Abbreviated Title: HPV-16 E7 TCR

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A Phase I/II Trial of T Cell Receptor Gene Therapy Targeting HPV-16 E7 for HPV-Associated Cancers

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Drug Name:	E7 TCR Transduced PBL	Fludarabine	Cyclophosphamide	Aldesleukin
IND Number:	16959	16959	16959	16959
Sponsor:	CCR, NCI	CCR, NCI	CCR, NCI	CCR, NCI
Manufacturer:	CC DTM	Generic	Generic	Generic
Supplier:	CC DTM	CC Pharmacy	CC Pharmacy	CC Pharmacy

Coordinating Center: NIH NCI Center for Cancer Research (CCR)
Participating Sites: See **Appendix A** for role of participating sites
Safety Monitoring Committee (SMC): NCI Intramural CCR SMC

PRÉCIS

Background:

- Metastatic or refractory/recurrent human papillomavirus (HPV)-16+ cancers (cervical, vulvar, vaginal, penile, anal, and oropharyngeal cancers) are incurable and poorly palliated by standard therapies.
- HPV-16+ cancers constitutively express the HPV-16 E7 oncoprotein, which is absent from healthy human tissues.
- Administration of T cell receptor (TCR) gene engineered T cells can induce objective tumor responses in certain malignancies including HPV-16+ cancers.
- T cells genetically engineered with a TCR targeting HPV-16 E7 (E7 TCR) display specific reactivity against HLA-A2+, HPV-16+ target cells.

Objectives:

Phase I Primary Objective

- To determine a safe dose for E7 TCR cells plus aldesleukin for the treatment of metastatic HPV-16+ cancers.

Phase II Primary Objective

- To determine safety and efficacy of E7 TCR cells plus aldesleukin for the treatment of metastatic HPV-16+ cancers.

Eligibility:

- Patients greater than or equal to 18 years old with metastatic or refractory/recurrent HPV-16+ cancer.
- Prior first line systemic therapy is required unless the patient declines standard treatment.
- Patients must be HLA-A*02:01-positive.

Design:

- This is a phase I/II clinical trial that will test the safety and efficacy of E7 TCR cells.
- All patients will receive a non-myeloablative lymphocyte-depleting preparative regimen of cyclophosphamide and fludarabine followed by a single infusion of E7 TCR cells. Cell infusion will be followed by high-dose aldesleukin.
- Re-enrollment will be allowed for a small number of subjects.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for 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 Phase I Primary Objective

- To determine a safe dose for E7 TCR cells plus aldesleukin for the treatment of metastatic HPV-16+ cancers.

1.1.2 Phase II Primary Objective

- To determine the safety and efficacy of E7 TCR cells plus aldesleukin for the treatment of metastatic HPV-16+ cancers.

1.1.3 Secondary Objective

- To assess progression-free survival.

1.1.4 Exploratory Objective

- To conduct exploratory immunologic studies to understand and improve the administered treatment.

1.2 BACKGROUND AND RATIONALE

Adoptive T cell therapy (ACT), the administration of autologous tumor-specific T cells, can mediate durable, complete regression of certain advanced-stage malignancies. (1) T cells for ACT can be directed against diverse tumors through T cell receptor (TCR) gene therapy in which peripheral blood T cells are transduced to express a TCR targeting a tumor antigen. This clinical protocol is for a treatment that targets HPV-16+ cancers with TCR gene therapy directed against HPV-16 E7. HPV-16 E7 is an ideal immunotherapeutic target that is constitutively expressed by and important for the survival of HPV-16+ cancers, and it is not expressed by healthy human tissues.(2) The rationale for this protocol is based on the application of the potent

immunotherapy of ACT to the attractive therapeutic target of HPV-16 E7 in advanced-stage HPV-16+ cancers including cervical, oropharyngeal, anal, vulvar, vaginal, and penile malignancies. HPV-Associated Malignancies

In the United States, there are more than 11,000 deaths from cancer at HPV-associated sites annually (**Table 1**). (3-6) Metastatic or recurrent/refractory malignancies from these sites are incurable and difficult to palliate. Responses to chemotherapy are variable but generally short-lived. (7-9) In a Gynecologic Oncology Group randomized trial comparing four cisplatin-based doublets as first line therapy for cervical cancer the response rates were 22 to 29 percent and median PFS was 4 to 6 months and median overall survival (OS) was 10 to 13 months. (10) The addition of bevacizumab to combination chemotherapy has been reported to increase overall survival by 3.7 months, but the vast majority of patients die of their disease within 2 years. (11) Randomized trials of second line therapy are lacking, but response rates for single agents are generally reported to be less than 20 percent. (12) For oropharyngeal cancer, estimates of the chemotherapy responsiveness can be inferred from data on the oropharyngeal site in subset analyses from clinical trials for head and neck cancers. In a pivotal clinical trial that established platinum, 5-flurouracil, plus cetuximab as first line therapy in head and neck cancer, patients with oropharyngeal tumors experienced PFS of 4 to 6 months and OS of 8 to 11 months. (8) As with cervical cancer, randomized trials of second line therapy following platinum treatment failure are lacking. A phase II trial of cetuximab, a monoclonal antibody targeting epidermal growth factor receptor (EGFR) reported a response rate of 13 percent with time to progression of 70 days; cetuximab now represents the most promising second line therapy for this disease. (13) Thus, second line therapies for HPV-associated tumors have low response rates and poor response duration, and novel therapies are needed.

Table 1: Estimated deaths from cancers at HPV-associated sites in the United States in 2014.

Adapted from: American Cancer Society. Cancer Facts & Figures 2014. Atlanta: American Cancer Society; 2014.

Site	Deaths
Oral cavity & pharynx (not tongue or mouth)	4,170
Cervix	4,020
Vulva	1030
Female genital	880
Penis	320
Anus	950
Total	11,370

1.2.1 Tumor-Infiltrating T Cells Targeting HPV Oncoproteins for HPV+ Cancers

We are conducting a clinical trial of autologous HPV-targeted tumor-infiltrating T cells for metastatic HPV+ cancers in which objective tumor responses have been observed in cervical, oropharyngeal, and anal cancers. In this protocol T cell cultures are generated from fragments of resected metastatic tumors. T cell cultures are screened for reactivity against HPV E6 and E7. Reactive cultures are preferentially selected for further expansion and administration to the patient. We have reported the results of treatment of nine patients with metastatic cervical cancer including two patients with ongoing complete responses after a single infusion of T cells (14). In these patients the frequency of HPV-reactive T cells in the infused cells correlated positively with clinical response to treatment. Patients who received T cells with little or no reactivity against the HPV oncoproteins did not experience objective tumor regression. In addition, the frequency of peripheral blood T cells with HPV reactivity one month after treatment was positively associated with clinical response. While these results suggest a possible role for HPV-specific T cell in mediating tumor regression limited conclusions can be drawn. The administered T cells were not purified for HPV reactivity; hence T cells with non-HPV reactivities were also given and may have played an important role in the clinical responses that were observed. Furthermore, the treatment is logistically complicated as it requires a surgical procedure, prolonged cell culture, and an HPV reactivity screening assay. Finally, not all tumor specimens yield T cells with HPV reactivity, and when this is the case it is not possible to confer reactivity. A more "off-the-shelf" cell therapy in which T cells are generated without an operation and have consistent reactivity against an HPV oncoprotein would be desirable.

1.2.2 Targeting of HPV oncoproteins with TCR Gene Therapy

The HPV-16 E6 and E7 oncoproteins are constitutively expressed by and important to the survival of HPV-16+ tumor cells, and absent from healthy human tissues. Approximately 65 percent of cervical, 70 percent of oropharyngeal, and 90 percent of anal cancers, as well as many vulvar, vaginal, and penile cancers, express these antigens, making them highly attractive therapeutic targets. (1, 2) T cells recognize epitopes of intracellular antigens that are presented by HLA molecules on the surface of tumor cells. CD8+ T cells, which specialize in direct recognition and killing of virally infected cells and tumor cells, require presentation of cognate peptide by a class I HLA molecule. HLA-A*02:01 is the most common class I HLA allele in the United States population, expressed by 40 to 50 percent of Caucasians.

We are presently conducting a clinical trial of TCR gene engineered T cells targeting an HLA-A2 restricted epitope of HPV-16 E6 (E6 TCR). In this protocol, T cells from the patient's peripheral blood are transduced with a retrovirus to express the E6 TCR. The transduced T cells are expanded *ex vivo* and administered back to the patient intravenously. Before cell administration the patient receives a lymphodepleting chemotherapy conditioning regimen to promote infused T cell engraftment. Following cell infusion the patient receives high-dose bolus aldesleukin, which is dosed to individual patient tolerance. We have, in a dose escalation trial, treated nine patients with this regimen. Dose limiting toxicity was not encountered (the maximum dose tested was in the range of 1×10^{11} to 2×10^{11} cells). Off target toxicity was not observed. Cytokine storm was not observed. Of the initial seven evaluable patients, one patient has experienced a partial tumor response that is ongoing after 6 months. (15)

1.2.3 E7 TCR discovery and characterization

$E7_{11-19}$ is a naturally processed epitope of HPV-16 E7 that binds to HLA-A*02:01 and that has been isolated from the surface of HPV-16+ HLA-A*02:01+ tumor cells. (16) We identified the nucleotide sequence of a TCR targeting $E7_{11-19}$ from the cervix-infiltrating T cells of a patient with cervical intraepithelial neoplasia who received a therapeutic cancer vaccine targeting HPV-16 E7. The nucleotide sequence 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 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. (17, 18) 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.

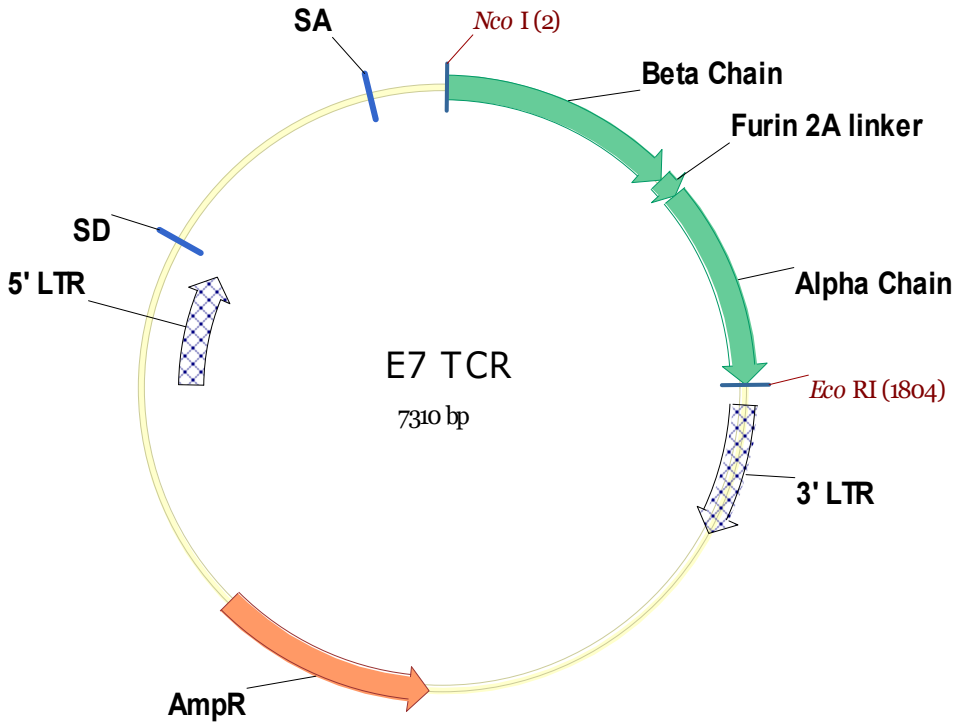


Figure 1: E7 TCR vector map. A TCR targeting $E7_{11-19}$ was isolated from the cervix-infiltrating lymphocytes of a patient with cervical intraepithelial neoplasia. The nucleotide sequence of the alpha and beta chains was determined, codon optimized for expression in human tissues, and the constant 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.(2) 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 2**). 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 2**).

