Abbreviated Title: E7 TCR Cell Induction Therapy
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Title: A Phase II Study of E7 TCR T Cell Induction Immunotherapy for Stage II and Stage III HPV-Associated Oropharyngeal Cancer

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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:

- Human papillomavirus (HPV)+ oropharyngeal cancer is an increasingly common type of cancer that frequently affects young patients.
- The treatment for locoregionally advanced cancer carries substantial life-long morbidity.
- Although the overall prognosis for HPV+ oropharyngeal cancer is favorable, about 20 percent of patients with stage II disease and 35 percent of patients with stage III disease will die within five years.
- Induction therapy is an area of active study in this type of cancer. The aims of induction therapy are to reduce the risk of disease recurrence and potentially to permit the study of de-intensified definitive treatment of locoregional disease.
- E7 TCR T cells, administered as a single infusion, have demonstrated safety and clinical activity in treatment-refractory metastatic HPV+ cancers.

Objectives:

- To determine the feasibility of systemic treatment with E7 TCR T cells for stage II or stage III HPV+ oropharyngeal cancer.

Eligibility:

- Patients greater than or equal to 18 years old with stage II or stage III HPV+ oropharyngeal cancer.
- The cancer must be HPV16+ and patient must be HLA-A*02:01+ HLA type.
- Patients must be treatment-naïve.

Design:

- This is a phase II, single arm, feasibility study of induction E7 TCR T cell therapy.
- Patients will receive a conditioning regimen of cyclophosphamide and fludarabine, a single infusion of E7 TCR T cells, and systemic aldesleukin.
- Patients will be referred for standard of care definitive therapy (chemoradiation or surgery) at the time of maximum tumor response

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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; an IRB 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 as defined in section **6.5** of E7 TCR T Cell therapy for the treatment of stage II and stage III HPV16+ oropharyngeal cancer

1.1.2 Exploratory Objectives

- To study tumor responses following E7 TCR T Cell induction therapy
- To study the toxicity of E7 TCR T Cell induction therapy
- To perform laboratory research to understand treatment response and to develop biomarkers that predict response
- To evaluate novel diagnostics for determination of patient eligibility in future trials

1.2 BACKGROUND AND RATIONALE

There are more than 16,000 cases of HPV+ oropharyngeal cancer in the United States each year, and the incidence is rising[1, 2]. Approximately 70 percent of oropharyngeal cancers are HPV+, and nearly all of them are caused by HPV type 16[3]. HPV+ oropharyngeal cancers have a better prognosis than their HPV- counterparts. However, about 20 percent of patients with stage II disease and 35 percent of patients with stage III disease will die within five years of diagnosis and staging[4]. In addition, treatment of stage II and stage III disease with surgery, chemotherapy, radiation, or a combination of these is highly morbid and can result in long-term impairment in swallowing, taste, mastication, and speech, as well as in tissue fibrosis and pain[5, 6]. Patients with HPV+ oropharyngeal cancers are typically younger and more likely to have nodal involvement than those with HPV- tumors, making the long-term morbidity of treatment a particular concern. Induction treatment strategies that have the dual aims of reducing the risk of distant disease recurrence and of decreasing locoregional disease to permit the future study of de-

intensified primary therapy are an area of active clinical investigation[7, 8]. In this clinical trial, we are studying induction therapy with E7 TCR T cells followed by standard therapy, which may involve surgery and risk-stratified adjuvant therapy or primary chemoradiation.

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[9]. 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[10, 11]. Patients 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. 5/18 patients with cervical cancer experienced tumor responses, and 2/11 patients with other HPV+ cancers experienced tumor responses. Two patients with cervical cancer had complete responses, and the other patients had partial responses. A patient 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. He is without disease years after treatment. The magnitude of the HPV-oncoprotein-reactivity of the infused treatment cells correlated with response in patients in this clinical trial. However, both viral and non-viral tumor antigens were targeted by the TIL administered to two patients with cervical cancer who each had a complete tumor response[10]. 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)[12]. The trial was a phase I/II, dose-escalation study. Patients had metastatic HPV16+ cancer that in most patients was refractory to multiple systemic agents. Patients 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. 2/12 patients experienced partial tumor responses; one subsequently had residual disease resected by surgery, and she is without evidence of cancer years later. Pre-treatment tumor biopsies from three patients were available to study to understand disease response and resistance. One patient 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 patient 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[13, 14]. A patient 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[15-18]. Awareness of this progressive resistance is driving a movement in oncology toward the earlier application of immunotherapy[19, 20].

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 patient with cervical intraepithelial neoplasia who received a therapeutic cancer vaccine targeting HPV-16 E7[21]. 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 patients.

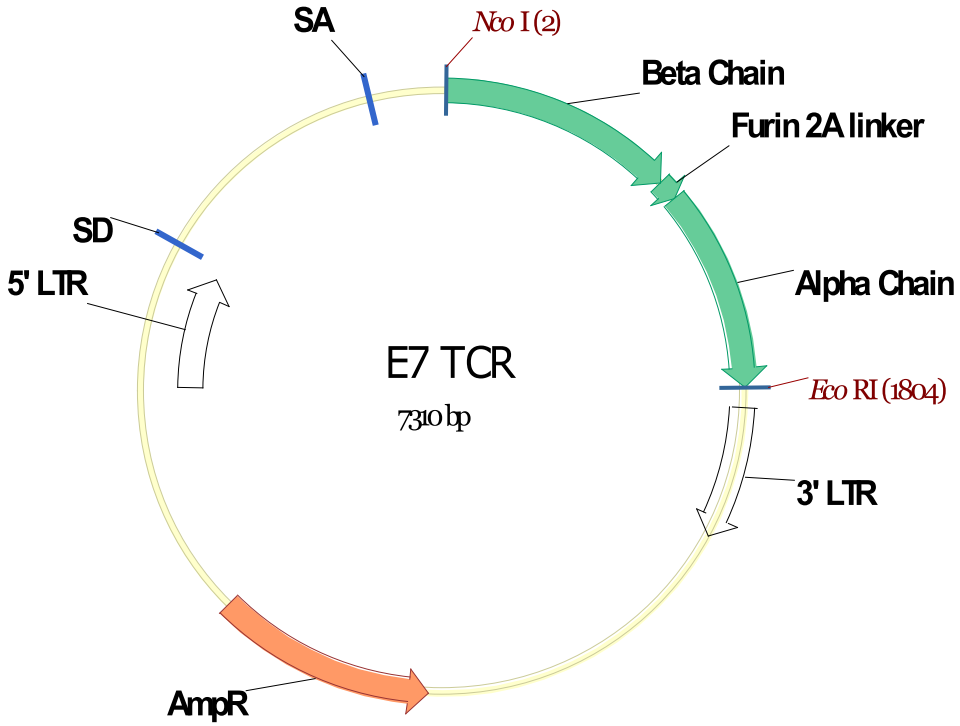


Figure 1: E7 TCR vector map. A TCR targeting $E7_{11-19}$ was isolated from the cervix-infiltrating lymphocytes of a patient with cervical intraepithelial neoplasia. The nucleotide sequence of the alpha and beta chains was determined, codon optimized for expression in human tissues, and the constant 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**).

