Abbreviated Title: Immunotherapy for vulvar HSIL
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Title: A Phase I Study of Immunotherapy with E6 T Cell Receptor T Cells for Vulvar High-Grade Squamous Intraepithelial Lesions

Principal Investigator: Christian S. Hinrichs, MD
Experimental Transplantation and Immunology Branch
National Cancer Institute (NCI)
10 Center Drive, Room 4B04
Bethesda, MD 20892
Phone: 240-760-6059
Email: Hinrichs@nih.gov

Investigational Agents:

Drug Name:	E6 TCR
IND Number:	16078
Sponsor:	Center for Cancer Research
Manufacturer:	CC DTM

Commercial Agents: Interleukin-2

PRÉCIS

Background:

- Vulvar high-grade squamous intraepithelial lesion (HSIL) is a premalignant epithelial lesion that is frequently multifocal and/or recurrent.
- The primary treatment is surgery, which may result in disfigurement and compromise of the urethra, anus, or clitoris. Recurrence after surgery is common and primarily treated with additional surgery.
- Vulvar HSIL is caused by chronic infection with the human papillomavirus (HPV) type 16 infection. In this clinical trial the HPV-16 infection is targeted with a single infusion of autologous T cells that have been genetically engineered to express an HPV-16 E6-specific T cell receptor (E6 TCR T cells).

Objective:

- Determine the safety of E6 TCR T cells for the treatment of vulvar HSIL.

Eligibility:

- Histologically confirmed diagnosis of HPV-16+ vulvar HSIL.
- Expression of the HLA-A2*02:01 allele.
- Measurable lesion(s) that are recurrent or cannot be resected with acceptable cosmetic or functional results.
- Age greater than or equal to 18 years old and less than or equal to 65 years old.
- Eastern Oncology Cooperative Group Performance Score of 0 or 1.

Design:

- This is a phase I clinical trial with a 3+3 dose escalation design.
- Subjects will receive E6 TCR T cells followed by up to two doses of aldesleukin 720,000 IU/kg IV.
- No conditioning regimen will be given, aldesleukin will be capped at a maximum of two doses, and E6 TCR T cell dosing will begin at dose level -1 from the previously determined safe dose.

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1. INTRODUCTION

1.1 STUDY OBJECTIVES

Objectives:

1.1.1 Primary Objective

- Determine the safety of E6 TCR T cells for the treatment of vulvar HSIL.

1.1.2 Secondary Objectives

- Assess clinical responses in subjects treated with E6 TCR T cells for vulvar HSIL.
- To evaluate a novel assay to genotype HPV from metastatic HPV+ cancers using a ddPCR-based blood test.

1.1.3 Exploratory Objectives

- Test for clearance of HPV infection from vulvar tissues.
- Evaluate the expansion, survival, trafficking, phenotype, and function of E6 TCR cells following infusion.
- Investigate the vulvar HSIL microenvironment including immune cell subsets and costimulatory/inhibitory molecules.
- Assess circulating HPV DNA levels associated with treatment.

1.2 BACKGROUND AND RATIONALE

Vulvar high-grade squamous intraepithelial lesion (HSIL) is a premalignant condition that is caused by high-risk human papillomavirus (HPV) infection. The primary treatment for vulvar HSIL is surgical. Surgery can result in substantial morbidity, particularly for lesions that are multifocal, extensive, or adjacent to important anatomic structures (e.g. the anus, clitoris, or urethra). Following surgery, disease recurrence is common due to persistence of the underlying HPV infection. Therefore, a systemic treatment directed against the viral infection at the foundation of the disease may be more effective. This research protocol seeks to target the HPV infection that is causing vulvar HSIL with immunotherapy. The immunotherapy consists of autologous T cells that are genetically engineered to recognize the HPV-16 E6 oncoprotein, which is expressed by HPV-16 infected cells. The protocol is designed as a one-time treatment to clear the HPV infection and thereby cause regression of the HSIL lesions.