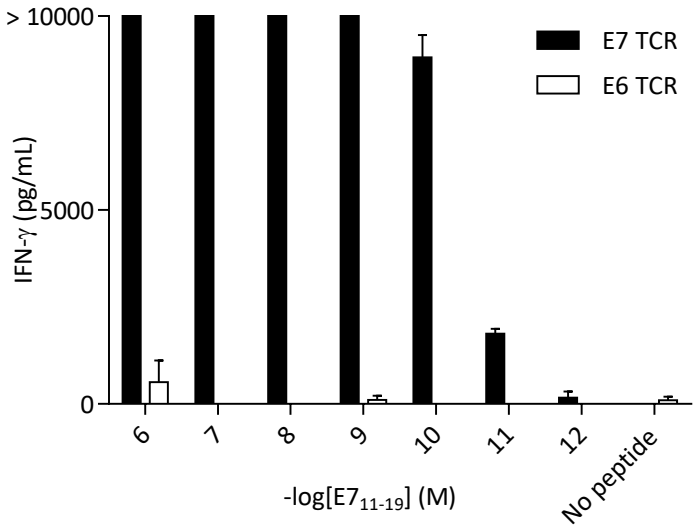


Figure 2: T cells transduced to express the E7 TCR demonstrated high avidity for the $E7_{11-19}$ peptide. T cells from PBMC were transduced to express the E7 TCR. Functional avidity was tested by coculture with T2 cells pulsed with titrated concentrations of $E7_{11-19}$ peptide.

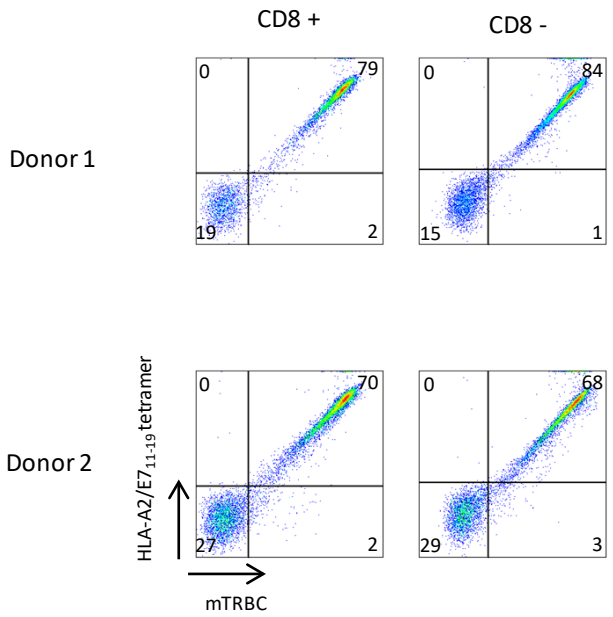


Figure 3: **Peripheral blood T cells transduced to express the E7 TCR display CD8-independent HLA-A*02:01/E7$_{11-19}$ tetramer binding.** T cells from PBMC were transduced to express the E7 TCR. Dot plots shown are gated on PI- lymphocytes and either CD8+ or CD8- cells as indicated above the dot plots.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

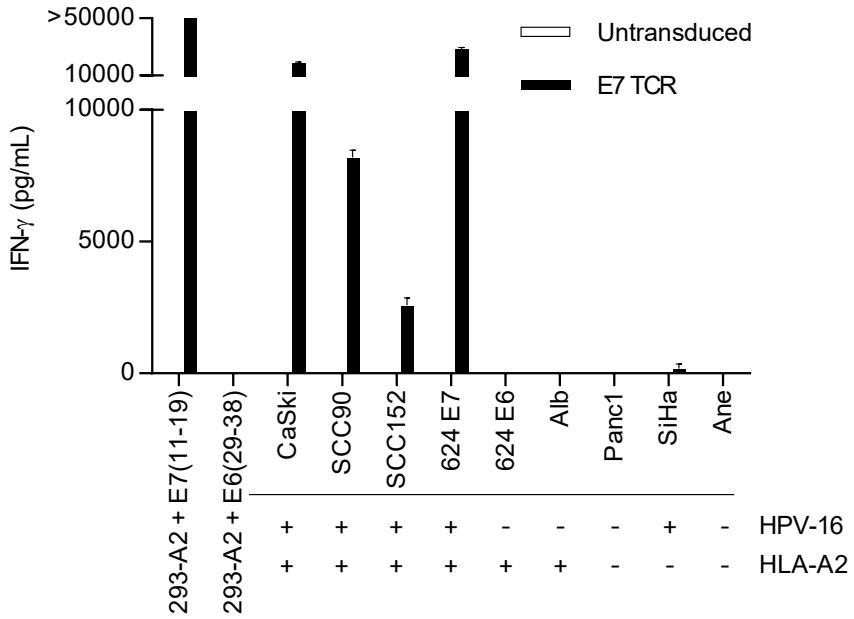
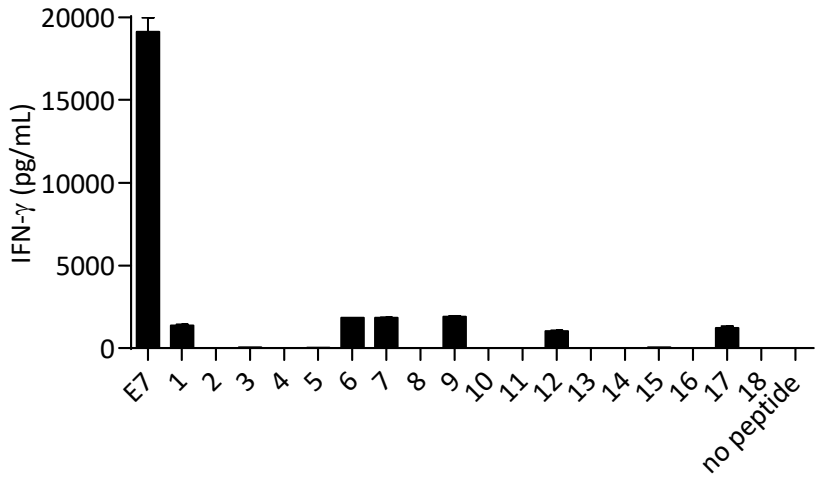


Figure 4: **T cells transduced to express the E7 TCR specifically recognized HPV-16+ HLA-A*02:01+ tumor lines** T cells transduced with E7 TCR were cocultured with targets expressing HPV-16 and HLA-A2 or with negative controls. Target cell line expression of HPV-16 and HLA-A2 is indicated below each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

A)



B)

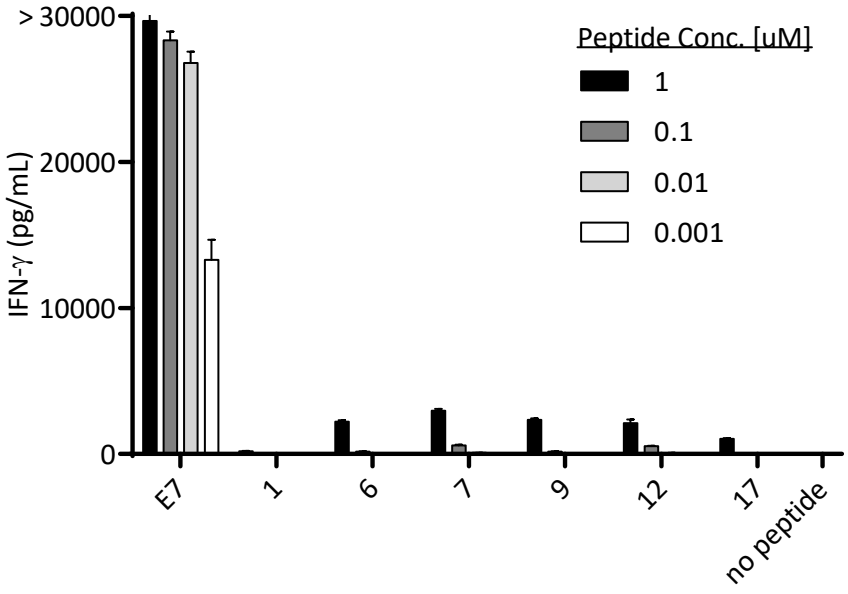


Figure 5: E7 TCR transduced T cells did not show cross reactivity against human peptides. E7 TCR transduced human T cells were tested for recognition of peptides identified by the BLAST search shown in **Table 2**. Target cells were T2 cells loaded with either the E7 peptide (E7) as a positive control, peptides identified by number in **Table 2**, or no peptide (A). Peptides which elicited a weak response by E7TCR were further tested for recognition at titrated concentrations (B).

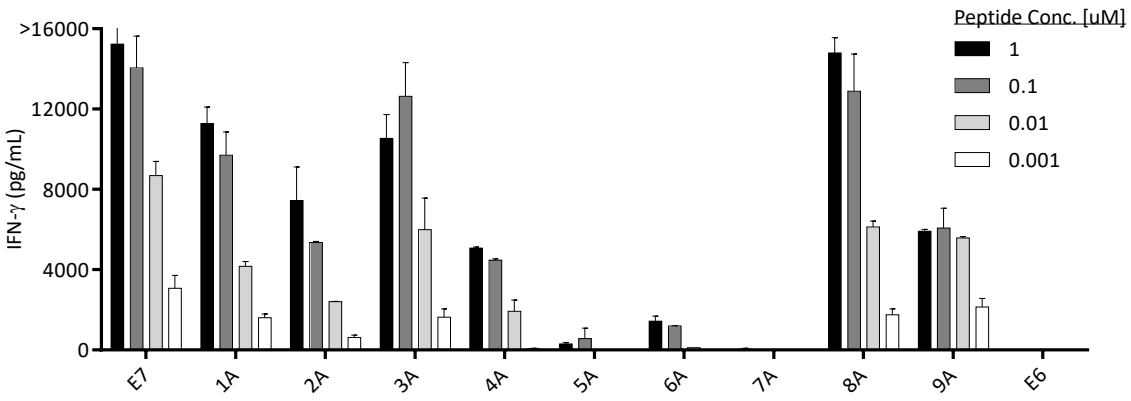


Figure 6: **Serial alanine substitutions to the $E7_{11-19}$ target peptide revealed positions 4-7 to be the most crucial for recognition by E7 TCR transduced T cells.** Human T cells were transduced to express the E7 TCR. The transduced cells with cocultured with T2 cells loaded with varying concentrations of $E7_{11-19}$ peptide (E7) or $E7_{11-19}$ with an alanine substitution at the position indicated by the x-axis labels. E6 peptide (E6) 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 2 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.