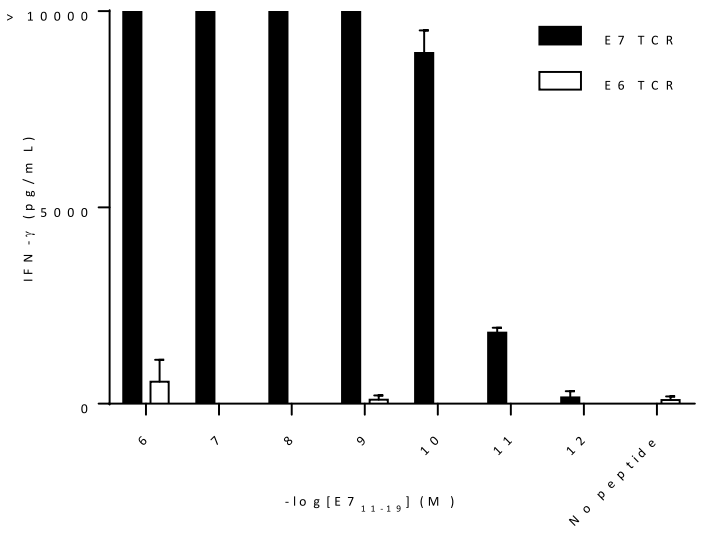


Figure 2: T cells transduced to express the E7 TCR demonstrated high avidity for the $E7_{11-19}$ peptide. T cells from PBMC were transduced to express the E7 TCR. Functional avidity was tested by coculture with T2 cells pulsed with titrated concentrations of $E7_{11-19}$ peptide.

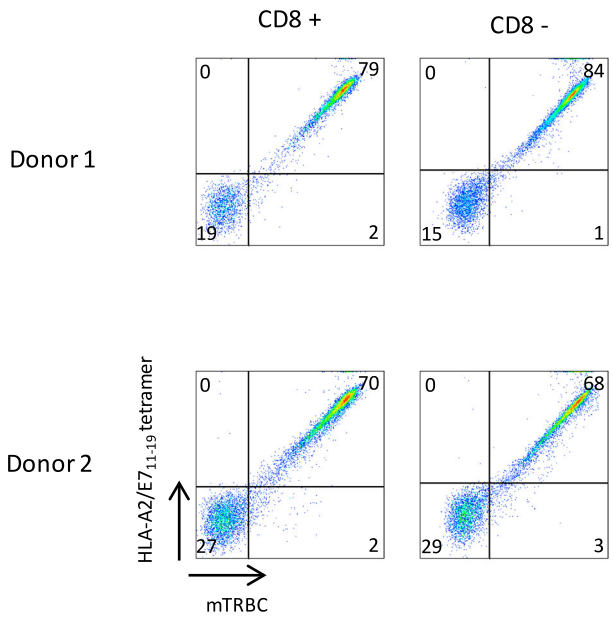


Figure 3: Peripheral blood T cells transduced to express the E7 TCR display CD8-independent HLA-A*02:01/E7$_{11-19}$ tetramer binding. T cells from PBMC were transduced to express the E7 TCR. Dot plots shown are gated on PI- lymphocytes and either CD8+ or CD8- cells as indicated above the dot plots.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

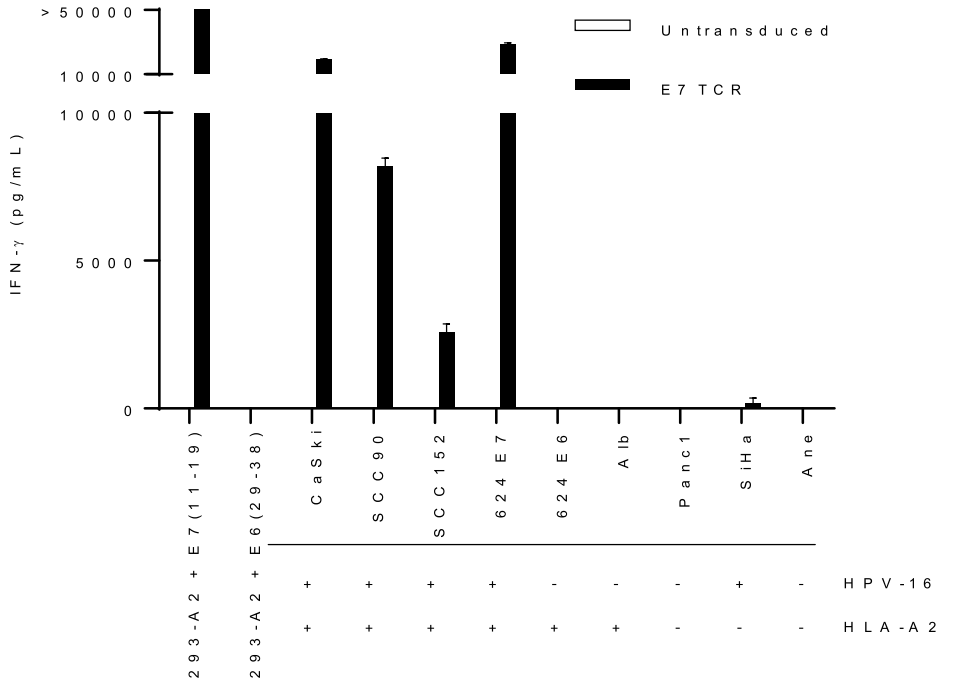


Figure 4: T cells transduced to express the E7 TCR specifically recognized HPV-16+ HLA-A*02:01+ tumor lines T cells transduced with E7 TCR were cocultured with targets expressing HPV-16 and HLA-A2 or with negative controls. Target cell line expression of HPV-16 and HLA-A2 is indicated below each label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