1.2.1 Vulvar high-grade squamous intraepithelial lesion (HSIL)

Vulvar HSIL is defined by the 2015 International Society for the Study of Vulvovaginal Disease (ISSVD) as an HPV-associated lesion of the vulva that has high malignancy potential. The term, vulvar HSIL, is equivalent to the previously used nomenclature of vulvar intraepithelial neoplasia, usual type [1]. It does not include low-grade squamous intraepithelial lesions without malignant potential such as condyloma acuminatum. It also does not include premalignant lesions that are not associated with HPV, which are classified as differentiated-type vulvar intraepithelial neoplasia. Multiple HPV types can cause vulvar HSIL but HPV-16 is the most prevalent [2]. Spontaneous regression of vulvar HSIL is rare, reportedly occurring in fewer than 1.5% of patients per year [3].

Patients with vulvar HSIL may present with symptoms of local itching, burning, or pain. Diagnosis is suggested by careful inspection of the lesion(s) and is confirmed by biopsy. Multicentric disease is common due to diffuse spread of HPV infection throughout vulvar epithelium. Vulvar HSIL transforms into vulvar squamous cell carcinoma (SCC) in 3 to 9% of patients. Therefore, all lesions require surveillance with physical exams and biopsies to rule out invasive cancer [4]. For lesions that do not regress, excision is required. The type of surgery depends on the extent and anatomic location of disease. Treatment of multifocal lesions, extensive disease, or lesions in proximity to the urethra, clitoris, or anus can result in deformity and functional impairment. Even with radical excision to negative margins, recurrence occurs in approximately 30% of patients [5]. Thus, surgery may be an unsatisfactory treatment option as it often fails to prevent disease recurrence, results in morbidity, and negatively impact psychological and sexual health [6].

1.2.2 Studies of the natural history and treatment history of vulvar HSIL

Studies of the natural history and treatment of vulvar HSIL are scientifically limited as they are mostly retrospective and have small sample sizes. One systematic review reported that 6.5% of 3322 patients with vulvar HSIL progressed to develop an invasive vulvar carcinoma [3] . In untreated patients, invasive vulvar carcinoma developed in nine percent of subjects within one to eight years. Invasive cancers are treated with radical local excision or modified radical vulvectomy, depending on the tumor stage. Locally advanced cancers are treated with chemoradiation. Salvage surgery with pelvic exenteration and colostomy or ureteral diversion may be employed. Five-year survival for local, regional, and distant vulvar cancers are 86%, 54%, and 16% respectively. A study based on data from the United States reported the incidence of vulvar HSIL to be 2.86. per 100,000 women [7]. Immunotherapy for vulvar HSIL

Vulvar HSIL is caused by HPV infection, and HPV infections can be recognized and attacked by the immune system. Hence, immunotherapy is an attractive treatment strategy for vulvar HSIL. Topical immunotherapy with imiquimod, an immune response modifier that activates dermal dendritic cells through Toll-like receptor 7, has been reported to have response rates of 13-81%, but vulvar inflammation and pain limit its use and it is not currently FDA-approved [8]. Nonetheless, it is a treatment option that some patients should consider as an alternative to this protocol. Experimental vaccines have been tested for the treatment of vulvar HSIL. A clinical trial of a vaccine targeting HPV-16 E6 and E7 showed promising results in a single-arm phase II study [9]. This vaccine is experimental and not available outside of a clinical trial. The preventative vaccines for HPV (e.g. Gardasil and Cervarix) are highly effective at preventing HPV infections but not considered effective for the treatment of VIN [10].

1.2.3 Targeting of HPV-associated cancers with adoptive T cell therapy

In a clinical trial of HPV-16 E6 TCR T cells for patients with metastatic cancers associated with HPV-16 (e.g. cervical cancer, anal cancer, head and neck cancer, etc.), subjects received a chemotherapy conditioning regimen followed by E6 TCR T cells and aldesleukin (protocol 15-C-0005). Two of 12 patients experienced objective tumor responses. No E6 TCR T cell dose-limiting toxicities, off-target toxicity, healthy tissue targeting, or cytokine storm were observed. The maximum administered dose was deemed the maximum tolerated dose. Separately, in a clinical trial of autologous tumor-infiltrating T cells generated from cultures with HPV reactivity for metastatic HPV+ cancers, objective tumor responses occurred in 3/9 patients with cervical

cancer [11]. Adoptive T cell therapy has not been reported for the treatment of premalignant HPV-related diseases.