1.2.4 Safety Considerations

The safety of infusion of large numbers of retrovirally modified tumor reactive T-cells has been demonstrated in clinical studies conducted at the NIH Clinical Center. (19, 20) Non-myeloablative chemotherapy conditioning and high-dose aldesleukin have well-characterized toxicity which is discussed in Section **14.1** of this study. (21) The chemotherapy used in this protocol has been administered to over 500 patients and all have reconstituted their hematopoietic system.

Patients at the NIH Clinical Center have been treated with up to 1.77×10^{11} gene engineered cells. The upper limit of the dose range for this trial was therefore set at 1×10^{11} transduced cells. The protocol is designed with a run in dose escalation but unexpected toxicities are unlikely because 1) the TCR targets a foreign viral protein that does not exist in the human genome so there is no chance of toxicity from targeting healthy tissue that unexpectedly expresses the target, 2) the TCR came from a human and therefore has been selected in thymus to not have autoreactivity, so autoimmunity is unlikely, 3) the TCR specificity has not been altered by affinity enhancement so there is no chance that cross reactivity against normal human proteins

has been introduced, and 4) testing against human peptides with similarity to the target epitope and with human peptides identified by alanine scanning revealed no cross reactivity (2).

Other protocols at the NIH Clinical Center have administered over 1 X 10^{11} tumor infiltrating lymphocytes (TIL) with widely heterogeneous reactivity including CD4, CD8, and NK cells without infusional toxicities. Experience at the NIH Clinical Center treating more than 200 patients with advanced cancers with genetically engineered T cells indicates that these cells do not have a significant risk of malignant transformation in this setting. While the risk of insertional mutagenesis is a known possibility using retroviral vectors, this has only been observed in the setting of infants treated for XSCID, WAS and X-CGD using retroviral vector-mediated gene transfer into CD34+ bone marrow cells. In the case of retroviral vector-mediated gene transfer into mature T cells there has been no evidence of long-term toxicities since the first NCI sponsored gene transfer study in 1989. Although continued follow-up of all gene therapy patients will be required, data suggest that administration of retrovirally transduced mature T cells is a safe procedure. While the risk of insertional mutagenesis is extremely low, the proposed protocol follows all current FDA guidelines regarding testing and follow up of patients receiving gene transduced cells.

Update with amendment H: The initial protocol included combination of E7 T cells with pembrolizumab in additional dose levels (dose level 4 and dose level 5). Clinical responses to E7 T cells without pembrolizumab were observed at the lower dose levels. The decision was made to remove dose level 4 and dose level 5 from the protocol due to the unknown safety of combination therapy and the apparent clinical activity of E7 T cells without pembrolizumab.

Because a safe dose of cells was determined in the phase I part of the trial, and some patients experienced a partial response, and each treatment is a single dose of E7 T cells, the protocol was amended to permit retreatment of patients with a partial response.

Update with amendment I: One dose-limiting toxicity occurred in the first 11 patients on this protocol. The 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. To increase safety, patients will be required to have a resting pulse oxygen measurement on room air as outlined in Sections **2.1.2.9**, **2.4.2** and **2.4.3.** The maximum tolerated dose found in the phase I portion of the study was 1 x 10^{11} E7 TCR T cells.

Update with amendment J: One patient treated on the phase II portion of the study experienced delirium and hypotension after E7 TCR T Cells and one dose of aldesleukin that required mechanical ventilation, vasopressors and hemodialysis. The patient also developed a HLH-like disorder characterized by fevers, prolonged low blood counts, and elevated ferritin and soluble CD25 that resolved following treatment with steroids. The patient also had delayed recovery of blood counts that was contributed to prior chemotherapy, prior pelvic radiation and poor nutrition. To further assess safety, patients will now also have a neurological assessment with neurological exam performed at baseline and daily during treatment with aldesleukin as outlined in Sections **2.2.2.2**, **3.3** and **0**.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Measurable (per criteria in Section **6.3**) metastatic or refractory/recurrent HPV-16+ cancer (determined by in situ hybridization (ISH) or a polymerase chain reaction (PCR)-based test).

2.1.1.2 Patients must be HLA-A*02 by low resolution typing, and HLA-A*02:01 by one of the high resolution type results.

2.1.1.3 All patients must have received prior first line standard therapy or declined standard therapy.

2.1.1.4 Patients with three or fewer brain metastases that have been treated with surgery or stereotactic radiosurgery are eligible. Lesions that have been treated with stereotactic radiosurgery must be clinically stable for one month before protocol treatment. Patients with surgically resected brain metastases are eligible.

2.1.1.5 Greater than or equal to 18 years of age

2.1.1.6 Able to understand and sign the Informed Consent Document.

2.1.1.7 Clinical performance status of ECOG 0 or 1, as shown in **Appendix E**.

2.1.1.8 Individuals must be willing to practice birth control from the time of enrollment on this study up to twelve (12) months after treatment. Individuals must be willing to undergo testing for HPV-16 prior to becoming pregnant after this period.

2.1.1.9 Individuals of childbearing potential must have a negative pregnancy test because of the potentially dangerous effects of the treatment on the fetus. Individuals of childbearing potential are defined as all individuals except individuals who are postmenopausal or who have had a hysterectomy. Postmenopausal will be defined as individuals over the age of 55 who have not had a menstrual period in at least one year. Because there is a potential risk for adverse events in nursing infants secondary to treatment of the mother with E7 TCR transduced PBL, breastfeeding should be discontinued if the individuals is treated with E7 TCR transduced PBL. These potential risks may also apply to other agents used in this study.

2.1.1.10 Serology:

- 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 are less responsive to the experimental treatment and more susceptible to its toxicities.)
- Seronegative for hepatitis B antigen, and seronegative for 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.

a. Hematology:

- Absolute neutrophil count greater than 1000/mm^3 without the support of filgrastim.

- WBC ≥ 3000/mm^3
- Platelet count ≥ 100,000/mm^3
- Hemoglobin > 8.0 g/dL

b. Chemistry:

- Serum ALT/AST ≤ 2.5 times the upper limit of normal
- Calculated creatinine clearance (CCr) ≥50 mL/min/1.73^2 using the Cockcroft-Gault equation
- Total bilirubin ≤ 1.5 mg/dL, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dL

c. More than four weeks must have elapsed since any prior systemic therapy at the time the patient receives the E7 TCR cells.

Note: Patients may have undergone minor surgical procedures within the past three weeks, as long as all toxicities have recovered to Grade 1 or less or as specified in the eligibility criteria in Section **2.1.1**.

2.1.2 Exclusion Criteria

2.1.2.1 Active systemic infections (for e.g.: requiring anti-infective treatment), coagulation disorders or other active major medical illnesses of the cardiovascular, respiratory or immune system, as evidenced by a positive stress thallium or comparable test, myocardial infarction, cardiac arrhythmias, severe obstructive or restrictive pulmonary disease. Patients with abnormal pulmonary function tests but stable obstructive or restrictive pulmonary disease may be eligible.

2.1.2.2 Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).

2.1.2.3 Concurrent opportunistic infections (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 experimental treatment and more susceptible to its toxicities).

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

2.1.2.5 Patients on immunosuppressive drugs including corticosteroids. With the exception of:

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

2.1.2.6 History of severe immediate hypersensitivity reaction to cyclophosphamide, fludarabine or aldesleukin.

2.1.2.7 Patients with a history of coronary revascularization or ischemic symptoms unless patient has a normal cardiac stress test.

2.1.2.8 Documented LVEF of less than or equal to 45% tested. The following patients will undergo cardiac evaluations

- Clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, second or third degree heart block or
- Age ≥ 50 years old

2.1.2.9 Any other condition, which would, in the opinion of the Principal Investigator, indicate that the subject is a poor candidate for the clinical trial or would jeopardize the subject or the integrity of the data obtained.

2.1.2.10 Subjects with baseline screening pulse oxygen level of < 95% on room air will not be eligible. If the underlying cause of hypoxia improves, then they may be reevaluated.

2.1.3 Recruitment Strategies

Patients for this protocol will be recruited via standard CCR mechanisms including posting to NIH websites (i.e., clinicaltrials.gov) and social media platforms, physician and self-referrals as well as various advertising venues. All advertisements, letters and other recruitment efforts 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

A waiver of consent for these activities has been requested in Section **12.6.2**.

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 patient has signed the consent.

2.2.2.1 Any time prior to starting the chemotherapy regimen:

a. HLA typing

b. HPV genotype testing of tumor. Archival tissue may be used or a new biopsy may be obtained.
c. Venous assessment (as per apheresis clinic policy)

2.2.2.2 Within 4 weeks prior to starting the chemotherapy regimen:

a. Complete history and physical examination, including weight, and vital signs noting in detail the exact size and location of any lesions that exist. (Note: Patient history may be obtained within 8 weeks.)
b. EKG
c. Pulmonary Function Testing for patients with a prolonged history of cigarette smoking (20 pack/year of smoking within the past 2 years) or symptoms of respiratory dysfunction. (Note: If performed prior to receiving treatment, this does not need to be repeated unless clinically indicated. Test results from outside NIH may be accepted per investigator discretion).
d. If available, previous CT of the chest, abdomen and pelvis, and brain MRI or PET to evaluate the status of disease. Additional scans and x-rays may be performed if required to determine patient eligibility or if clinically indicated based on patients' signs and symptoms. (Note: Brain MRI from outside the CCR may be accepted per investigator discretion if performed within 3 months of initiation of chemotherapy regimen).

2.2.2.3 Within 3 months prior to starting the chemotherapy regimen:

e. Cardiac evaluation for patients who are greater than or equal to age 50, or who have a history of ischemic heart disease, chest pain, or clinically significant atrial and/or ventricular arrhythmias including but not limited to: atrial fibrillation, ventricular tachycardia, heart block. Patients with a LVEF of less than or equal to 45% will not be eligible. Patients under the age of 50 who present with cardiac risk factors may undergo cardiac evaluation as noted above (e.g., diabetes, hypertension, obesity.)
f. HIV antibody titer and HbsAG determination, anti-HCV. (Note: may be performed within 3 months of chemotherapy start date but must be within one month before initial apheresis for generation of a cell product).
g. Anti CMV antibody titer, HSV serology, and EBV panel. (Note: patients who are known to be positive for any of the above do not need to be retested).

2.2.2.4 Within 14 days prior to starting the chemotherapy regimen:

a. Blood tests:
   - 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).
   - Thyroid Panel
   - CBC with differential and TBNK PT/PTT

b. Urinalysis and culture if indicated

2.2.2.5 Within 7 days prior to starting the chemotherapy regimen:

a. Beta-HCG pregnancy test (serum or urine) on all individuals of child-bearing potential
b. ECOG performance status of 0 or 1

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 at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

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 Procedures

Cohorts

Number	Name	Description
1	*Phase I: Dose Escalation Cohort*	*Up to 18 evaluable patients with HPV-16 positive cancer to determine MTD of E7 cells + aldesleukin*
2	*Phase II Cohort*	*Patients with HPV-16 positive cancer enrolled after the RP2D of 1 x 10^{11} E7 cells + aldesleukin has been determined (total enrollment including Phase I not to exceed 40)*

Arms

Number	Name	Description
1	*Dose level 1 through 3*	*Non-myeloablative, lymphocyte depleting preparative regimen, followed by E7 TCR Cells at escalating doses, followed by aldesleukin*
2	*Phase II arm*	1 x 10^{11} *E7 Cells that was determined in Phase I + aldesleukin*

Arm Assignment

Patients in Cohort 1 will be directly assigned to Arm 1, and patients in Cohort 2 will be directly assigned to Arm 2.