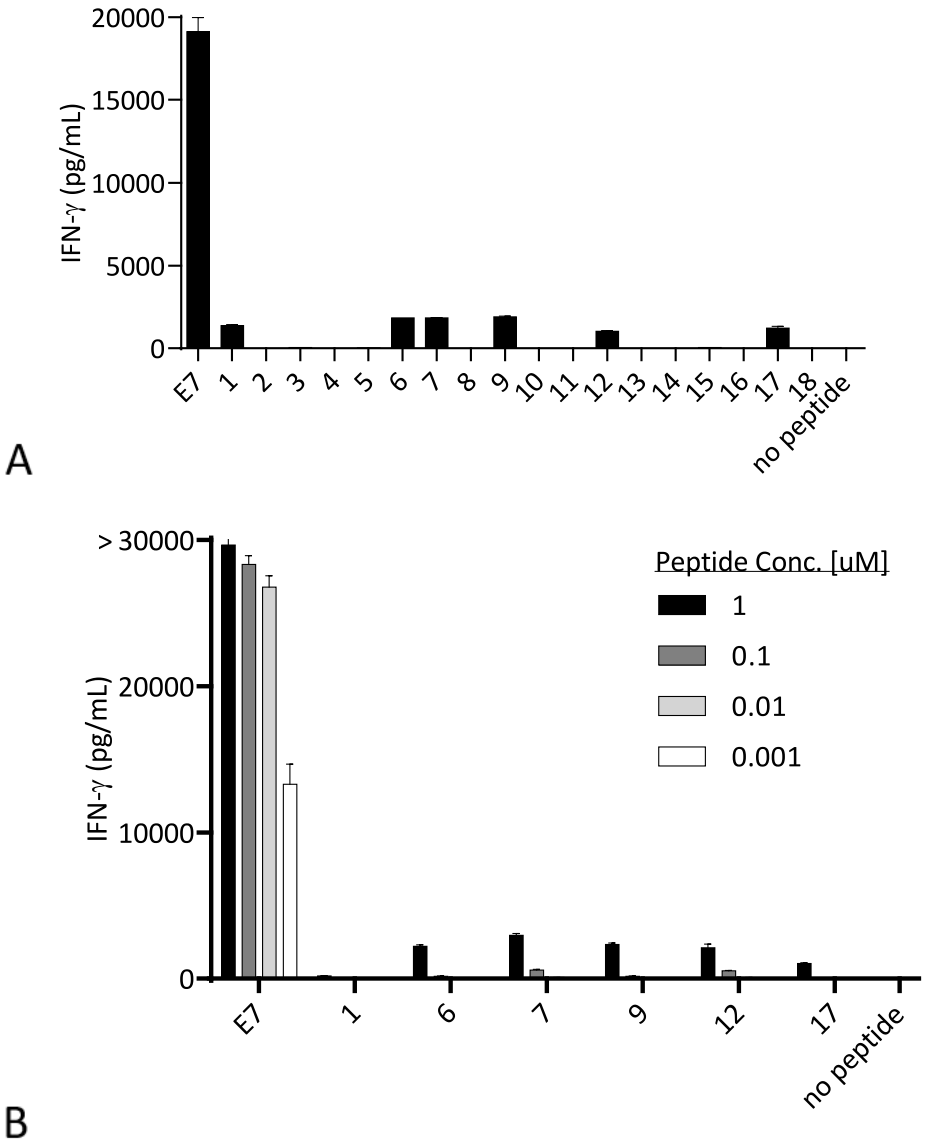


Figure 5: E7 TCR transduced T cells did not show cross reactivity against human peptides. E7 TCR transduced human T cells were tested for recognition of peptides identified by the BLAST search shown in **Table 1**. Target cells were T2 cells loaded with either the E7 peptide (E7) as a positive control, 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).

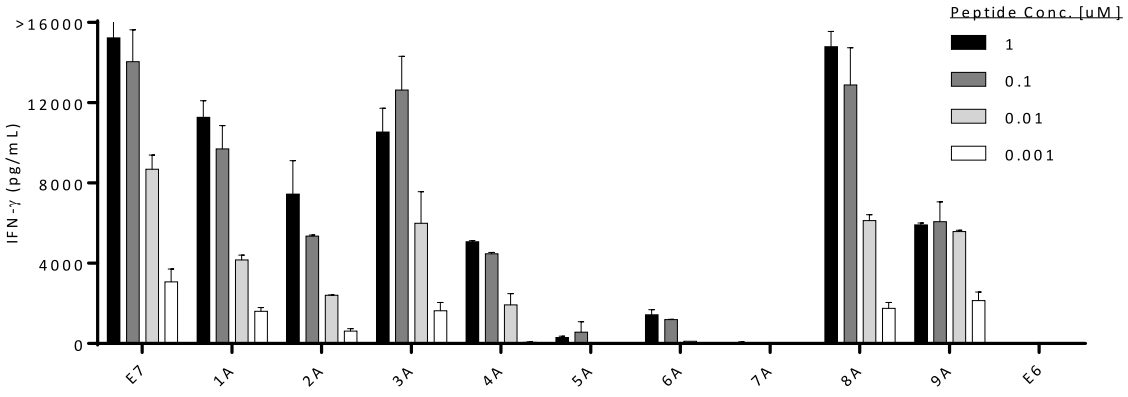


Figure 6: Serial alanine substitutions to the $E7_{11-19}$ target peptide revealed positions 4-7 to be the most crucial for recognition by E7 TCR transduced T cells. Human T cells were transduced to express the E7 TCR. The transduced cells were cocultured with T2 cells loaded with varying concentrations 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 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 clinical trial of HPV-16 E7 TCR T cells for patients with metastatic HPV16+ cancers (e.g. cervical cancer, anal cancer, head and neck cancer, etc.). This was a phase I/II dose-escalation study. Patients 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. 4 of 12 patients experienced objective tumor responses. Two other patients experienced unconfirmed partial responses. Six patients did not respond to treatment. One dose-limiting toxicity occurred. This patient had impaired lung function from rapidly progressing cancer in the lungs and 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, 1×10^{11} TCR+ E7 T cells, was selected as the maximum dose for the phase II portion of the study.

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 1×10^{11} tumor infiltrating lymphocytes (TIL) with widely heterogeneous reactivity including CD4, CD8, and NK cells. Experience at the NIH Clinical Center treating more than 200 patients with advanced cancers with genetically engineered T cells have not identified a risk of malignant transformation in this setting. 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 patient who received CD19 CAR-T cells (it did not cause malignant transformation and may have enhanced the efficacy of the T cells[22].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 patients will be required, data support the safety of retrovirally transduced mature T cells[23]. While the risk of insertional mutagenesis is low, the proposed protocol follows all current FDA guidelines regarding testing and follow up of patients receiving gene transduced cells[24]. To increase the safety of this protocol, the dose of E7 TCR T cells will be up to 3.0×10^{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 patients in the prior phase I/II study and the maximum number of doses of aldesleukin will be decreased by 50% (from 12 doses in the prior pahse I/II study to 6 doses).

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Histologically or cytologically confirmed stage II or stage III (AJCC 8th edition) oropharyngeal squamous cell carcinoma that has not been treated.