1.2.4 E6 TCR discovery and characterization

HPV-16 E6 is a viral oncoprotein that is constitutively expressed by and important to the survival of HPV-16+ infected cells, and it is absent from healthy human tissues. We isolated a TCR from the tumor-infiltrating T cells of a patient with metastatic anal cancer that recognized the $E6_{29-38}$ epitope (described below).

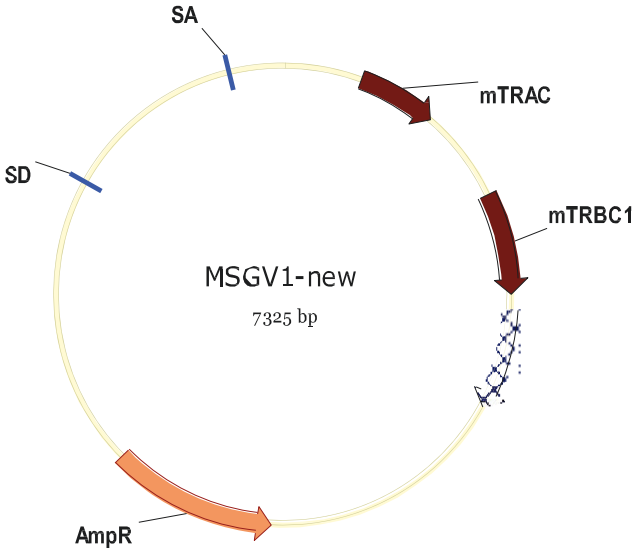


Figure 1. PG13-oDCA2-E6-C12 TCR vector map. *An MSGV1 retroviral vector encoding the TCR was constructed. This retroviral vector consists of 7,325 base pairs and includes a 5'LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites (SA). Alpha and beta chains of the E6 TCR are linked by a P2A peptide.*

Peripheral blood T cells transduced to express the E6 TCR displayed high avidity for the $E6_{29-38}$ peptide (**Figure 2**) and CD8-independent HLA-A*02:01/$E6_{29-38}$ tetramer binding (**Figure 3**). They specifically recognized a panel of HPV-16+ HLA-A*02:01+ cervical and oropharyngeal cancer cell lines. Thus, TCR gene engineered T cells expressing the E6 receptor can be used to target HPV-16+ HLA-A*02:01+ cells.