2.4 BASELINE EVALUATIONS

The following are baseline evaluations that are not included in the screening process. Some of the screening evaluations may be used as baseline.

2.4.1 Within 4 weeks prior to starting the chemotherapy regimen:

a. Patients with lesions that can be biopsied under sedation and/or local anesthesia (skin lesions, visceral lesions approachable by CT, USG or MRI guided core biopsy) and who are willing to have biopsies performed, will have a baseline pre-treatment biopsy followed by two additional biopsies, one following treatment (6 weeks post treatment preferred) and the second at the time of progression. Refer to Section **5.1.3** for guidelines for handling specimens.
b. CT of the chest, abdomen and pelvis.
c. PET or brain MRI may be performed if clinically indicated to evaluate the status of disease.
d. Evaluation with colonoscopy, esophagogastroduodenoscopy or other form of endoscopy may be performed to evaluate for the presence of disease in the GI tract or if clinically indicated.
e. Neurological assessment with a neurological exam.

2.4.2 Within 24 hours before starting intravenous fluid hydration for chemotherapy

a. Pulse oxygen level on room air will be assessed at a timepoint within 24 hours before starting intravenous fluid hydration for chemotherapy. If this value is < 95% hydration then chemotherapy will be held until it is ≥ 95%, or it is determined that the patient's hypoxia is irreversible, in which case the patient will be considered ineligible.

2.4.3 Within 24 hours before infusion of E7 T cells

a. Pulse oxygen level on room air will be assessed at a timepoint within 24 hours before infusion of E7 T cells. If this value is < 95% T cell infusion will be held until it is ≥ 95%. Cell infusion may be delayed for up to 48 hours.

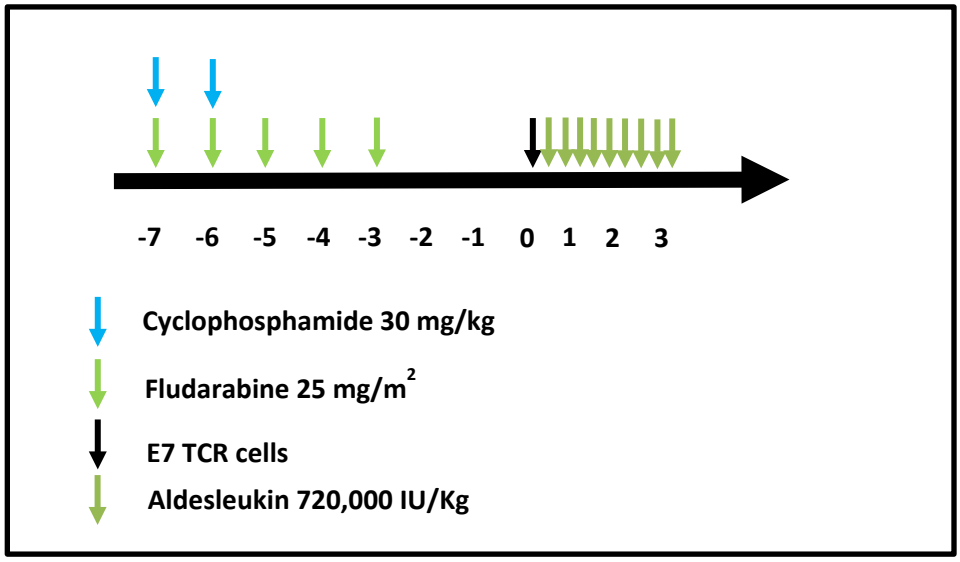
3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a phase I/II clinical trial that will test the safety and efficacy of E7 TCR cells.

A small number of subjects may be eligible for re-enrollment, and would be required to meet all eligibility criteria at the time of re-enrollment. Patients will be assigned a new sequential study number for the re-enrollment study period. Any cryopreserved cells produced from a patient who was removed from the study can be used to treat that patient after re-enrollment. We do not anticipate changes in the risk profile for the initial versus re-enrollment

Schema:



*Details regarding the number of patients in each dose level group are provided in Section **3.1.5**.

3.1.1 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. 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).

3.1.2 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 16-C-0061 and banked for research if a patient is co-enrolled. E7 TCR cells can be produced in approximately 21 to 27 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) will be retained in the DTM; the remaining cells will first be frozen in 10 vials at 100 x 10^6 cells per vial with excess frozen at 300 x 10^6 cells/vial. Cells will be frozen in the DTM and then transferred to the Blood Processing Core (BPC).

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. When a patient is no longer eligible for retreatment on this protocol due to meeting any of the off-study criteria listed in Section **3.8.2**, any remaining cryopreserved pretreatment PBMC collected on this protocol will be transferred from the Department of Transfusion Medicine to the Principal Investigator of this protocol for storage in the Blood Processing Core and possible use in research and banked according to protocol 16-C-0061 (Tissue Procurement Protocol).

3.1.3 Treatment Phase

PBMC will be obtained by leukapheresis (approximately $2x10^9$ to $1x10^{10}$ cells are obtained). PBMC will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth. Transduction is initiated by exposure of approximately $10x10^6$ to $500x10^6$ cells to supernatant containing the E7 TCR retroviral vector. These transduced cells will be expanded and tested for their anti-tumor activity. Successful TCR gene transfer will be determined by FACS analysis for the TCR protein. Successful TCR gene transfer for each transduced peripheral blood lymphocyte (PBL) population will be defined as greater than 10% TCR positive cells. 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 -7 to -3 before the intravenous infusion of *in vitro* tumor reactive TCR gene-transduced PBL plus IV high dose aldesleukin, as indicated in Section **3.2.4**. 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 at the first follow-up visit.

3.1.4 Dose Limiting Toxicities (DLTs)

All Grade 3 and greater toxicities occurring within 30 days of the cell infusion with the exception of:

- Cytokine Release Syndrome (CRS) that resolves ≤ grade 2 within 14 days of the last dose of aldesleukin
- Autoimmune toxicity that resolves to ≤ grade 2 within 14 days for starting symptom treatment (e.g. steroids)
- Cardiac, gastrointestinal, dermatological, hepatic, pulmonary, renal, hematologic, neurologic toxicity, or toxicity in **Appendix C** attributable to aldesleukin that resolves to ≤ grade 2 within 14 days of the last dose of aldesleukin
- Transient grade 3 hypoxia associated with cell infusion that corrects to ≤ grade 2 with supplemental oxygen and/or that resolves to ≤ grade 2 within 24 hours or before starting aldesleukin.
- Hemorrhage due to underlying cancer or prior radiotherapy
- Infection that is controlled within 7 days of onset
- Hematological toxicities because they are expected from the conditioning regimen and their duration is unpredictable

- If a DLT is clearly due to progressive disease the subject will be replaced and the DLT will not be included.

Note: Any adverse event that leads to a discontinuation of T-cell infusion will be considered a DLT.

3.1.5 Dose Escalation

Phase I of the protocol will follow a 3+3 phase 1 dose escalation design. At each dose level, three patients will be treated initially*. Should none of the first 3 patients treated on a dose level experience a DLT enrollment can start on the next higher dose level. Should 1 of 3 patients experience a dose limiting toxicity on a particular dose level, three more patients would be treated at that dose level to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher level. If 1/6 patients have a DLT at a particular dose level, accrual can proceed to the next higher dose level. If a level with 2 or more DLTs in 3-6 patients has been identified, 3 additional patients will be accrued at the next-lowest dose for a total of 6, in order to further characterize the safety of the maximum tolerated dose. The maximum tolerated dose is the dose at which a maximum of 1 of 6 patients has a DLT. If 2 DLTs occur on dose level 1, study will be stopped.

With Amendment G, an additional three patients have been added to dose level three to allow for collection of additional data on E7 TCR T cells without pembrolizumab (no DLT has been encountered).

With Amendment H, a total of 40 patients will be treated in Phase II, including the 3 additional patients added via amendment G, to dose level 3. The maximum tolerated dose was not reached in the Phase I portion, so the maximum administered safe dose will be designated as the recommended Phase II dose (RP2D). These patients will also be evaluated for safety and efficacy (see Section **8**).

There will be a 12 day delay in treatment between the patients within each dose level. Therefore, after a patient in a dose level starts chemotherapy, the next patient in the same dose level will not start chemotherapy until at least 12 days later to allow more time for the analysis of adverse events. There will also be a 4 week delay between each dose level in order to further increase patient safety.

The total number of anti-HPV-16 E7 engineered PBL cells transferred for each dose level will be in the ranges of:

- **Dose level 1** 1×10^9 transduced E7 TCR cells
- **Dose level 2** 1×10^{10} transduced E7 TCR cells
- **Dose level 3** 1×10^{11} transduced E7 TCR cells

The cell dose administered will be in a range of +/- 30% of the target dose above. The number of transduced cells will be quantified by multiplying the frequency of cells expressing the mouse TCR constant region (determined by flow cytometry) by the number of cells produced. If fewer than the target number of cells are generated the patient will still be treated but toxicity data from the patient will not be used in determining the MTD. Participants in the phase II portion of the study who receive treatment with a cell dose more than 30% below the RP2D will be evaluable for safety but will not be included in the primary efficacy analysis.

With Amendment I, a total of 40 patients will be treated in Phase II, including the 3 additional patients added via amendment G, to dose level 3. The maximum tolerated dose was reached in the Phase I portion. The dose that will be used in the Phase II portion of the study is 1 x 10^{11} transduced E7 TCR cells (Dose level 3). These patients will also be evaluated for safety and efficacy (see Section **8**).

3.1.6 Safety Protocol Stopping Rules

The study will be halted (immediately stop accrual and treatment) if any of the following safety conditions are met during phase I portion and we will promptly investigate and submit an amendment to the IRB and FDA if necessary:

1. If one or more deaths (other than death related to progressive disease) occurs within 30 days of treatment regimen.
2. If two or more patients develop a Grade 3 or greater toxicity at any point in the study not attributable to the chemotherapy preparative regimen or aldesleukin (or circumstances unrelated to the study) that does not resolve to Grade 2 within 10 days.
3. If 2 DLTs occur on dose level 1.

3.2 DRUG ADMINISTRATION

Treatment schedule will be according to the following schedule (See Schedules **3.2.4**). (Times are offered as examples and may be changed as long as a similar time relationship between administrations of the drugs is maintained. Study medication start times for drugs given once daily may be within 2 hours of the scheduled time [once it is established at the 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 -7 through Day -3:

The following will comprise a course of therapy:

Drug	Dose	Route and Administration	Treatment Days
Cyclophosphamide	30 mg/kg	IV infusion over 1 hour (± 15 min)	Once daily x 2 doses Days -7 and -6
Fludarabine	25 mg/m^2	IV infusion over 30 minutes (± 15 min) To be administered following completion of cyclophosphamide.	Once daily x 5 doses Days -7, -6, -5, -4, -3

Dose modifications: Cyclophosphamide and fludarabine will be dosed on actual body weight unless the

patient has a body mass index (BMI) > 35. Note: Chemotherapy infusions maybe slowed or delayed as medically indicated.

