **96**(6):487-488.

30. Bubley GJ, Carducci M, Dahut W, Dawson N, Daliani D, Eisenberger M, Figg WD, Freidlin B, Halabi S, Hudes G *et al*: **Eligibility and response guidelines for phase II clinical trials in androgen-independent prostate cancer: recommendations from the Prostate-Specific Antigen Working Group**. *J Clin Oncol* 1999, **17**(11):3461-3467.

31. Vergote I, Rustin GJ, Eisenhauer EA, Kristensen GB, Pujade-Lauraine E, Parmar MK, Friedlander M, Jakobsen A, Vermorken JB: **Re: new guidelines to evaluate the response to treatment in solid tumors [ovarian cancer]. Gynecologic Cancer Intergroup**. *J Natl Cancer Inst* 2000, **92**(18):1534-1535.

32. Wahl RL, Jacene H, Kasamon Y, Lodge MA: **From RECIST to PERCIST: Evolving Considerations for PET response criteria in solid tumors**. *J Nucl Med* 2009, **50 Suppl 1**:122S-150S.

33. O JH, Wahl RL: **PERCIST in Perspective**. *Nucl Med Mol Imaging* 2018, **52**(1):1-4.

34. O JH, Lodge MA, Wahl RL: **Practical PERCIST: A Simplified Guide to PET Response Criteria in Solid Tumors 1.0**. *Radiology* 2016, **280**(2):576-584.

16 APPENDICES

16.1 APPENDIX 1: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
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. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about 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.

16.3 APPENDIX 2: ADVERSE EVENTS OCCURRING IN ≥ 10% OF PARTICIPANTS TREATED WITH ALDESLEUKIN (N=525)

Body System	% Participants	Body System	% Participants
Body as a Whole		*Metabolic and Nutritional Disorders*	
Chills	52	Bilirubinemia	40
Fever	29	Creatinine increase	33
Malaise	27	Peripheral edema	28
Asthenia	23	SGOT increase	23
Infection	13	Weight gain	16
Pain	12	Edema	15
Abdominal pain	11	Acidosis	12
Abdomen enlarged	10	Hypomagnesemia	12
Cardiovascular		Hypocalcemia	11
Hypotension	71	Alkaline phosphatase incr	10
Tachycardia	23	*Nervous*	
Vasodilation	13	Confusion	34
Supraventricular tachycardia	12	Somnolence	22
Cardiovascular disorder[a]	11	Anxiety	12
Arrhythmia	10	Dizziness	11
Digestive		*Respiratory*	
Diarrhea	67	Dyspnea	43
Vomiting	50	Lung disorder[b]	24
Nausea	35	Respiratory disorder[c]	11
Stomatitis	22	Cough increase	11
Anorexia	20	Rhinitis	10
Nausea and vomiting	19	*Skin and Appendages*	
Hemic and Lymphatic		Rash	42
Thrombocytopenia	37	Pruritus	24
Anemia	29	Exfoliative dermatitis	18
Leukopenia	16	*Urogenital*	

a Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.

b Lung disorder: physical findings associated with pulmonary congestion, rales, rhonchi.

c Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.

[1]Source: Proleukin® Prescribing Information – June 2007

16.4 APPENDIX 3: EXPECTED IL-2 TOXICITIES AND THEIR MANAGEMENT

Toxicity	Grade	Supportive Medications	Stop Cycle*	Stop Treatment**
Chills	3	IV Meperidine 25-50mg IV q1hr, prn	No	No
Fever	3	Acetaminophen 650mg po q4hr; Indomethacin 50-75mg po q8h	No	No
Pruritus	3	Hydroxyzine HCl 10-20mg po q6h, prn; Diphenhydramine HCl 25-50mg po q4h prn	No	No
Nausea/Vomiting/Anorexia	3	Ondansetron 10mg IV q8hr prn, Granisetron 0.01 mg/kg IV qday prn, Droperidol 1mg IV a4-6h prn; Prochlorperazine 25mg PR prn or 10mg IV q6hr prn	No	No
Diarrhea	3	Loperamide 2mg po q3h prn; Diphenoxylate HCl 2.5mg and Atropine sulfate 25mcg po q3h prn; Codeine sulfate 30-60mg po q4h prn	If uncontrolled after 24h despite all supportive measures	No
Malaise	3 or 4	Bedrest	If other toxicities occur simultaneously	No
Hyperbilirubinemia	3 or 4	Observation	If other toxicities occur simultaneously	No
Anemia	3 or 4	Transfusion with PRBCs	If uncontrolled despite all supportive measures	No
Thrombocytopenia	3 or 4	Transfusion with platelets	If uncontrolled despite all supportive measures	No