2.1.1.2 HPV16+ tumor and HLA-A*02:01+ HLA type (HLA-A*02 is also acceptable for determination of eligibility).

2.1.1.3 Measurable disease by RECIST 1.1 criteria.

2.1.1.4 Patient age 18 and older. Because no dosing or adverse event data are currently available on the use of E7 TCR T Cells in patients <18 years of age, children are excluded from this study. This reflects the age range of patients 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. Postmenopausal will be defined 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 and men must agree to use adequate contraception (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 or her partner 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. Patients 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 patient 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 Followup Protocol , which

will follow patients for up to 15 years per Food and Drug Administration (FDA) requirements.

2.1.1.11 Patients 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 patients with Gilbert's Syndrome who must have a total bilirubin < 3.0 mg/dL
• AST(SGOT)/ALT(SGPT)	Serum ALT/AST < 2.5 X ULN
• creatinine clearance	Calculated creatinine clearance (CrCl) >50mL/min/1.73 m2 for patients 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 Patients who are receiving any other investigational agents.

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

2.1.2.3 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.

2.1.2.4 There is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with E7 TCR T Cells. For this reason, women may not breastfeed while receiving study treatment and for one year after the study treatment ends. These potential risks may also apply to other agents used in this study.

2.1.2.5 Patients 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. Patients who have decreased immune competence may be less responsive to the treatment.

2.1.2.6 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;

- Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication)

2.1.2.7 Patients 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.

2.1.2.8 Patients with a second active invasive cancer are not eligible if it may confound assessment of response to the current therapy.

2.1.2.9 Patients who do not have a local physician to provide standard therapy post treatment.

2.1.3 Recruitment Strategies

Patients for this protocol will be recruited via standard CCR mechanisms as well as various advertising venues (e.g., referrals from physicians, drawing from populations in NIH Clinics, posting on NIH websites and NIH social media forums, etc.). All recruitment advertisements and letters, if they include more than that listed publicly (e.g., on clinicaltrials.gov) 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. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a participant has signed the consent.

NOTE: Results from outside laboratories are accepted for screening evaluations provided that they were performed in the appropriate timeframe.

- HLA-A*02:01 testing. Tests from CLIA approved laboratories outside the NIH are acceptable. (Any time prior to initiation of study therapy) (HLA-A*02 is also acceptable for determination of eligibility any time prior to enrollment, but HLA-A*02:01 is required prior to initiation of treatment).

- HPV16 genotype testing. Tests from CLIA approved laboratories outside the NIH are acceptable. (Any time prior to initiation of study therapy). p16+ by IHC is also acceptable for determination of eligibility but genotyping is required prior to initiation of treatment.
- 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. (Within 3 months prior to treatment, results not required for enrollment but required for treatment)
- Anti CMV antibody titer, HSV serology, and EBV panel (unless known to be positive by a prior test). (Within 3 months of treatment, results not required for enrollment but required for treatment)
- History and physical exam including review of the medical record to confirm the diagnosis and stage. (Within 4 weeks prior to initiation of study therapy)
- 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. (Within 4 weeks of enrollment, results not required for eligibility but required for treatment)
- Pregnancy test (Within 3 months prior to treatment)
- Confirmation of oropharyngeal cancer (pathology report from outside institution is sufficient))
- Electrocardiogram (ECG) (Within 3 months prior to treatment)

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	Patients	Eligible patients with oropharyngeal cancer

Arms

Number	Name	Description
1	Arm 1	Up to $3x10^{10}$ E7 TCR T cells (based on the number of cells that can be generated in the shortened manufacturing process) will be administered intravenously over 20 to 30 minutes on day 0.

Arm Assignment

Patients in Cohorts 1 will be directly assigned to Arm 1.

2.4 BASELINE EVALUATION

2.4.1 Within 30 days prior to apheresis

1. Complete physical examination, including weight and vital signs
2. Vein assessment per apheresis clinic policy.
3. 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
4. CBC with differential and platelet count
5. PT/PTT
6. Urinalysis

2.4.2 Any time prior to starting cyclophosphamide

1. Baseline tumor biopsy (optional)
2. Baseline research blood draw

2.4.3 Within 4 weeks prior to starting cyclophosphamide

1. 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.
2. 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.
3. Chest x-ray
4. ECG
5. Cardiac and/or pulmonary testing (e.g. cardiac stress test, echocardiogram, or pulmonary function tests) for patients at elevated risk such as those with COPD, diabetes, hyperlipidemia, hypertension, a history of smoking, or age >60. Patients with cardiac ischemia will not be treated. Patients with a clinically significant arrhythmia or LVEF <45% will be evaluated by a cardiologist. Patients with a FEV1/FVC<70% or FEV1< 80% will be evaluated by a pulmonologist.
6. TBNK
7. Thyroid panel

2.4.4 Within 1 week of starting cyclophosphamide

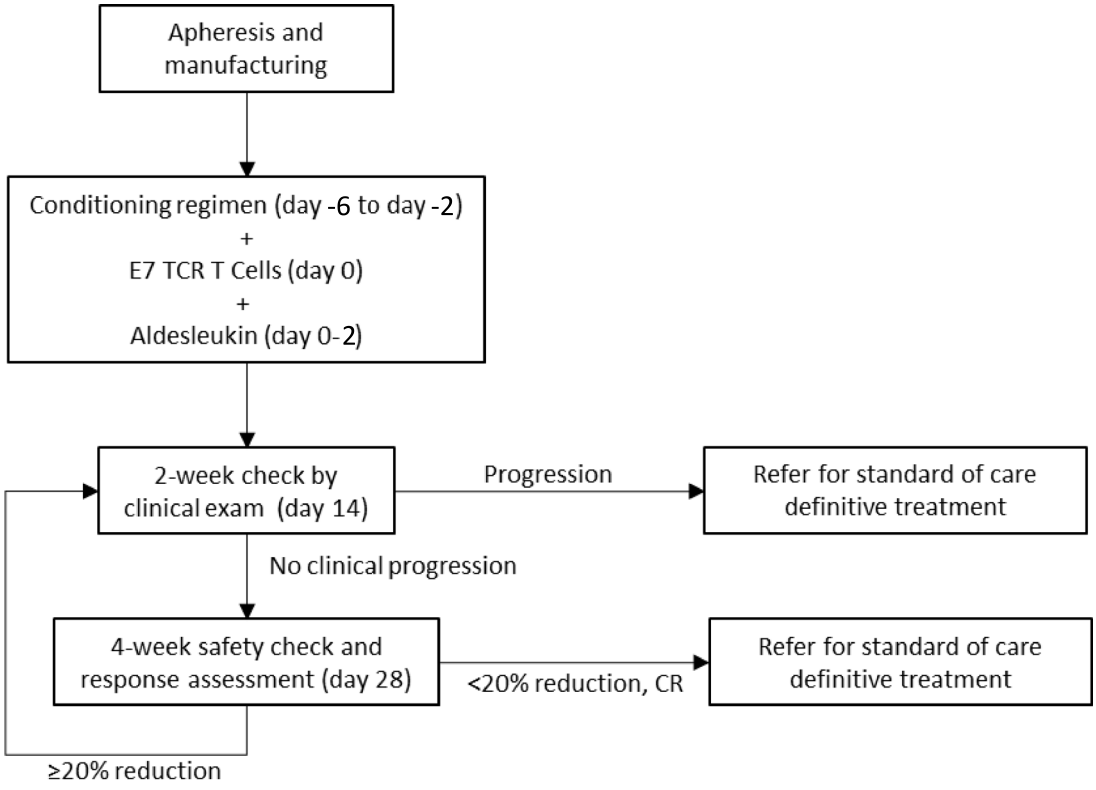
1. Complete physical examination, including weight and vital signs
2. Chemistries: Chemistries Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT,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