3.2.2 Day -7 and -6

- 9 am: Hydrate (if applicable). Begin hydration with 0.9% sodium chloride injection with or without 10 meq/L of potassium chloride at approximately 1.5 ml/kg/hour (recommend starting at least 6 hours pre-cyclophosphamide and continue hydration until 24 hours after last cyclophosphamide infusion). The hydration rate will be capped at 100 mL/hr. Furosemide may be given once daily on cyclophosphamide treatment days to promote diuresis. Furosemide may be held if hydration is not employed or based on clinical factors. 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 may be administered. The rate of hydration and total time of hydration may be reduced or increased based on urine output and other clinical considerations per the primary investigator.
- 2 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 12 hours X 3 days),Olanzapine (5 to 10 mg PO once daily for 5 days), and Aprepitant (125 mg PO on day -7, 80 mg PO daily on days -6 and -5) will be utilized for prophylaxis for chemotherapy induced nausea and vomiting. Modifications to the antiemetic regimen may be made per PI discretion but corticosteroids should be avoided.
- 4 pm: Cyclophosphamide 30 mg/kg/day X 2 days IV in 250 ml D5W with mesna 15 mg/kg/day over 1 hour (± 15 min) X 2 days. If the patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Table 3**.

Mesna will be administered at 7.5 mg/kg IV infusion over 1 hour concurrent with cyclophosphamide and then 1.5 mg/kg/hour IV infusion over 23 hours to be started after the completion of cyclophosphamide.

3.2.3 Day -7 to Day-3

- Fludarabine 25 mg/m^2/day IVPB daily over 30 minutes (± 15 min) for 5 days. To be administered following completion of cyclophosphamide. (*The fludarabine will be started approximately 1-2 hours after the cyclophosphamide and mesna on Days -7 and -6*).If the patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in **Table 3**. (Fludarabine will be dose adjusted for renal impairment. Renal function may be determined based on estimated equations (e.g. CKI-EPI, Cockroft Gault) or by measured and timed urine collections based on the clinical judgement of the investigator team.

Creatinine clearance	Fludarabine daily dose
≥ 80 ml/min/1.73 m^2	25 mg/m^2/dose
50-79 ml/min/1.73 m^2	20 mg/m^2/dose
30-49 ml/min/1.73 m^2	15 mg/m^2/dose
< 30 ml/min/1.73 m^2	Hold dose

3.2.4 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.

Day 0 (two to four days after the last dose of fludarabine):

- E7 TCR T cells will be administered intravenously via infusion pump over approximately 20 to 30 minutes via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.
- Aldesleukin as described in Section **3.3** below.

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

- Fluconazole: can be used at the discretion of the treating clinician
- Valacyclovir po or Acyclovir PO or IV: will be administered until CD4 counts > 200/mm^3 X 2

Day 1-4 (Day 0 is the day of cell infusion):

- Aldesleukin as described in Section **3.3** below.

TMP/SMX may be administered at 160mg/800mg every other day at the time of count recovery.

Dose 1-3 Schedule

Therapy	**Day**											
	-7	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
Cyclophosphamide 30 mg/kg IV once daily x 2 days	X	X										
Ondansetron 0.15 mg/kg IV every 12 hours x 3 days	X	X	X									
Olanzapine 5-10mg PO once daily x 5 days	X	X	X	X	X							
Aprepitant 125 mg PO x 1 day, then 80 mg PO daily x 2 days	X	X	X									
Mesna 7.5 mg/kg IV infusion over 1 hour concurrent with	X	X										

Therapy	Day											
	-7	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**
cyclophosphamide then 1.5 mg/kg/hour IV infusion over 23 hours												
Fludarabine 25 mg/m^2 IV once daily x 5 days	X	X	X	X	X							
E7 TCR cells								X				
Aldesleukin								X	X	X	X	X
Valacyclovir PO or Acyclovir PO or IV								X	X	X	X	X

3.3 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 four days (maximum 12 doses). The start of aldesleukin treatment may be delayed up to 3 days after cell infusion if medically necessary. Doses will be preferentially administered every eight hours; however, up to 24 hours may elapse between doses depending on 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 D**. Toxicities will be managed as outlined in **Appendix C**. 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 is strongly recommended to be signed by the patient to identify a surrogate to make decisions if a patient becomes unable to make decisions. Neurological assessment will be performed daily on patients who are receiving aldesleukin.

3.4 POTENTIAL REPEAT TREATMENT

Patients who were enrolled before the RP2D was established and have experienced a PR may be retreated at the RP2D (1 x 10^{11} transduced E7 TCR cells). Additionally, patients who experienced a partial or complete response (at the RP2D Dose level 3) by RECIST criteria and subsequently progress by RECIST criteria may receive a second treatment course. Patients must continue to meet the original eligibility criteria to be considered for retreatment. Research assessments will be performed at the same time intervals used for initial treatment. Patients will be treated at the RP2D (1 x 10^{11} transduced E7 TCR cells). Patients who develop grade 3 or 4 toxicity due to cell infusion will not be retreated. A maximum of 1 retreatment course may occur. The second treatment will not begin prior to 6-8 weeks after the last dose of aldesleukin.

Note: Response data for all treatments will be captured in the database however only the response data from the first treatment will be used in the determination of response.

3.5 ON STUDY EVALUATION

Note: Refer to Section **5** for research evaluations

3.5.1 Prior to starting the preparative regimen

- Apheresis as indicated
    - Within 14 days prior to starting the preparative regimen, patients will have a complete blood count (CBC with differential), electrolytes, BUN, creatinine, liver function tests, TBNK, and serum chemistries performed. If any results are beyond the criteria established for eligibility, the patient will not proceed until the abnormalities can be resolved.

3.5.2 During the preparative regimen: (day -7 to cell infusion)

- Tests will be performed every 1-2 days, including Complete Blood Count (CBC with differential) 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
- E7 TCR assay may be collected 1-2 times per week following cell infusion
- Urinalysis (every 1-2 days)

3.5.3 After Cell Infusion

3.5.3.1 Daily (Day +1 to Day +7)

- Following cell administration - Vital signs will be monitored hourly (+/- 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated
- CBC
- Chemistries: Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin
- Once total lymphocyte count is greater than 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (within one business day, while the patient is hospitalized)
- Other tests will be performed as clinically indicated including Calcium total, Magnesium total (Mg), Phosphorus, LD, Total Protein, Total CK, Uric Acid

3.5.4 During Hospitalization

3.5.4.1 Every 1-2 days

- A review of systems and physical exam as clinically indicated
- Tests will be performed as clinically indicated including Complete Blood Count (CBC with differential), 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

- Once total lymphocyte count is greater than 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly Monday through Thursday (within one business day, while the patient is hospitalized).
- Surveillance blood cultures may be drawn at the discretion of the investigator and continue as clinically indicated.
- Other tests will be performed as clinically indicated.

3.6 POST TREATMENT EVALUATION (FOLLOW-UP)

At each scheduled evaluation for response, patients will undergo:

- Physical examination
- 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
- Complete blood count
- Thyroid panel as clinically indicated
- TBNK, until CD4 > 200/mm 3 x 2
- Toxicity assessment, including a review of systems
- CT of the chest, abdomen and pelvis as clinically indicated. If clinically indicated, other scans or x-rays may be performed, e.g. PET, brain MRI, bone scan
- 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 will 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 16-C-0061 (Tissue Procurement Protocol). PBMC from apheresis will be stored in 10 vials at 100x106 cells per vial with remaining cells stored at 300x106 cells per vial.

3.6.1 Post Cell Product Administration

All patients will return to the NIH Clinical Center for evaluation 40 days (+/- 2 weeks) following administration of the cell product. Patients discharged with Grade 3 or greater significant adverse events should be evaluated by their referring physician within 2 weeks of discharge.

3.6.2 Stable Disease, Partial Response, Complete Response or Unresolved Toxicities

Patients who experience stable disease, a partial response, or a complete response or have unresolved toxicities will be evaluated for response as noted below:

- Week 12 (+/- 2 weeks)
- Every 3 months (+/- 1 month) x3
- Every 6 months (+/- 1 month) x 5 years

- As per PI discretion for subsequent years

Note: Patients may be seen more frequently as clinically indicated

3.6.3 Telephone Follow-up

Patients who are unable or unwilling to return for follow up evaluations will be followed via phone or e-mail contact and if there are any safety issues, will be advised to have a follow-up visit with their local oncologist. Patients may be asked to send laboratory, imaging and physician exam reports performed by their treating physician.

3.6.4 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 protocol, 20C0051. After the patient comes off study, health status data will be obtained from surviving patients via telephone contact or mailed questionnaires for a total of 15 years after cell infusion.

3.7 COST AND COMPENSATION

3.7.1 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 are performed outside the NIH Clinical Center, participants may have to pay for these costs.

3.7.2 Compensation

Participants will not be compensated on this study.

3.7.3 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.

3.8 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 40 days following the last dose of study therapy.

3.8.1 Off Treatment Criteria

Patients will be taken off treatment (and followed for survival) for the following:

- Completion of treatment
- Grade 3 or greater autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs).
- If a subject experiences Grade 3 or higher toxicity due to cell infusion (reaction to cellular product or infusion reaction), the patient will receive no further treatment.
- Patient requests to be withdrawn from active therapy
- Investigator Discretion

- Positive pregnancy test

3.8.2 Off Study Criteria

Patients will be taken off study for the following:

- Screen failure
- The patient voluntarily withdraws
- There is significant noncompliance
- Progressive disease
- General or specific changes in the patient's condition which render continued participation unacceptable in the judgment of the investigator.
- Death
- Study closure
- Completion of study follow-up period
- Patient meets criteria listed in Section **2.4.2**
- Patient lost to follow-up
- Investigator discretion

Note: patients who are taken off study for study closure may be followed on the Long-term Gene Therapy Follow-up protocol (20C0051).

3.8.3 Lost to Follow-up

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

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

- The site will attempt to contact the participant and reschedule the missed visit within 1-2 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

3.9 SCHEDULE OF EVALUATIONS

For the treatment schedule, please refer to Section 3.2.4.

Note: the retreatment schedule (see Section 3.4) will be identical to the schedule for patients being treated for the first time, including eligibility assessments. A maximum of one course of treatment will be allowed on retreatment.