Toxicity	Grade	Supportive Medications	Stop Cycle*	Stop Treatment**
Edema/Weight gain	3	Diuretics prn	No	No
Hypotension	3	Fluid resuscitation, Vasopressor support	If uncontrolled despite all supportive measures	No
Dyspnea	3 or 4	Oxygen or ventilator support	If requires ventilator support	No
Oliguria	3 or 4	Fluid boluses or dopamine at renal doses	If uncontrolled despite all supportive measures	No
Increased Creatinine	3 or 4	Observation	Yes (Grade 4)	No
Renal Failure	3 or 4	Dialysis/CVVH	Yes	Yes
Pleural Effusion	3	Thoracentesis	If uncontrolled despite all supportive measures	No
Bowel Perforation	3	Surgical intervention	Yes	Yes
Confusion	3	Observation	Yes	No
Somnolence	3 or 4	Intubation for airway protection	Yes	Yes
Arrhythmia	3	Correction of fluid and electrolyte imbalances; chemical conversion or electrical conversion therapy	If uncontrolled despite all supportive measures	No
Elevated Troponin Levels	3 or 4	Observation	Yes	If changes in LV function have not improved to baseline by next dose

Toxicity	Grade	Supportive Medications	Stop Cycle*	Stop Treatment**
Myocardial Infarction	4	Supportive care	Yes	Yes
Elevated Transaminases	3 or 4	Observation	For Grade 4 without liver metastases	If changes have not improved to baseline by next dose
Hyperbilirubinemia	3 or 4	Observation	For Grade 4 without liver metastases	If changes have not improved to baseline by next dose
Electrolyte Imbalances	3 or 4	Electrolyte replacement	If uncontrolled despite all supportive measures	No
Neutropenia	4	Observation	No	No

*Unless the toxicity is not reversed within 12 hours.

**Unless the toxicity is not reversed to Grade 2 or less by next treatment.

16.5 APPENDIX 4: INTERLEUKIN-2 TOXICITIES OBSERVED IN PARTICIPANTS TREATED AT THE NIH

TABLE 8. *Toxicity of Treatment with Interleukin-2*

Interleukin-2 Plus	Alone	TNF	a-IFN	MoAB	CYT	LAK	TIL	Total
Number of Patients	155	38	128	32	19	214	66	652*
Number of Courses	236	85	210	35	30	348	95	1039
Chills	75	16	68	8	8	191	33	399
Pruritus	53	9	26	2	2	82	6	180
Necrosis	3	—	2	—	—	—	—	5
Anaphylaxis	—	—	—	1	—	—	—	1
Mucositis (requiring liquid diet)	6	1	7	—	2	12	2	30
Alimentation not possible	1	—	1	—	—	2	—	4
Nausea and vomiting	162	42	117	14	20	263	48	666
Diarrhea	144	38	98	15	13	250	38	596
Hyperbilirubinemia (maximum/mg %)								
2.1–6.0	126	49	97	21	18	190	46	547
6.1–10.0	49	3	12	8	9	72	26	179
10.1+	26	1	4	3	1	40	8	83
Oliguria								
<80 ml/8 hours	81	37	67	14	9	114	25	347
<240 ml/24 hours	19		2	3	1	12	5	42
Weight gain (% body weight)								
0.0–5.0	106	23	65	8	9	117	49	377
5.1–10.0	78	41	111	22	10	148	26	436
10.1–15.0	43	17	26	3	9	62	15	175
15.1–20.0	7	3	8	1	1	15	3	38
20.1+	2	1		1	1	6	2	13
Elevated creatinine (maximum/mg %)								
2.1–6.0	148	43	121	20	14	237	54	637
6.1–10.0	21	1	14	3	—	34	12	85
10.1+	5	—	1	1	—	2	1	10
Hematuria (gross)	—	—	—	—	—	2	—	2
Edema (symptomatic nerve or vessel compression)	4	—	6	—	—	7	—	17
Tissue ischemia	—	—	—	—	1	1	—	2
Resp. distress:								
not intubated	17	1	9	4	1	28	7	67
intubated	15	—	6	3	—	12	5	41
Bronchospasm	2	—	2	—	1	4	—	9
Pleural effusion (requiring thoracentesis)	4	1	—	1	2	8	1	17
Somnolence	29	2	22	6	2	45	8	114
Coma	9	1	8	—	2	8	5	33
Disorientation	52	3	50	7	4	89	10	215
Hypotension (requiring pressors)	119	16	40	17	12	259	45	508
Angina	5	1	8	—	—	8	—	22
Myocardial infarction	4	—	1	—	—	1	—	6
Arrythmias	15	2	13	3	—	39	6	78
Anemia requiring transfusion (number units transfused)								
1–15	77	16	53	9	6	176	40	377
6–10	22	1	5	3	2	53	9	95
11–15	4	—	1	—	—	15	4	24
16+	1	—	1	—	—	11	1	14
Thrombocytopenia (minimum/mm^3)								
<20,000	28	1	2	4	6	71	19	131
20,001–60,000	82	11	62	14	12	150	30	361
60,001–100,000	53	36	76	11	8	79	22	285
Central line sepsis	13	—	7	1	4	36	2	63
Death	4	—	1	—	—	3	2	10

* Eleven patients are in two protocols.