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a phase II feasibility study. The study schema is as follows:

3.1.1 Protocol Schema



Assessments:

- 2-week (+/- 4 days) checks for clinical progression by clinical exam will be performed at the NIH.
- 4-week (+/- 4 days) response and safety assessments will be performed at the NIH Clinical Center.
- The 2-week check by clinical exam and 4-week response assessments will continue until criteria are met to refer for definitive treatment (see below).
- The 2-week clinical check will include a physical examination, adverse event assessment and an exam by an otolaryngologist to assess for possible clinical progression. The exam may include flexible laryngoscopy performed in the clinic.
- The 4-week safety check will include a physical examination, adverse event assessment and an exam by an otolaryngologist. The exam may include flexible laryngoscopy performed in the clinic.

Tumor measurements at each response assessment will be performed as described in section **6.3**.

- Patients with all of the following will be observed and reevaluated at 4-week intervals (with a 2-week clinical check):
  - 20% or greater partial reduction in the sum of the diameters of the index lesions taking as reference the last response assessment. For the first 4-week visit, the reference measurements will be from baseline.
  - No new lesions
  - No lesion with an increase of more than 20% with an absolute increase of at least 5 mm.
  - No unambiguous progression of non-index lesions
  - No CR
- Patients with any of the following will be referred for definitive treatment:
  - Less than 20% reduction in the sum of the diameters of the index lesions
  - One or more new lesions
  - An increase in any measurable lesion by more than 20% with an absolute increase of at least 5 mm
  - Unambiguous progression of a non-index lesion
  - CR

3.1.2 Leukapheresis

The patient 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 patient 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. The leukapheresis collection will be obtained at least 21 days prior to the cell infusion (or any time prior to treatment if collected on protocol 16C0061). 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 enrolled on that study.

3.1.3 E7 TCR T cell preparation

After cells are obtained by apheresis (either on this protocol or protocol 16C0061 if the patient 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 patient 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 patient is co-enrolled. E7 TCR cells can be produced in approximately 11 to 15 days. Cell products may be cryopreserved during production to accommodate patient 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 ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF) the following day. The contact in the ETIB PDCMF is Jeremy Rose (240-858-3244).

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 ETIB PDCMF and possible use in research and banked according to protocol 16C0061 (Tissue Procurement Protocol) if subject co-enrolled on that study.

3.1.4 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. Patients will receive up to 3 x 10^{10} E7 TCR T cells (i.e. TCR+ cells). There is no lower limit of the cell dose as patients 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, patients 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**. Patients will receive one course of treatment. The start date of the course will be the start date of the chemotherapy; the end date will be the day of the first post-treatment evaluation and the first safety and response assessment follow-up visit.

3.2 DRUG ADMINISTRATION

Treatment schedule will be according to the following schedule (See Schedules **3.2**). (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 first administration]. All other medications may be given +/- one 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 patient clinical factors. Patients at risk of adverse clinical consequences

from volume overload (e.g. patients 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:

3.2.2 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 1.5 mL/kg/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 patient 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 hr X 2 days. If the patient 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 (see section**13.3**) over 23 hours after each cyclophosphamide dose. If the patient 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 patient 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*)

3.2.3 Cell infusion and other treatment administration

The E7 TCR cells will be delivered to the patient care unit by an authorized staff member. Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), an identification of the product and documentation of administration are entered in the patient's chart, as is done for blood banking protocols. The dose of E7 TCR T cells will be up to 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 **3.2.4** below.

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

- Fluconazole: can be used at the discretion of the treating clinician
- Valacyclovir p.o. or Acyclovir IV: see section **4.2.2**.

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

- Aldesleukin as described in section **3.2.4** below.

TMP/SMX will be administered at 160mg/800mg every other day starting at or around the time of discharge

Day	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Therapy:											
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		
Valacyclovir po or Acyclovir IV							X	X	X	X	X

3.2.4 Aldesleukin: Intravenous Administration

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 of cell infusion and continuing for up to three days (maximum 6 doses). The start of aldesleukin treatment may be

delayed up to 2 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 patient 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 patients 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 4**. Toxicities will be managed as outlined in **Appendix 3**. 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 patient to identify a surrogate to make decisions if a patient becomes unable to make decisions.

3.3 SAFETY PROTOCOL STOPPING RULES

The study will be halted (immediately stop accrual and treatment) if any of the following safety conditions are met until an appropriate evaluation of the cause of the toxicity is determined and a plan of correction if necessary is established:

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

3.4 STUDY CALENDAR

Procedure	Screening[28]	Baseline[4]	Before treatment[27]	Preparative regimen[7]	Day 0	During hospitalization[8]	Follow-up Period		
							Check by clinical exam[24]	Safety check and response assessment[1,11]	Annual followup[25]
Medical History	X	X							X
Physical Exam	X	X			X	X[23]	X	X	
Vital Sign	X	X			X[22]	X	X	X	
ECOG Performance Score	X	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	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]					X[15]	

Procedure	Screening[28]	Baseline[4]	Before treatment[27]	Preparative regimen[7]	Day 0	During hospitalization[8]	Follow-up Period		
							Check by clinical exam[24]	Safety check and response assessment[1,11]	Annual followup[25]
Biopsy		X						X[26]	
Correlative Research Studies		X	X		X[14]	X[14]	X	X	
E7 TCR assay[17]						X	X	X	
Cardiac evaluation[12]		X							
Chest x-ray		X							
ECG	X	X							
Chest CT and MRI or PET[21]	X	X						X	
Pulmonary Function Test[3]		X							
ENT Evaluation								X	
Response Evaluation								X	
Adverse Events			X	→	→	→	→	→	→
Infusion of transduced cells[6]					X				
Additional apheresis or blood draw[13]								X	