Procedure	Screening/ Baseline[2]	Before treatment	Preparative regimen[5]	Day 0	During hospitalization[6]	Follow-up[9] (End of Treatment)
Medical history	X					
Physical	X			X	X	X
ECOG performance status	X					
Neurological Assessment	X			X[19]	X[19]	
NIH Advance Directives Form[16]						
EKG	X					
Chest CT and MRI or PET	X					X
Pulmonary Function Test[1]	X					
Cardiac evaluation[10]	X					
Viral titers	X					
Biopsy	X					X[12]
Blood chemistries[8]	X	X	X		X	X
Complete blood count (CBC with diff.)	X	X	X		X	X
Thyroid panel	X					X[13]
HLA typing	X					
TBNK		X			X[7]	X
Urinalysis	X		X			
Pregnancy test[3]	X					
Leukapheresis		X[17]				X[15]
Infusion of transduced cells[4]				X		
Additional apheresis or blood draw[11]						X
Research blood	X	X		X[14]		X
E7 TCR assay[18]					X	X

1. For patients with a prolonged history of cigarette smoking, as indicated in Section **2.2.1**
2. Exact timeline is indicated in Section **2.1.3**
3. For women of child-bearing potential as defined in Section **2.1.1.9**
4. See other treatments in Schedules, Section **3.2.4**
5. On days -7 to -1, every 1 -2 days as clinically indicated
6. Every 1 to 2 days while hospitalized
7. Once total lymphocyte count is greater than 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (within one business day, while the patient is hospitalized)
8. 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
9. 40 days (+/- 2 weeks) after cell infusion, additional visits as indicated in Section **3.6**
10. For patients who are greater than or equal to 50 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 **2.2.2.2**
11. As described in Section **3.6**.
12. Following treatment (6 weeks post treatment preferred) and at disease progression only.
13. As clinically indicated
14. Monday, Wednesday, Friday during hospitalization once ALC > 200/mm^3
15. If the patient is unable to undergo apheresis, approximately 96 ml of blood may be obtained. Apheresis will only be done in the first follow-up visit; in the following visits, blood will be collected.
16. As indicated in Section **12.3**, all subjects will be offered the opportunity to complete an NIH advanced 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.
17. This can occur at any time prior to treatment on protocol 16C0061 if the patient is co-enrolled on that protocol. See Section **3.1.1** for further details. For patients undergoing retreatment, retreatment will only occur if there are enough cells stored from their prior course. See Section **3.4** for details on retreatment.
18. Clinical assay performed by the NCI Flow Cytometry Laboratory in the Laboratory of Pathology. The assay may be collected at follow-up visits.
19. Performed daily on patients while receiving aldesleukin.

4 CONCOMITANT MEDICATIONS/MEASURES

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

4.1 PROHIBITED MEDICATIONS

Current use of immunosuppressive medication, EXCEPT for the following:

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

4.2 INFECTION PROPHYLAXIS

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

4.2.1 Pneumocystis Jirovecii Pneumonia

Patients may receive the fixed combination of trimethoprim and sulfamethoxazole [SMX] as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) P.O. daily three times a week on non-consecutive days, beginning between days -8 and -5.

Pentamidine may be substituted for TMP/SMX-DS in patients with sulfa allergies. It may 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.

Prophylaxis for Pneumocystis and Herpes will continue for 6 months post chemotherapy. 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.

4.2.3 Fungal Prophylaxis (Fluconazole)

Patients may start Fluconazole 400 mg po. starting on the day of cell infusion and continue until the absolute neutrophil count is greater than $1000/mm^3$. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in patients unable to take it orally.

4.2.4 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics as per current institutional guidelines for fever. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

4.2.5 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.

4.2.6 Neutrophil Recovery

Patients may receive filgrastim (or filgrastim biosimilar) for count recovery when clinically indicated per NIH CC Pharmacy guidelines.

4.3 OTHER CONCOMITANT MEDICATIONS TO CONTROL ALDESLEUKIN SIDE EFFECTS

Concomitant medications to control side effects of aldesleukin 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) famotidine (20mg IV q12h)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 CORRELATIVE STUDIES FOR RESEARCH

5.1 BIOSPECIMEN COLLECTION

Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

5.1.1 Pre-cell infusion evaluations

- At baseline/screening 12 CPT tube and 2 SST tubes may be collected. One CPT tube may be used to collect 4mL of plasma, which may 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 aliquoted into four vials of 0.5-1mL each. All samples will be processed in the Blood Processing Core (BPC).
- At day -7 prior to cell infusion 6 CPT tubes and 1 SST tube may be collected. One CPT tube may be used to collect 4mL of plasma, which may be frozen in 4mL vials. PBMC

from the remainder of the CPT tubes may be frozen in aliquots of 10 x 10^6 cells/vial. Serum from SST tubes may be aliquoted into four vials of 0.5-1mL each. All samples will be processed in the Blood Processing Core.

5.1.2 Post cell infusion evaluations

- 2 SST tubes (4mL) may be collected daily for serum starting on the day of chemotherapy and continuing through the end of hospitalization. Serum will be processed in the Blood Processing Core 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 BPC on Monday, Wednesday, and Friday x 5 days, then weekly (while the patient is hospitalized).
    - 6 CPT tubes (8mL each). One CPT tube daily may be used to collect 4mL of plasma, which may 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 CPT tube may be used to collect 4mL of plasma, which may 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 BPC.
- Serum samples may be provided to the laboratory of Liang Cao, Genetics Branch, CCR for testing of plasma or serum for HPV DNA.

5.1.3 Tumor Biopsies

- Tumor may be biopsied pre- and post-treatment in order to test for HPV-16, HLA expression, and other characteristics that may affect response to therapy. Biopsies are not required; a maximum of three biopsies, with three cores/attempts, may be performed.
- Tissue may be obtained pre-treatment, following treatment (6 weeks post treatment preferred) and at the time of progression.
- Tissue may be obtained via CT, MRI or US guided biopsy under IV sedation as appropriate.
- Specimens may be transported by the assigned research nurse to the Center for Immuno-Oncology (CIO) cellular therapy laboratory for sample labeling. Contact: Scott Norberg, Bldg 10, phone 301-275-9668.
- Following labeling, samples may be transported by an assigned lab member to the Blood Processing Core where they will be frozen in optimal cutting temperature compound.
- Some of these samples will be archived and analyzed under another protocol 16-C-0061 (Tissue Procurement Protocol) if the subject is also enrolled on that study.

5.1.4 Immunological Testing

- Apheresis may be performed prior to and about four to six weeks after the treatment. Apheresis product will be transferred to the Blood Processing Core. 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 will be transferred directly to the Blood Processing Core 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 by blinded scoring of the intensity and frequency of staining. Flow cytometry data will be analyzed with FlowJo software. Biopsies - will only be performed if minimal morbidity is expected based on the procedure performed and the granulocyte and platelet count.
- 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. (14, 15)
- The laboratory studies are considered exploratory. Statistical analysis will 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 16-C-0061 (Tissue Procurement Protocol).

5.1.5 Data sent to Rutgers Cancer Institute of New Jersey

Clinical outcome data and/or adverse event data may be sent to Rutgers to assist with the safety analysis. Data will be deidentified. Identifiable information will only be provided, if needed, to interpret data.

5.2 GENE-THERAPY-SPECIFIC FOLLOW-UP

FDA-required follow-up for participants who have received gene therapy will be performed as indicated in the long term gene therapy follow-up protocol (20C0051). Participants will be co-enrolled on 20C0051. A baseline (pre-treatment) sample will be collected on protocol 20C0051 to improve analysis of the long term follow up testing.

5.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

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

5.3.1 Samples Managed by the Blood Processing Core (BPC)

5.2.1.1 Blood Collection

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

For sample pickup, page 102-11964.

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

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

The samples will be processed, barcoded, and stored in the BPC until requested by the investigator.

5.3.1.1 Sample Data Collection

Patient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the CIO, may be archived by the Blood Processing Core (BPC) will be barcoded, with data entered and stored in Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined BPC lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by the BPC. These computers all have a password restricted login screen.

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

5.3.2 CIO - Cellular Therapy Laboratory

Samples transferred to the CIO cellular therapy laboratory will be barcoded and tracked with Labmatrix.

Laboratory research data will be stored on the NCI secure server 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.

5.3.3 Protocol Completion/Sample Destruction

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

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

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

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed or returned to the patient, if so requested. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.1**.

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

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The NIH PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR 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. 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).

Data from analyses performed at participating site will be provided to the NIH team by secure email and entered into the study database.

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

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. All study related adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of attribution will be captured in the study database up to 40 days following last administration of investigational agent. Document AEs from the first study

intervention, Study Day 0, through 40 days after the study therapy was last administered. Beyond 40 days after the last administration, only adverse events which are serious and related to the study intervention need to be recorded.

In addition, all incidences of intubation including the duration of and reason for intubation will be captured in the database.

6.1.2 Reporting of Laboratory Events

An abnormal laboratory value will be considered an AE 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_ Coded, linked or identified 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

For the purposes of this study, patients should be re-evaluated for response as indicated in Section **3.6.2**. In addition to a baseline scan, confirmatory scans should also be obtained 4 to 8 weeks following initial documentation of objective response.

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 (22). In cases where patients have isolated bony metastases that do not meet RECIST criteria, Positron Emission Tomography Response Criteria in Solid Tumors (PERCIST) (version 1.0) guidelines will be used (23). 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. Normalization of standardized uptake value on FDG-PET is used in PERCIST. For patients that have both bony lesions and lesions that meet RECIST criteria, only RECIST criteria will be used to evaluate best overall response.

6.3.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with E7 TCR transduced PBL.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.3.2 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: $\geq$ 20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under: as $\geq$ 10 mm
    - Scan slice thickness > 5 mm: double the slice thickness
- With calipers on clinical exam: $\geq$ 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 $\geq$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.

Measurable bone metastases: Any size bone lesions with a baseline SUL (standard uptake value, corrected for lean body mass) on FDG-PET of 1.5 x mean liver SUL + 2 standard deviations of mean SUL is considered measurable. Bone lesions are only measured in patients with isolated bone metastases.

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. 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.

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. Lesions that have been previously treated with loco-regional therapy or in a previously irradiated area can be considered target lesions if they have demonstrated unequivocal progression by radiographic imaging.

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.

6.3.3 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.

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). 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.

FDG-PET: Patients with isolated bony metastases will have FDG-PET to determine if lesions meet criteria to be followed by PERCIST version 1.0. While FDG-PET response assessments in solid tumors being followed by RECIST need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression of lesions (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.

6.3.4 Response Criteria

6.3.4.1 Evaluation of Target Solid 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.

6.3.4.2 Evaluation of Bony Lesions in Patients with Isolated Bone Metastases

Complete Response (CR): Normalization of all bony lesions (target and nontarget) to SUL less than mean liver SUL and equal to normal surrounding tissue SUL. Verification with follow-up study in 1 month if anatomic criteria indicate disease progression.

Partial Response (PR): > 30% decrease in SUL peak; minimum 0.8 unit decrease. Verification with follow-up study if anatomic criteria indicate disease progression.

Progressive Disease (PD): > 30% increase in SUL peak (minimum 0.8 unit increase in SUL peak), > 75% increase in TLG of the 5 most active lesions, Visible increase in extent of FDG uptake, or new lesions. Verification with follow-up study if anatomic criteria indicate complete or partial response.

Stable Disease (SD): Does not meet other criteria.

6.3.4.3 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).

6.3.4.4 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 Tumors (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.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

6.3.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or 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.

6.3.6 Progression free survival:

Progression-free survival (PFS) is measured from the start of treatment until the time of disease progression or death from any cause.

6.4 TOXICITY CRITERIA

Careful evaluation to ascertain the toxicity, immunologic effects and anti-tumor efficacy of the treatment regimens will be performed. This study will utilize the CTCAE version 4.0 for toxicity and adverse event reporting. A copy of the CTCAE version 4.0 can be downloaded from the website http://ctep.cancer.gov. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING/IRB 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 at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements. Note: Only IND Safety Reports that meet the definition of an unanticipated problem or present new information that might affect the willingness of participants to enroll or remain on the study 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 at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements..