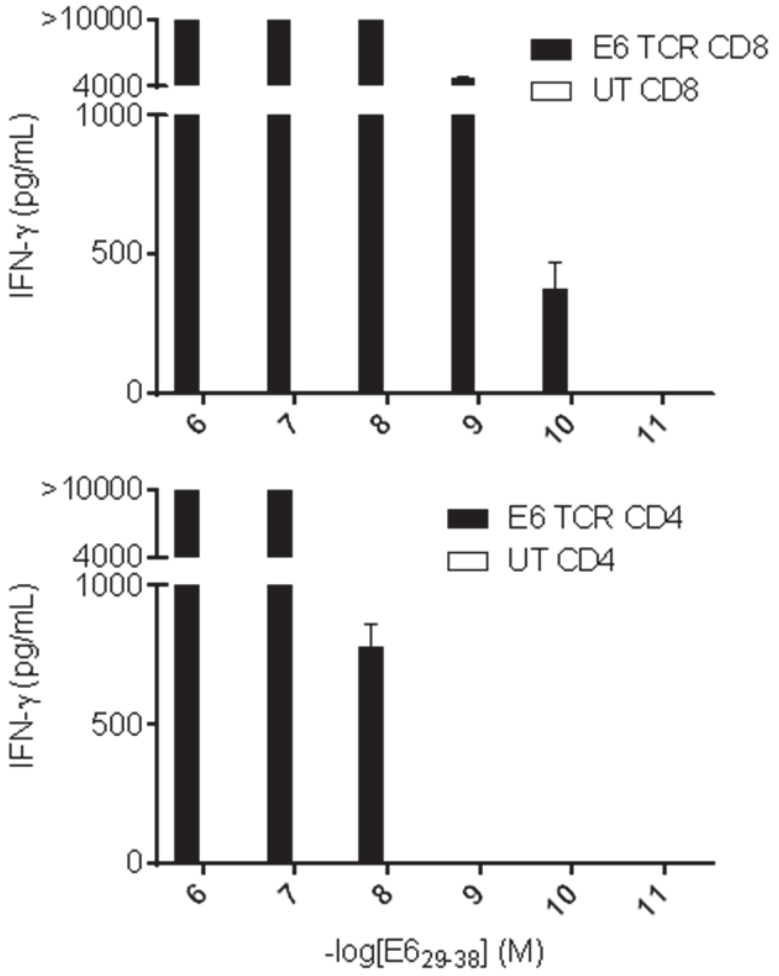


Figure 2. T cells transduced to express the E6 TCR demonstrated high avidity for the E6$_{29-38}$ peptide. *CD4 and CD8 T cells from PBMC were transduced to express the E6 TCR. Functional avidity was tested by coculture with T2 cells pulsed with titrated concentrations of E6$_{29-38}$ peptide.*

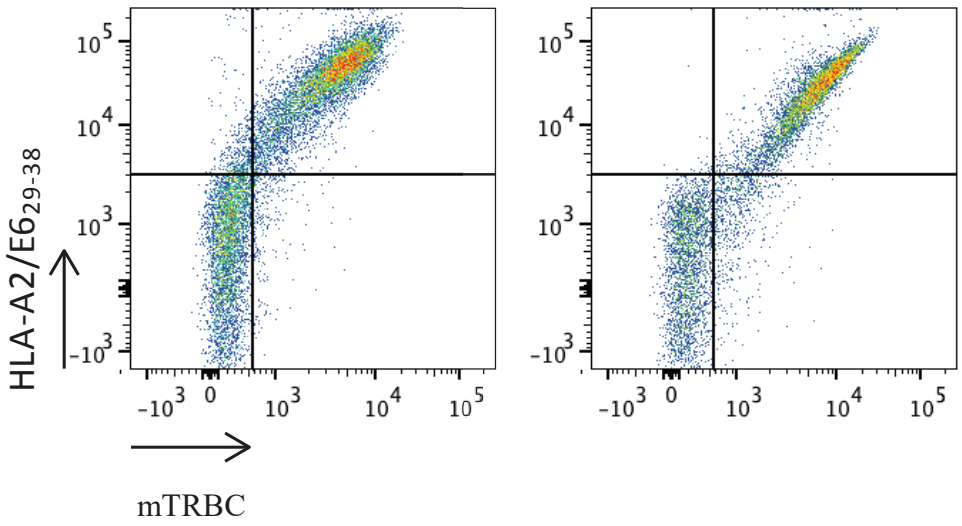
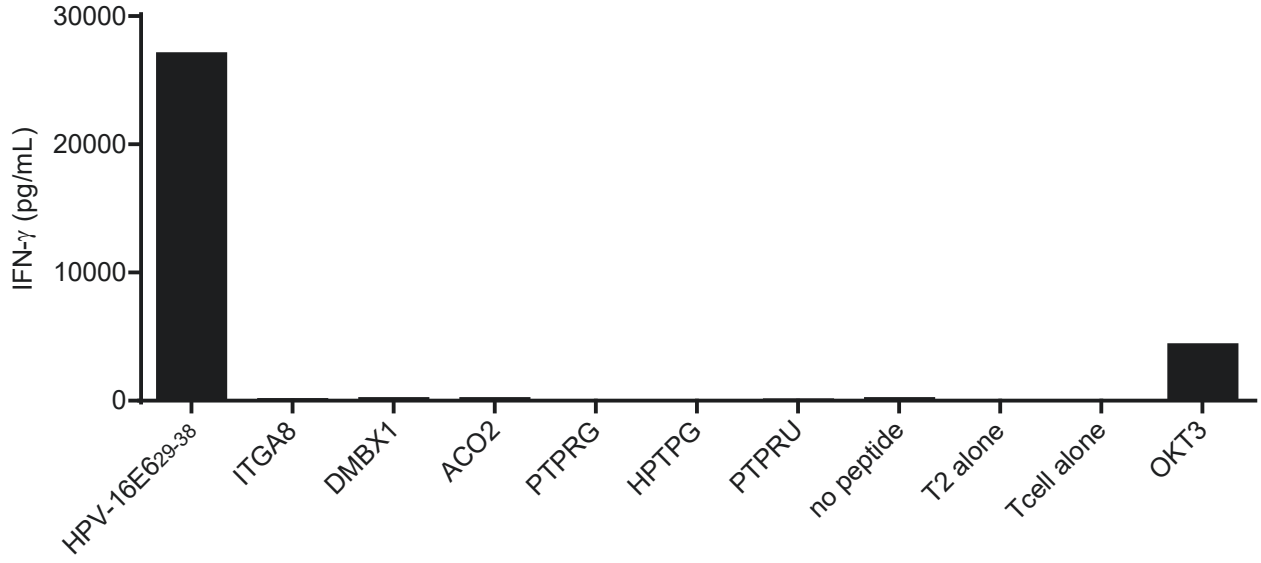