1. End of treatment visit will occur 28 days +/- 4 days after the E7 TCR T Cell infusion, or when the patient comes off treatment if before 28 days. If the patient 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, patients 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 patients with a prolonged history of cigarette smoking, as clinically indicated in Section **2.4**
4. Exact timeline is indicated in Section **2.4**

5. For women of child-bearing potential as defined in Section **2.1.1.6**
6. See other treatments in Section **3.2**
7. On days -6 to -1, every 1-2 days as clinically indicated
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 patient 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. 28 days (+/- 4 days) after cell infusion, additional visits as indicated in Section **3.2.3**
12. For patients 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. Patients with a LVEF of less than or equal to 45% will not be eligible, as noted in section **2.4**
13. An approximately 5 liter apheresis may be performed at the first follow up visit, if the patient is unable to undergo apheresis, approximately 96 mL of blood may be obtained. Subsequently, approximately 60 mL of blood may be obtained at follow up visits for at least three months. 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 patient is unable to undergo apheresis, approximately 96 mL of blood may be obtained. Apheresis may be done in the first month follow-up visit; in the following visits, blood will be collected.
16. This can occur at any time prior to treatment on protocol 16C0061 if the patient is co-enrolled on that protocol. See Section **3.1.2** for further details.
17. Clinical assay performed by the NCI Flow Cytometry Laboratory in the Laboratory of Pathology.
18. p16+ by IHC is also acceptable for enrollment but not for the treatment.
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. 14 days (+/- 4 days) after cell infusion, additional visits as indicated in Section **3.2.3.** Patient may be seen by ENT.
25. Patients will be contacted (either by phone or visit to NIH) every 1 year (+/- 1 months) following completion of protocol therapy for a total of 5 years to determine the dates and types of additional therapies (if any) that patients received after definitive treatment and the status of their disease.
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 patients receiving gene transfer is required by the FDA and must continue even after the patient comes off the study. Long-term follow-up will be done under a different long-term gene therapy follow-up protocol.

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

Participants will not be compensated on this study.

3.6.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. 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 28 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
- Inability to generate a cell product. A second attempt may be made to generate a cell product from the patient. If the second attempt fails, that patient will be removed from the study and replaced with another patient.
- The investigators decide to end the study
- The investigators decide it is in the patient's best interest
- Substantial patient non-compliance that prevents compliance with the study requirements.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 PROHIBITED MEDICATIONS

Patients needing systemic steroid therapy may not participate in this study.

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.

As described below (Sections **4.2.1** and **4.2.2**), 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 patient misses less than 20% of their doses for either medication a protocol deviation does not need to be filed.

4.2.1 Pneumocystis Jirovecii Pneumonia

Patients will 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 will be substituted for TMP/SMX-DS in patients with sulfa allergies. It will be administered aerosolized at 300 mg per nebulizer within one week of chemotherapy start date.

4.2.2 Herpes Virus Prophylaxis

Patients 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.

4.2.3 Fungal Prophylaxis (Fluconazole)

4.2.4 The decision if to administer fungal prophylaxis (e.g., fluconazole) will be at the discretion of the treating clinician and documented in the medical record Empiric Antibiotics

Patients 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 patients with unexplained fever or infectious complications.

4.3 BLOOD PRODUCT SUPPORT

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

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

Note: Patients may be transfused at a higher platelet count as clinically indicated, e.g.:

- 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

Patients 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 patients require steroid therapy, they will be taken off treatment. Patients who require transfusions will receive irradiated blood products. Ondansetron 0.15 mg/kg/dose IV every 8 hours will be administered for nausea and vomiting. Additional anti-emetics will 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

The amount of blood that may be drawn from adult patients 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 ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF). 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 ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF).

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 ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF) 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 ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF) on Monday, Wednesday, and Friday x 5 days, then weekly (while the patient is hospitalized). Send to the PDCMF lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 240-858-3244.
    - 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 ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF).

5.1.3 Tumor Biopsies

- Biopsy of oropharyngeal primary tumors are optional and will be performed at baseline and approximately 4 weeks following T cell infusion. Biopsies will be performed in OP5 clinic under local anesthesia by an otolaryngologist. Direct or indirect visualization may be used. The oropharynx may be sprayed with topical lidocaine. If the tumor remains sensate, additional lidocaine can be injected into the tumor at the site of intended biopsy. Cup forceps may be used to perform no more than 5 biopsies per tumor site. A flexible endoscope (passed transnasally) may be used for visualization when necessary. In cases where the tumor cannot be adequately visualized for biopsy in the clinic, the subject may

undergo direct laryngoscopy with biopsy in the operating room under general anesthesia (approximately 30 minutes).

- Specimens will be transported by the assigned research nurse to Dr. Christian Hinrichs' lab for sample labeling. Contact: Scott Norberg, Bldg 10, room 4B-04, phone 484-707-8595.
- Following labeling, samples will be transported by an assigned lab member to the ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF) where they can be frozen in optimal cutting temperature compound. Contact: the PDCMF lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 240-858-3244.
- 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 4 weeks after the treatment. Apheresis product will be transferred to the ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF), PDCMF lab; Attention Jeremy Rose, Bldg 10, room 12C216. Contact phone: 240-858-3244. 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 ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF) 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. [10, 12]

- 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	ETIB Preclinical Development and Clinical Monitoring Facility
Serum	8-16 mL	SST	<4 weeks prior to cell infusion, day-6	ETIB Preclinical Development and Clinical Monitoring Facility
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	ETIB Preclinical Development and Clinical Monitoring Facility
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	ETIB Preclinical Development and Clinical Monitoring Facility
Oropharyngeal Biopsy (optional)	N/A	N/A	Prior to starting chemotherapy and approximately 4 weeks following T cell infusion	Processed in Christian Hinrichs Lab, then transported to ETIB Preclinical Development and Clinical Monitoring Facility

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 patient 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 patient is off study.

- Patients' 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 long-term gene therapy follow up protocol if the patient 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 IRB notification and an executed MTA.