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

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

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

7.4 NCI GUIDANCE FOR REPORTING EXPEDITED ADVERSE EVENTS FOR MULTI-CENTER TRIALS

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802: Non-compliance in Human Subjects Research, found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements. The participating site PI must immediately report to the coordinating center PI any deaths possibly related to the research within 24 hours of PI awareness of the event. The Site PI must also report any other events required by Policy 801 to the coordinating center PI within 7 days of PI awareness.

A reporting form will be provided with the participating site documents for such reports.

7.5 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.5.1 Serious Adverse Event Reports to IBC

The NIH Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of E7 TCR transduced PBL 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 transduced PBL, but are not fatal or life-threatening, much 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.

7.5.2 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 NIH 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.

7.5.2.1 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.

7.5.2.2 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.

7.6 NIH Required Data and Safety Monitoring Plan

7.6.1 Principal Investigator/Research Team

The clinical research team will meet on a regular biweekly basis 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. Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timelines.

The NIH Principal Investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The site Principal Investigator will personally conduct or supervise the investigation at the respective site and provide appropriate delegation of responsibilities to other members of the research staff.

7.6.2 Safety Monitoring Committee (SMC) – NCI CCR SMC

This protocol will require oversight from the Safety Monitoring Committee (SMC). 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. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal Investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a 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 or 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 4.0.

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/display/CCRCRO/Forms+and+Instructions

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

8.4 WAIVER OF EXPEDITED REPORTING TO CCR

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)
Blood and lymphatic system disorders	Anemia	1-3	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	Platelet count decreased	1-3	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	Platelet count decreased	4 if < 14 days	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
Investigations	White blood cell decreased	1-4	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)
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)

CTCAE System Organ Class	Adverse Event	Grade	Prolongation of Hospitalization	Expected Frequency	Attribution
Blood and lymphatic system disorders	febrile neutropenia (without an associated infection)	3	Expected	100%	Commercial Product (Cyclophosphamide and fludarabine)

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.

8.6 Reporting Pregnancy

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

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 becomes 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 should be followed up and documented.

8.6.2 Paternal exposure

Patients should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of E7 TCR Transduced PBL.

Pregnancy of the patient's partner is not considered to be an AE. The outcome of all pregnancies occurring from the date of the first dose until 4 months after the last dose should, if possible, be followed up and documented. Pregnant partners may be offered the opportunity to participate in

an institutional pregnancy registry protocol (e.g., the NIH IRP pregnancy registry study) to provide data about the outcome of the pregnancy for safety reporting purposes.

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 in expedited manner to the FDA in accordance to 21 CFR 31.2.32. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

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

8.8 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

> Non-NIH participating sites not using the CCR Protocol Deviation Tracking System (PDTS) will report any protocol deviation on the OSRO Site Protocol Non-Adherence/Deviation Log, or a site-generated protocol deviation report approved by OSRO. The Non-Adherence/Deviation Log should be maintained in the site essential documents file and submitted to OSRO via OSROMonitoring@mail.NIH.gov on the **first business day of each month throughout the study**.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and,
- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

For Phase I, the objective of this study is to determine a safe dose for E7 TCR cells plus aldesleukin for the treatment of metastatic HPV-16+ cancers. The study may require up to 6 patients per dose level. For purposes of sample size estimation, we will assume that as few as 9 and no more than 18 patients will be required to perform the initial safety evaluation for E7 during the phase I portion. As of March 2nd, 2018, a total of 9 patients have been treated with no dose-limiting toxicities encountered.

With Amendment H

A total of 40 patients will be treated, including the 3 additional patients added to dose level 3 via amendment G to confirm safety.

These patients will be evaluated for efficacy and for safety as follows. With 40 patients treated at a single dose level, there will be 90% power to rule out a 10% overall response rate (PR +CR) in favor of a targeted alternative response rate of 25%, with a one-sided 0.10 significance level exact binomial test. These levels were selected to account for a variety of tumor types which may have varying degrees of response. In practice, if there are 7 responses out of 40 patients (17.5%), then the lower one-sided exact 90% confidence bound about this fraction is 10.0%, which

matches the level to be ruled out. Thus, 7 responses in 40 patients would be considered a minimally acceptable overall response rate for the cohort. After the trial, the results obtained will be reported along with a one-sided lower 90% confidence interval bound as well as two-tailed 80% and 95% confidence intervals.

In addition, to ensure that enrollment to the RP2D level is not continued if response rates are insufficient, an early stopping rule will be imposed. If after 21 evaluable patients there are 0 or 1 responses, then no further patients will be enrolled as soon as this can be determined because the upper one-sided exact 90% confidence interval bound on 1/21 is 17.3%, which is marginally inconsistent with the intended 7/40 (17.5%) minimally necessary to consider the results to be positive overall.

This cohort of 40 patients may consist of patients with any of 6 possible tumor types, but all of which are HPV-16+. As such, they will be considered together for a primary evaluation because of this common characteristic. In addition to evaluating the results in the full set of 40 patients, there will be individual exploratory evaluations of response for the patients according to their type of tumor along with 80% and 95% two-sided confidence intervals. The results obtained in this exploratory fashion may be used to guide parameter selection for subsequent trials if appropriate.

As indicated in Section **3.4**, patients may be re-enrolled on the study as new patients to allow re-treatment, and these patients will be considered both times in the total accrual ceiling for the study. This re-enrollment of patients will not be used in the analysis of the 40-patient cohort.

Provided that about 2 patients every month will be enrolled onto this trial, with a 4-week time frame between each dose level, approximately 1 ½ - 2 years may be needed to accrue the maximum number of patients. Participants in the phase II portion of the study who receive treatment with a cell dose more than 30% below the RP2D will be evaluable for safety but will not be included in the primary efficacy analysis.

With up to 40 evaluable participants needed for treatment, as well as planning for a small number of inevaluable participants (15), we intend to initiate intervention in up to 55 participants. Note: To allow for screen failures (305), a total of 360 will be set for the purposes of the NIH accrual ceiling.

11 COLLABORATIVE AGREEMENTS

De-identified samples may be provided to Kite Pharma for assistance in performing the research studies described in Section **5.1**. Kite Pharma is collaborating in the development of the E7 TCR. A CRADA between NCI and Kite Pharma is now terminated(CRADA # 03022). Kite Pharma provided funding for this study.

11.1 MULTI-INSTITUTIONAL GUIDELINES

Documents requiring submission to the reviewing IRB as provided in the Participating Site Information Sheet should be provided to the coordinating center for submission to the IRB.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

The patients to be entered in this protocol have metastatic or recurrent/refractory locally advanced HPV-associated cancer that is refractory to standard therapy, and limited life expectances.

Subjects from both genders 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 gender 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 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **12.5**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) to assess ongoing capacity of the subjects and to identify an LAR as needed.

Please see Section **12.6.1** for consent procedure.

12.4 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 a 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 metastatic 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 D**.

12.4.1 Cell Infusion

The cells have a type of virus (retrovirus) put into them that recognizes the HPV E7 protein. Although this retrovirus is not active, there is the rare possibility that it may cause infection. The cells could also cause subjects to develop another type of cancer, such as leukemia or lymphoma. These specific gene-modified cells have not been given before so we do not have much information about the side effects.

Potential risks include:

- Fever, chills and shortness of breath, which may last for a few hours (common)
- Lung congestion causing shortness of breath

- Severe reaction to the cells which would include very low blood pressure and damage to heart, lung, and/or kidneys
- As this is a new experimental therapy which has not been given to patients, side effects that we do not anticipate that may cause conditions to deteriorate may be encountered. Any new information that becomes available during the course of this study will be shared.
- Experience with diverse types of cell therapy including tumor-infiltrating lymphocytes, CAR-T cells, and TCR-T cells, indicates that the risk of cell therapy may include cytokine release syndrome (where the T cell therapy causes the release of chemicals called cytokines that aggressively ramp up the immune system), autoimmunity (an immune reaction against normal tissues) and neurotoxicity (damage to the nervous system). Cytokine release syndrome may cause mild symptoms such as fever, fatigue, headache, rash, joint stiffness, and muscle aches, or severe symptoms such as low blood pressure, high fever, uncontrolled systemic inflammatory response, hemophagocytic lymphohistiocytosis (a rare condition in which certain types of white blood cells build up in organs and destroy other blood cells), shock, vascular leakage (where fluids and proteins leak out of small blood vessels and into surrounding tissues), disseminated intravascular coagulation (a serious disorder where proteins that control blood clotting become overactive), and multi-organ system failure.
- A patient treated on the phase I portion of the protocol, who had breathing problems from advanced cancer in the lungs, developed severe breathing, blood pressure, and kidney toxicity that required temporary support with a breathing machine, blood pressure medicines, and dialysis and this resulted in injury to her toes and feet.

12.4.2 Biopsy

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

12.4.3 Blood Sampling

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting. Up to 200 ml of blood may be collected at any visit on study (at screening this maximum at a single visit is 100 ml of blood), but no more than 473 ml per 8 weeks period.

12.4.4 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.

12.4.5 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.

12.4.6 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.

12.4.7 Pulmonary Function Tests (PFT)

PFTs are safe for most participants; however, some may experience dizziness, shortness of breath and fainting. In rare PFTs may lead to a collapsed lung. In participants with asthma, PFTs may precipitate an asthma attack.

12.4.8 CT and PET Scans

During a CT scan and PET, participants are briefly exposed to much more radiation than they would be during a plain X-ray. Radiation exposure potentially increases the risk of developing cancer. Although rare, the intravenous (IV) contrast material involved in some CT and PET 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 are to tell the study doctor if they've ever had a prior reaction to contrast material during medical tests.

12.4.9 MRI & Gadolinium-enhanced MRI

12.4.9.1 MRI

Participants might be at risk for injury from the MRI magnet if they have some kinds of metal in their body. It may be unsafe for them to have an MRI scan if they have:

- pacemakers or other implanted electrical devices,
- brain stimulators,
- some types of dental implants,
- aneurysm clips (metal clips on the wall of a large artery),
- metal prostheses (including metal pins and rods, heart valves, and cochlear implants),
- permanent eyeliner,
- tattoos,
- an implanted delivery pump,
- or shrapnel fragments. Welders and metal workers may have small metal fragments in the eye.

Participants will be screened for these conditions before having any MRI scan. If they have a question about metal in their body, they should tell us. They will be asked to fill out an MRI screening form before each MRI scan they have.

In addition, all magnetic objects (like watches, coins, jewelry, and credit cards) must be removed before they enter the MRI scan room.

If participants are afraid of confined (small, cramped) spaces, they may get anxious during an MRI. If they have back problems, they may have back pain or discomfort from lying in the scanner.

The noise from the scanner is loud enough to damage hearing, especially if they already have hearing loss. We will give them hearing protection. If the hearing protection comes loose during the scan, they should let us know right away.

There are no known long-term risks of MRI scans.

12.4.9.2 Gadolinium-enhanced MRI

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 (NSF)". This condition always involves the skin and can also involve the muscles, joints and internal organs. NSF has resulted in a very small number of deaths. A blood test of 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 kidney function is below the safe level.

Most of the gadolinium contrast leaves the body in the urine. However, the FDA has 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 in the body than others. In this study, we will use the gadolinium contrast drugs that are less likely to remain in the body. We will also give participants additional information called a "Medication Guide." Upon request, we will give individual information about retained gadolinium we see on their studies.