Figure 3. Peripheral blood T cells transduced to express the E6 TCR display CD8-independent HLA-A*02:01/E629-38 tetramer binding. *T cells from PBMC were transduced to express the E6 TCR. Dot plots shown are gated on PI- lymphocytes.*

In contrast to TCRs that have had unexpected cross-reactivity against normal human proteins, this TCR was isolated directly from a human T cell. Hence, it was subjected to thymic selection and is unlikely to possess avid reactivity against self-antigens. The complementarity determining regions of the TCR have not been modified, so 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 10 amino acids are shared with any human protein. There is no cross reactivity of this TCR against epitopes of these human proteins (**Figure 4**).



Gene Abbreviation	Sequence	Full Name of Protein
HPV16 $E6_{29-38}$	TIHDIILECV	HPV-16 $E6_{29-38}$
ITGA8	TISDTILEVG	Integrin alpha-8
DMBX1	TFQDIILEAR	Diencephalon/mesencephalon homeobox 1
ACO2	IIHQIILENY	Aconitate hydratase, mitochondrial
PTPRG	FIHDFILEAI	Protein tyrosine phosphatase
HPTPG	IIHDFILEAT	Receptor-type tyrosine-protein phosphatase gamma
PTPRU	FIHDAILEAC	Receptor-type tyrosine-protein phosphatase kappa or mu or U

Figure 4. E6 TCR transduced T cells did not recognize T2 cells pulsed with peptides sharing ≥ 6 amino acids with peptides from human proteins identified by BLAST search. *T2 cells pulsed with the peptides indicated in the table were cocultured with T cells expressing the E6 TCR.*

1.2.5 High-dose Interleukin-2 (Aldesleukin)

Interleukin-2 (IL-2) is a T cell growth factor that has been administered as a single agent and in combination with adoptive T cell therapy for the treatment of cancer [12]. As a single agent it can induce regression of metastatic melanoma and renal cell carcinoma with overall response rates around 20% [13]. Although high quality published data comparing adoptive T cell therapy with and without IL-2 are lacking, data from animal models indicate that IL-2 enhances the anti-tumor function of adoptively transferred T cells [14, 15].

Single-agent high-dose aldesleukin for invasive cancers is administered at a fixed dose (600,000 or 720,000 IU/Kg) but a variable number of doses are given depending on individual tolerance of the drug. Aldesleukin infusions are continued until adverse events prohibit further drug administration. To increase that safely of the present protocol, aldesleukin dose number will be capped at two doses, a number of doses that has been well tolerated in prior studies [16, 17]. In prior studies, the toxicity of aldesleukin was not increased by coadministration of autologous T cells [18, 19].

1.2.6 Safety Considerations

HPV-16 E6 TCR T cells target a foreign viral protein that is not present in human tissue. The TCR was discovered from the study of tumor infiltrating T cells isolated from a human and therefore has undergone thymic selection to not have human autoreactivity. The nucleotide sequence of the E6 TCR was codon optimized for expression in human tissues and the TCR constant regions replaced with their murine counterparts to improve TCR alpha/beta chain pairing and TCR expression. The alpha/beta TCR sequence was cloned into the MSGV1 retroviral vector, which has been tested extensively in clinical trials. TCR avidity enhancement was not performed and wild-type CDR regions are present, therefore there is no chance that off-target reactivity has been artificially introduced. The clinical safety of E6 TCR T cells has been studied in a protocol for the treatment of metastatic HPV-16 cancers (15-C-0005). In that study,

a conditioning chemotherapy regimen that enhances T cell survival and function, and increases the toxicity of the treatment was employed, and high-dose aldesleukin was administered to individual patient tolerance. Twelve patients were treated and no dose-limiting toxicity for E6 TCR T cell infusion was encountered. The E6 TCR T cells did not demonstrate off-target toxicity, targeting of healthy tissues, or cytokine storm. The maximum administered dose was thus declared to be the maximum tolerated dose (1.7×10^{11} cells).