5.4.1 Preclinical Development and Clinical Monitoring Facility (PDCMF)

- Patient blood and tissue samples, collected for the purpose of research under applicable IRB approved protocols of the Genitourinary Malignancies Brand (GMB) or Experimental Transplantation and Immunology Branch (ETIB), may be archived by the ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF). All data associated with archived clinical research samples is entered into Labmatrix. Access is limited to PDCMF staff and GMB/ETIB clinical staff, requiring individual login and password. All staff in the PDCMF laboratory receive annually updated NIH/CIT training and maintain standards of computer security.
- The data recorded for each sample includes the patient ID, trial name/protocol number, date drawn, treatment cycle/post-transplant time point, cell source (e.g. peripheral blood, marrow,) as well as box and freezer location. Patient demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI/ETIB clinical records. As of January 2007, all newly received samples receive a unique bar code number, which is included in the sample record in the PDCMF database. Only this bar code is recorded on the sample vial and the vials will not be traceable back to patients without authorized access to the PDCMF database. All non-coded samples previously archived will be stripped of identifiers prior to distribution for any use other than as a primary objective of the protocol under which they were collected.
- Samples are stored in freezers. All samples will be labeled solely with a bar code (which includes the date, and serially determined individual sample identifier). The key will be available to a restricted number of GMB/ETIB investigators and associate investigators on the protocol. Coded samples will be stored frozen at -20°C, -80°C or liquid nitrogen vapor phase to -180°C according to the stability requirements in a single location under the restricted control of the PDCM Facility of ETIB.

  These freezers are located onsite at the Preclinical Service laboratory (12C216) (-85°C freezer) or in ETIB common equipment space (CRC/3-3273).Access to samples from a protocol for research purposes will be by permission of the Principal Investigator of that protocol in order to be used (1) for research purposes associated with protocol objectives for which the samples were collected, or (2) for a new research activity following submission and IRB approval of a new protocol and consent, or (3) for use only as unlinked or coded samples under the OHSRP Exemption Form guidelines stipulating that the activity is exempt from IRB review. Unused samples must be returned to the PDCMF

laboratory. Samples, and associated data, will be stored permanently unless the patient withdraws consent. If researchers have samples remaining once they have completed all studies associated with the protocol, they must be returned to the PDCMF laboratory.

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 patient and their samples.

5.4.3 Protocol Completion/Sample Destruction

- Once research objectives for the protocol are achieved, researchers can request access to remaining samples, providing they have both approval of the Principal Investigator of the original protocol under which the samples or data were collected and either an IRB approved protocol and patient consent or an OHSRP exemption indicating that the activity is exempt from IRB review.
- The laboratories will report to the Principal Investigator any destroyed samples, if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container), lost in transit between facilities or misplaced by a researcher.
- Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples that meet expedited reporting requirements (see section **7.2.1**) will also be reported.

5.5 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

- Genomic studies will be conducted under protocol 16C0061 (Tissue Procurement Protocol) onto which patients will be 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 patient code identification numbers with the personal identifiers will be stored in a secure data base. 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 patient 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. Patients will be followed for adverse events for 28 days following cell infusion, or until off-study, or until they initiate a new treatment, whichever comes first. For patients who start definitive treatment prior to 28 days from cell infusion, only adverse events and serious adverse events that are possibly related to E7 TCR T cells will be recorded.

6.1.2 Recording 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 patient's outcome.

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:

_x_ Coded, linked data in an NIH-funded or approved public repository.

_x_ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)

_x_ Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

_x_ An NIH-funded or approved public repository: Clinicaltrials.gov

_x_ BTRIS (automatic for activities in the Clinical Center)

_x_ Approved outside collaborators under appropriate individual agreements.

_x_ Publication and/or public presentations.

When will the data be shared?

_x_ Before publication.

_x_ At the time of publication or shortly thereafter.

6.3 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 patients with solid tumors [25]. 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**.

6.3.1 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 patient, 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.

6.3.2 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 patient 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.[26-28] 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.[29]

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 optional 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.

6.3.2.1 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.

6.3.2.2 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 patient 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).

6.3.2.3 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 patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients 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: Patients 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.

6.3.3 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.

6.4 TOXICITY CRITERIA

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

6.5 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 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 each 4-week response assessment. An increase in either T stage or N stage will be scored as 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.
3) If any patient starts the conditioning regimen chemotherapy but does not receive the E7 TCR T Cells, it will be considered a feasibility failure.

7 NIH REPORTING REQUIREMENTS/DATA 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 REPORTING

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/IRB 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 should 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 on a regular basis (approximately weekly) when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

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 patient 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 SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient 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 patient/subject 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 patient 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 patient 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 WAVIER OF EXPEDITED REPORTING TO CCR

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)

CTCAE System Organ Class	Adverse Event	Grade	Prolongation of Hospitalization	Expected Frequency	Attribution
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)
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 patients that start standard of care treatment prior to 28 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 patients 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 patient 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.

8.6.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of E7 TCR T cells.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 4 months after the last dose should, if possible, 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.

9 CLINICAL MONITORING

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary and secondary study endpoints; adherence to the protocol, regulations, ICH E6, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESES

10.1.1 Primary efficacy endpoints

The primary objective of this protocol is to determine if it is feasible to administer E7 TCR T cells prior to definitive therapy in patients with oropharyngeal cancer.

The endpoint to be measured is the fraction of subjects for whom E7 TCR induction therapy is feasible as defined in Section 6.5.

10.1.2 Secondary efficacy endpoints

None

10.1.3 Sample size determination

This study evaluates the fraction of patients who can receive induction therapy with E7 TCR cells without failing the feasibility criteria in section 6.5. For a given patient, this will be considered a success. It would be desirable if the fraction of patients who are able to achieve success were consistent with 80-85%. In this pilot trial of 15 evaluable patients who undergo the E7 TCR cell administration if 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 patients 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 patient per month may enroll onto this trial. Thus, accrual is expected be completed in approximately 1.5 years. To allow for a small number of ineligible and inevaluable patients, the accrual ceiling will be set at 180 patients.

10.1.4 Populations for analysis

All subjects who receive E7 TCR T cell administration under this protocol will be included in the analyses.

Feasibility Analysis: All patients who receive induction E7 TCR T cells and who can be evaluated for the two feasibility criteria in Section 6.5 will be evaluable for feasibility.

Toxicity Analysis: All patients will be evaluable for toxicity from the time of their treatment with E7 TCR T cells.

Response Analysis: Patients who receive E7 TCR T cell therapy and either experience clinical progression at the 2-week clinical check or are evaluated at the 4-week response assessment will be evaluable for response. Response will be classified according to the definitions below.

10.2 STATISTICAL ANALYSES

10.2.1 General approach

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

10.2.2 Analysis of the primary efficacy endpoints

The fraction who achieve a success among those who receive E7 TCR T cell administration and who can be evaluated for the two feasibility criteria in Section 6.5, with 95% confidence intervals on the fraction reported as well.

10.2.3 Analysis of the secondary efficacy endpoints

None

10.2.4 Safety analysis

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

10.2.5 Baseline descriptive statistics

Demographic and clinical characteristics of all patients will be reported.