12.4.10Radiation Exposure

Including assessments at screening, this research study involves up to 3 CT CAP scans, 4 CT guided biopsies, and 3 PET scans collected for research purposes.

Subjects undergoing these scans and biopsies will be exposed to 8.3 rem per year. The CT scans, CT guided biopsies, and PET scans, in this study will expose the research participant to 27.7 years' worth of background radiation. This level of exposure results in an increased risk of cancer.

12.5 RISKS/BENEFITS ANALYSIS

The success of this effort cannot be predicted at this time. Since all patients in this protocol have metastatic or recurrent/refractory locally advanced HPV16-associated cancer and limited life expectancies the potential benefit is thought to outweigh the potential risks.

12.5.1 Adult Patients (including who are or may become unable to consent)

As outlined above, although there is prospect of direct benefits to individual subjects. We believe that procedures performed on this study will pose more than a minimal risk to the patients.

12.6 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable 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 used in compliance with local policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location, but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

12.6.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in Section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.6**.

12.6.2 Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in Section **2.2.1** may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

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

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

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

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

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

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

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

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council for 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 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 stored at the NCI. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by

clinical sites and by NCI research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NCI.

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

14 PHARMACEUTICAL INFORMATION

Note: The package insert/prescribing information for each commercial supply used should supersede any information included below.

14.1 INTERLEUKIN-2

14.1.1 Other Names

Aldesleukin, Proleukin, Recombinant Human Interleukin 2

14.1.2 How Supplied

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

14.1.3 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 with 24 hours.

14.1.4 Storage

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

14.1.5 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 30C. As there are no formal stability studies of aldesleukin diluted with HSA, the expiration time will be limited to 4 hours, per pharmacy guidance.

14.1.6 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.

14.1.7 Toxicities:

Expected toxicities of aldesleukin are listed in the product label and in **Appendix C**. 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 B**. Additional Grade 3 and 4 toxicities seen with aldesleukin are detailed in **Appendix C**.

14.2 FLUDARABINE

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 How Supplied

It will be purchased by the NIH Clinical Pharmacy Department from commercial sources. Fludarabine is supplied in a 50 mg vial either as a fludarabine phosphate powder in the form of a white, lyophilized solid cake or as an intravenous solution in a 25 mg/ml concentration.

14.2.3 Stability

For the intravenous powder for solution, 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 of 5% dextrose in water or 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 3**.

Table 3. 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.

Unless otherwise specified in this protocol, actual body weight is used for dose calculations of treatment agents. In patients who are determined to be obese (BMI >35), the practical weight (see 3 below) will be used.

14.3 CYCLOPHOSPHAMIDE

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 How Supplied

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 8C.

14.3.4 Administration

It will be diluted in 250 mL of 5% dextrose in water (D5W) or 0.9% sodium chloride, USP 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 3**.

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)

The procedure for the expanding the human PBL is 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.

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 https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF (section IV).

14.5 MESNA

14.5.1 Other Names

Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891

14.5.2 How Supplied

Mesna will be obtained commercially by the Clinical Center Pharmacy Department and is supplied as a 100 mg/mL solution.

14.5.3 Storage

Intact ampules are stored at room temperature.

14.5.4 Stability

Diluted solutions (1 to 20 mg/mL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48 to 72 hours in D5W, 48 to 72 hours in D5W/0.45% NaCl, or 24 hours in 09% NaCl.

14.5.5 Administration

Dilute to concentrations less than or equal to 20 mg Mesna/mL fluid in D5W or 0.9% NaCl and to be administered intravenously as a continuous infusion. If patient is obese (BMI >35) drug dosage will be calculated using practical weight as described in **Table 3**.

14.5.6 Toxicities

Include nausea, vomiting, and diarrhea.

14.6 FILGRASTIM

Note: Filgrastim or its biosimilar may be used interchangeably on this protocol.

14.6.1 Other Names

Granulocyte Colony-Stimulating Factor, G-CSF, Filgrastim, Neupogen

14.6.2 How supplied

Filgrastim will be obtained commercially by the Clinical Center Pharmacy Department and is supplied in 300 ug/mL and 480 ug/1.6mL vials.

14.6.3 Storage

G-CSF should be refrigerated and not allowed to freeze. The product bears the expiration date. The product should not be shaken.

14.6.4 Stability

It is generally stable for at least 10 months when refrigerated.

14.6.5 Administration

The appropriate dose is drawn up into a syringe. G-CSF will be given as a daily subcutaneous injection.

14.6.6 Toxicities

The side effects of G-CSF are skin rash, myalgia, and bone pain, an increase of pre-existing inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

14.7 TRIMETHOPRIM AND SULFAMETHOXAZOLE DOUBLE STRENGTH (TMP/SMX DS)

14.7.1 How supplied

TMP/SMX DS will be obtained by the Clinical Center Pharmacy Department from commercial sources.

It may be used for the prevention of PCP pneumonia.

14.7.2 Administration

The oral dose is 1 tablet PO daily 3 times a week (MUST be on non-consecutive days) beginning at or near the time of discharge from the hospital and continuing for at least 6 months and until the CD4 count is greater than 200 on 2 consecutive lab studies.

14.7.3 Toxicities

Like other sulfa drugs, TMP/SMX DS can cause allergies, fever, photosensitivity, nausea, and vomiting. Allergies typically develop as a widespread itchy red rash with fever 8 to 14 days after beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur.

14.8 AEROSOLIZED PENTAMIDINE IN PLACE OF TMP/SMX DS

Patients with sulfa allergies may receive aerosolized Pentamidine 300 mg per nebulizer at or around the time of discharge from the hospital and continued monthly until the CD4 count is

above 200 on 2 consecutive follow up lab studies and for at least 6 months post chemotherapy. Pentamidine Isethionate will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of PCP infections. It is supplied in 300 mg vials of lyophilized powder and will be administered via nebulizer. Toxicities reported with the use of Pentamidine include metallic taste, coughing, bronchospasm in heavy smokers and asthmatics; increased incidence of spontaneous pneumothorax in patients with previous PCP infection or pneumatoceles, or hypoglycemia.

14.9 HERPES VIRUS PROPHYLAXIS

14.9.1 Valacyclovir (Valtrex)

Valacyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It may be used orally to prevent the occurrence of herpes virus infections in patients with positive HSV serology. It is supplied in 500 mg tablets. Valacyclovir will be started the day after the last dose of fludarabine at a dose of 500 mg orally daily if the patient is able to tolerate oral intake. See package insert for dosing adjustments in patients with renal impairment. Common side effects include headache, upset stomach, nausea, vomiting, diarrhea, or constipation. Rare serious side effects include hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

14.9.2 Acyclovir

Acyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It may be used to prevent the occurrence of herpes virus infections in patients who cannot take oral medications. It is supplied as powder for injection in 500 mg/vials. Reconstitute in 10 mL of sterile water for injection to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7 mg/mL or less and used within 12 hours. IV solutions should be diluted to a concentration of 7 mg/mL or less and infused over 1 hour to avoid renal damage. 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. Stomach upset, headache or nausea, rash or hives; peripheral edema; pain, elevated liver function tests; and leukopenia, diarrhea, lymphadenopathy, myalgias, visual abnormalities and elevated creatinine have been reported. Hair loss from prolonged use has been reported. Acyclovir will not be used concomitantly with other nucleoside analogs that interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

14.10 FUNGAL PROPHYLAXIS

14.10.1 Fluconazole

Fluconazole will be obtained by the Clinical Center Pharmacy Department from commercial sources. It can be used as prophylaxis against fungal infections. It is available in 200 mg tablets. It can cause headache, nausea, vomiting, diarrhea, or abdominal pain, and liver damage which may be irreversible. It can cause rashes and itching, which in rare cases has caused Stevens Johnson Syndrome. It has several significant drug interactions. The package insert should be consulted prior to prescribing. For IV administration in patients who cannot tolerate the oral preparation, Fluconazole comes in 2 mg/mL solution for injection, and prepared according to

Clinical Center Pharmacy standard procedures. It should be administered at a maximum IV rate of 200 mg/hr.

14.11 SUPPORT MEDICATIONS

14.11.1 Ondansetron hydrochloride

Ondansetron hydrochloride will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to control nausea and vomiting during the chemotherapy preparative regimen. It can cause headache, dizziness, myalgias, drowsiness, malaise, and weakness. Less common side effects include chest pain, hypotension, pruritus, constipation and urinary retention. Consult the package insert for specific dosing instructions.

14.11.2 Furosemide

Furosemide will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to enhance urine output during the chemotherapy preparative regimen with cyclophosphamide. Adverse effects include dizziness, vertigo, paresthesias, weakness, orthostatic hypotension, photosensitivity, rash, and pruritus. Consult the package insert for a complete list of all side effects.

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16 APPENDICES

16.1 APPENDIX A: PARTICIPATING SITE ROLES AND OVERSIGHT PLAN

The study will also be conducted at a participating site under a reliance agreement. The role of the participating site is generally limited to sample and data analysis as described in Section **5.1.5**. Enrollment and routine follow up per protocol will not be conducted at participating site.

Data will be collected from the sites as outlined in Section **6.1** and expedited reporting will be per Section **7.4**. Protocol and regulatory compliance will be ensured as described in Section **7.6.1** and **13.2**.

IRB determinations will be forwarded to the participating site PI/study coordinator by a member of the study team.

16.2 APPENDIX B: ADVERSE EVENTS OCCURRING IN ≥10% OF PATIENTS TREATED WITH ALDESLEUKIN (N=525)[1]

Body System	% Patients	Body System	% Patients
Body as a Whole		*Metabolic and Nutritional Disorders*	
Chills	52	Bilirubinemia	40
Fever	29	Creatinine increase	33
Malaise	27	Peripheral edema	28
Asthenia	23	SGOT increase	23
Infection	13	Weight gain	16
Pain	12	Edema	15
Abdominal pain	11	Acidosis	12
Abdomen enlarged	10	Hypomagnesemia	12
Cardiovascular		Hypocalcemia	11
Hypotension	71	Alkaline phosphatase incr	10
Tachycardia	23	*Nervous*	
Vasodilation	13	Confusion	34
Supraventricular tachycardia	12	Somnolence	22
Cardiovascular disorder[a]	11	Anxiety	12
Arrhythmia	10	Dizziness	11
Digestive		*Respiratory*	
Diarrhea	67	Dyspnea	43
Vomiting	50	Lung disorder[b]	24
Nausea	35	Respiratory disorder[c]	11
Stomatitis	22	Cough increase	11
Anorexia	20	Rhinitis	10
Nausea and vomiting	19	*Skin and Appendages*	
Hemic and Lymphatic		Rash	42
Thrombocytopenia	37	Pruritus	24
Anemia	29	Exfoliative dermatitis	18
Leukopenia	16	*Urogenital*	
		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 C: EXPECTED IL-2 TOXICITIES AND THEIR MANAGEMENT

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

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

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

*Unless the toxicity is not reversed within 12 hours.

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

16.4 APPENDIX D: INTERLEUKIN-2 TOXICITIES OBSERVED IN PATIENTS TREATED AT THE NIH CLINICAL CENTER

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.

16.5 APPENDIX E: ECOG PERFORMANCE STATUS SCALE

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.

* As published in Am. J. Clin. Oncol.: Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.