In the present protocol, the safety margin of the treatment has been increased by the following measures: 1) No conditioning chemotherapy will be given. 2) Aldesleukin dose number will be capped at two. 3) E6 TCR T cell dose will begin at dose level -1.

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 insertional mutagenesis is a known hypothetical risk, there has never been a reported vector-mediated gene transfer into mature T cells since the first NCI sponsored gene transfer study in 1989 [20]. This proposed protocol follows all current FDA guidelines regarding testing and follow up of patients receiving gene transduced cells.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have vulvar HSIL as confirmed by pathology report from a CLIA-certified laboratory.

2.1.1.2 Vulvar HSIL must be HPV-16+ by a PCR, RNA, or in situ hybridization test from a CLIA certified laboratory.

2.1.1.3 Patients must have measurable lesion(s) as defined in section **6.3.2** and one or more of the following criteria:

a. Failure of surgery to control disease (i.e. positive margins or recurrence of HSIL after surgery).
b. Multifocal or extensive disease for which surgery would result in major deformity that is not be acceptable to the patient.
c. Disease for which surgery would have a risk of functional impairment that is not be acceptable to the patient (i.e. involve partial or complete excision of the clitoris, anus, vagina, or urethra).

2.1.1.4 Patients may have received any previous therapy, including surgical excision, but must have histologically documented recurrence on new biopsy and a measurable lesion that meets the above criteria.

2.1.1.5 The presence of disease that can be biopsied for research purposes is not an inclusion criterion.

2.1.1.6 Patients must have the HLA-A*02:01 allele

2.1.1.7 Age ≥18 years and ≤ 65 years. As age increases, the ability to tolerate the toxicities of aldesleukin decreases, so the patient population for this study will include up to and including 65 years of age to increase safety.

2.1.1.8 ECOG performance status of 0-1 as defined by **Appendix A.** Able to understand and sign the Informed Consent Document.

2.1.1.9 Women of child-bearing potential must have a negative pregnancy test. Women of child-bearing potential are defined as all women who are not post-menopausal or who have not had a hysterectomy. Postmenopausal will be defined as women over the age of 55 who have not had a menstrual period in at least 1 year.

2.1.1.10 The effects of E6TCR T Cells on the developing human fetus are unknown. For this reason, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.

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

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

2.1.1.13 Must be willing to participate in Gene Therapy Long Term Followup Protocol (15-C-0141), which will follow patients for up to 15 years per Food and Drug Administration (FDA) requirements.

2.1.1.14 Patients must have normal organ and marrow function as defined below:

• leukocytes	≥3,000/mcL
• absolute neutrophil count	≥1,000/mcL
• platelets	≥150,000/mcL
• hemoglobin	≥10.0 g/dL
• total bilirubin	within normal institutional limits except in patients with Gilbert's Syndrome who must have a total bilirubin < 3.0 mg/dL
• AST(SGOT)/ALT(SGPT)	Serum ALT/AST < 3X ULN
• creatinine	< 1.5X baseline, < 1.5X ULN OR
• creatinine clearance	≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal (by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation)

2.1.2 Exclusion Criteria

2.1.2.1 Patients who are receiving any other investigational agents

2.1.2.2 Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with E6 TCR, breastfeeding should be discontinued if the mother is treated with E6 TCR. These potential risks may also apply to other agents used in this study.

2.1.2.3 Uncontrolled intercurrent illness including, but not limited to, any ongoing or active infection (e.g. requiring anti-infective therapy), coagulation disorders, cardiovascular disorders, respiratory disorders, cancer, or psychiatric illness/social situations (within the last six months) that would limit compliance with study requirements.

2.1.2.4 Any form of systemic immunodeficiency, including acquired deficiency such as HIV or primary immunodeficiency such as Severe Combined Immunodeficiency Disease. The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune competence may be less responsive to the treatment.

2.1.2.5 Concurrent systemic steroid therapy if greater than the equivalent of 5 mg prednisone PO daily. Patients previously on steroids must be off steroids for four weeks prior to treatment.