10.2.6 Planned interim analyses

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

10.2.7 Subgroup analyses

None will be performed.

10.2.8 Tabulation of individual participant data

None will be provided

10.2.9 Exploratory analyses

Change in tumor size following induction therapy will measured by criteria in Section **6.3**, with the significance of the difference tested by a Wilcoxon signed rank test.

Statistical tests performed on research samples 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 patients to be entered in this protocol have stage II and III HPV-associated oropharyngeal squamous cell carcinoma and are more likely to have persistent or recurrent disease after standard therapy.

Subjects from both sexes and 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 patient 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 patient, 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 patient population.

12.3 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The experimental treatment has a chance to provide clinical benefit though it is not known if it will do so.

Over 400 patients have been treated in the Surgery Branch, NCI with 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 patients. In 93 patients 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 patients treated in subsequent randomized trial 2 treatment related deaths occurred, both due to the TBI component of the treatment regimen.

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 patient's airway. It is important to note that although these patients require significant supportive measures during this period, all toxicities are reversible, and the overwhelming majority of patients 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.

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 patients 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 patients who received 1,039 treatment courses are listed in **Appendix 4**.

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 Electrocardiogram (ECG)

An electrocardiogram or ECG is a test that records the electrical activity of the heart. It is used to measure the rate and regularity of heartbeats as well as the size and position of the heart chambers, and the presence of any damage to the heart. For this test, participants will be asked to lie down, and small patches that have an adhesive edge with a gel in the middle, called electrodes, will be placed on the arms, legs, and chest. The areas where the electrodes are placed will be cleaned and, if needed, some hair may be shaved or clipped to allow for better attachment of the electrodes. The adhesive from the patches may irritate the skin.

12.3.1.4 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 the arms.

12.3.1.5 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 the veins. It may be necessary to remove the catheter.

12.3.1.6 X-Ray

An x-ray examination exposes participants to a small amount of radiation, corresponding to one-fifth of the dose a person gets each year from natural sources, such as the sun and the ground. This small amount of radiation is not considered dangerous.

12.3.1.7 CT scan, PET, and MRI

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.

Risks for gadolinium enhanced MRI scans:

The risks of an IV catheter include bleeding, infection, or inflammation of the skin and vein with pain and swelling.

Mild symptoms from gadolinium infusion occur in fewer than 1% of those who receive it and usually go away quickly. Mild symptoms may include coldness in the arm during the injection, a metallic taste, headache, and nausea. In an extremely small number, fewer than one in 300,000 people, more severe symptoms have been reported including shortness of breath, wheezing, hives, and lowering of blood pressure. Participants should not receive gadolinium if they previously had an allergic reaction to it. They will be asked about such allergic reactions before gadolinium is given.

People with kidney disease are at risk for a serious reaction to gadolinium contrast called "nephrogenic systemic fibrosis" which has resulted in a very small number of deaths. A blood test of the kidney function may be done within the month before an MRI scan with gadolinium contrast. Participants will not receive gadolinium for a research MRI scan if the kidney function is not normal or if they received gadolinium within the previous month.

Most of the gadolinium contrast leaves the body in the urine. However, the FDA recently issued a safety alert that indicates small amounts of gadolinium may remain in the body for months to years. The effects of the retained gadolinium are not clear. At this time, retained gadolinium has not been linked to health risks in people whose kidneys work well. Some types of gadolinium contrast drugs are less likely to remain than others. In this study, we will use the gadolinium contrast drugs that are less likely to remain.

12.3.1.8 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 patients 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 the body.

The chest X-ray, PET and CT scans in this study may expose patients 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%).

12.4 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) or Investigational Device Exemption (IDE) 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/each 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 Center for Cancer Research (NCI CCR). 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 CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NCI CCR.

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 AND INVESTIGATIONAL DEVICE INFORMATION

14.1 INTERLEUKIN-2 (ALDESLEUKIN, PROLEUKIN, RECOMBINANT HUMAN INTERLEUKIN 2)

14.1.1 Source

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 8C) 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 3**. 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 2**. Additional Grade 3 and 4 toxicities seen with aldesleukin are detailed in **Appendix 3**.

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

It 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 Stability

Following reconstitution with 2 mL of sterile water for injection to a concentration of 25 mg/mL, the solution has a pH of 7.7. The fludarabine powder is stable for at least 18 months at 2 to 8C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Since no preservative is present, reconstituted fludarabine will typically be administered within 8 hours. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribonucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

14.2.4 Storage

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

14.2.5 Administration

Fludarabine is administered as an IV infusion in 100 mL 0.9% sodium chloride, USP over 15 to 30 minutes. The doses will be based on body surface area (BSA). If patient is obese (BMI >35), drug dosage will be calculated using practical weight as described in **Table 2**.

Table 2. Modification of Dose Calculations* in patients 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.

14.2.6 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 patients 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 patient 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

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 patient's body weight. If patient 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 patients; 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 patients receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients 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 -80C 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 patient PBL. There will be no re-use of the same unit of supernatant for different patients. 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 stage II or III HPV+ oropharyngeal cancer. 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/ETIB 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.

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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.

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

Body System	% Patients
Body as a Whole	
Chills	52
Fever	29
Malaise	27
Asthenia	23
Infection	13
Pain	12
Abdominal pain	11
Abdomen enlarged	10
Cardiovascular	
Hypotension	71
Tachycardia	23
Vasodilation	13
Supraventricular tachycardia	12
Cardiovascular disorder[a]	11
Arrhythmia	10
Digestive	
Diarrhea	67
Vomiting	50
Nausea	35
Stomatitis	22
Anorexia	20
Nausea and vomiting	19
Hemic and Lymphatic	
Thrombocytopenia	37
Anemia	29
Leukopenia	16

Body System	% Patients
Metabolic and Nutritional Disorders	
Bilirubinemia	40
Creatinine increase	33
Peripheral edema	28
SGOT increase	23
Weight gain	16
Edema	15
Acidosis	12
Hypomagnesemia	12
Hypocalcemia	11
Alkaline phosphatase incr	10
Nervous	
Confusion	34
Somnolence	22
Anxiety	12
Dizziness	11
Respiratory	
Dyspnea	43
Lung disorder[b]	24
Respiratory disorder[c]	11
Cough increase	11
Rhinitis	10
Skin and Appendages	
Rash	42
Pruritus	24
Exfoliative dermatitis	18
Urogenital	
Oliguria	63

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

16.3 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	No

Toxicity	Grade	Supportive Medications	Stop Cycle*	Stop Treatment**
			supportive measures	
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.

16.4 APPENDIX 4: INTERLEUKIN-2 TOXICITIES OBSERVED IN PATIENTS 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.