2.1.2.6 Any history of clinically significant cardiac arrhythmia, coronary revascularization, ischemic symptoms, or previously documented LVEF of less than or equal to 45%. A cardiac stress test is required for all patients greater than 50 years old. A cardiac stress test may also be performed for any clinical concern. Patients with cardiac ischemia are not eligible.

2.1.2.7 Patients with any active invasive cancer are not eligible.

2.1.2.8 Patients vulvar HSIL that is not HPV-16+ or is associated with multiple types of high-risk HPV are not eligible.

2.2 SCREENING EVALUATION

2.2.1 Any time prior to starting the therapeutic regimen:

a. HLA-Typing
b. Review of most recent cervical cytology and HPV testing. Cervical cytology testing will be performed if clinically indicated.
c. If a pathology report confirming vulvar HSIL is not available, if available results are unclear, or there is a lack of necessary information in the available results, a vulvar biopsy will be performed to confirm diagnosis.
d. Cervical high-risk HPV testing
e. Venous assessment (as per apheresis clinic policy)
f. One 10 mL tube of blood will be collected for the optional ddPCR-based research blood test.

2.2.2 Within 4 weeks prior to starting the therapy unless otherwise indicated:

a. Complete history and physical examination, noting in detail the exact size and location of all lesions. (Note: Patient history may be obtained within 8 weeks prior to starting therapy.)
b. Chest x-ray
c. EKG
d. Cardiac stress test for patients over the age of 50.
e. HIV antibody titer, HbsAG determination, and anti-HCV antibody. (Note: may be performed within 3 months of therapy start date).
f. Past medical history including family history, social history, allergies, and current medications.

2.2.3 Within 14 days prior to starting the therapy:

a. Chem 20 equivalent: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (Bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid).
b. Thyroid Panel
c. CBC with differential and TBNK
d. PT/PTT

2.2.4 Within 7 days prior to starting the therapy regimen:

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

2.3 REGISTRATION PROCEDURES

Registration will be a two-part process as patients are screened on this protocol. Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. To initially register a subject after the participant has signed the consent, complete the top portion of the registration Eligibility Checklist from the website (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) indicating that the patient is being registered for screening and send via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. Once eligibility is confirmed after completion of screening studies, complete the remainder of the form which is the eligibility checklist, indicating that the patient is being registered for treatment and email the completed registration checklist to the CRO at NCI Central Registration Office ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

Subjects that do not meet screening criteria should be removed from the study following the procedure in section **3.6.3**.

2.3.1 Treatment Assignment and Randomization/Stratification Procedures for registration purposes only

Cohorts

Number	Name	Description
1	Cohort 1	Patients with HPV 16 positive vulvar HSIL enrolled to determine the MTD
2	Cohort 2	Patients with HPV 16 positive vulvar HSIL enrolled after MTD is determined

Arms

Number	Name	Description
1	Arm 1	Patients will undergo leukapheresis, then treatment with E6 TCR cells (at escalating doses) + aldesleukin
2	Arm 2	Patients will undergo leukapheresis, then treatment with E6 TCR cells (at the MTD) + aldesleukin

Stratifications

Not applicable to this protocol.

Randomization and Arm Assignment

This is not a randomized study. Patients in Cohort 1 will be directly assigned to Arm 1. Patients in Cohort 2 will be directly assigned to Arm 2.

2.4 BASELINE PROCEDURES

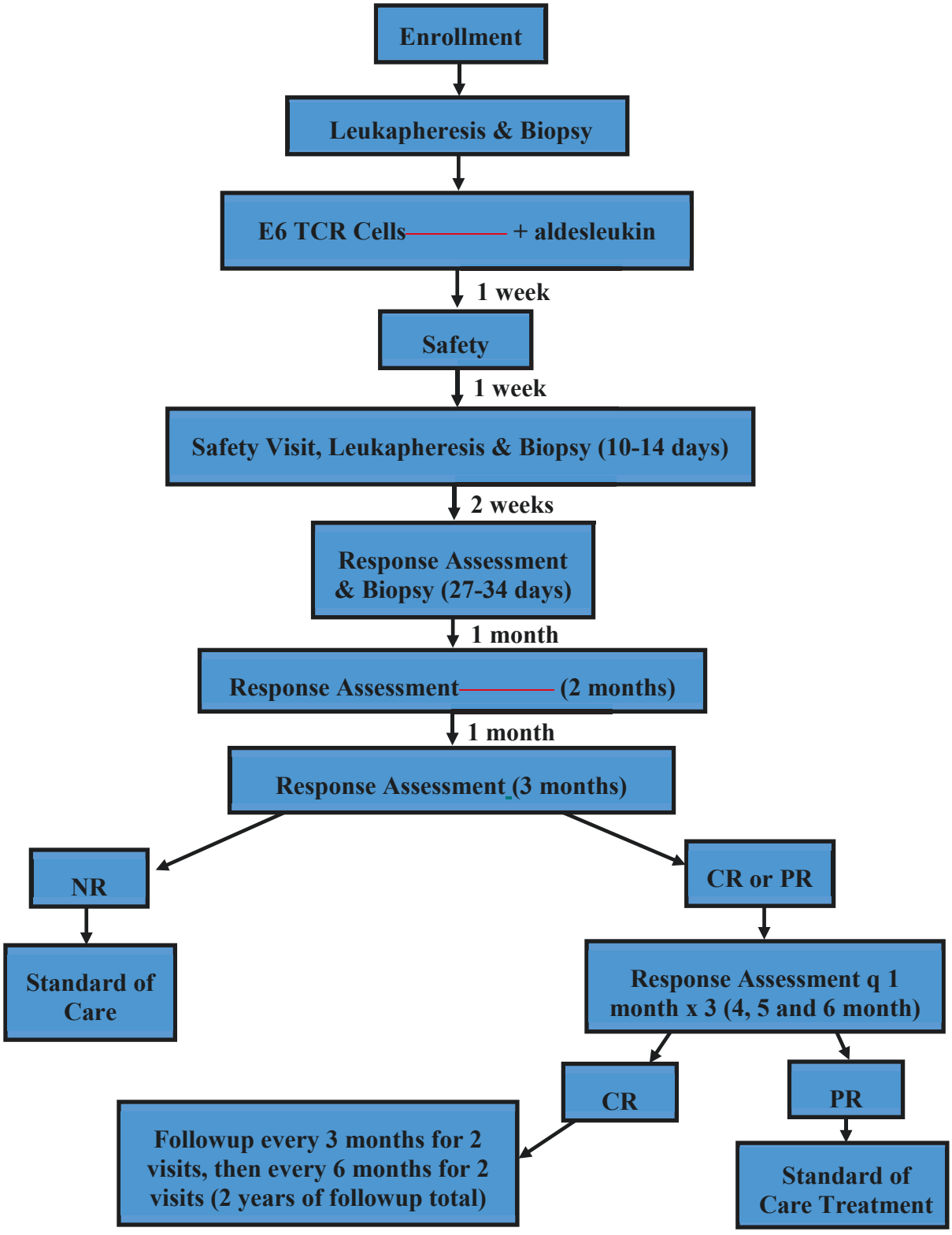
a. Baseline photographs and optional biopsies of HSIL lesions to be performed under local anesthetic in the clinic within 8 weeks prior to therapy. Patients will be required to sign a separate consent for all biopsies conducted during the study. Refer to Section **5.4** for guidelines for handling specimens.
b. Peripheral blood lymphocytes (PBLs), plasma, and serum collected at time of baseline biopsy. As noted in **5.1.3**, Biopsies will not be performed if they are not technically feasible, if they will interfere with response assessment, or if a patient declines consent.
c. Replication Competent Retrovirus (RCR) testing.
d. Leukapheresis as described in section **3.1.2**.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a phase I clinical trial with a 3+3 dose escalation design.

3.1.1 Protocol schema



3.1.2 Leukapheresis

The patient will undergo a 10-15 liter leukapheresis (generally, 12 liters will be processed to target yield of 6-10 $x10^9$ lymphocytes) in the Department of Transfusion Medicine (DTM) Dowling Apheresis Clinic according to DTM standard operating procedures. 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 16-C-0061 (ETIB Tissue Procurement Protocol).

3.1.3 E6 TCR T cell preparation

After cells are obtained by apheresis, further cell processing to generate E6 TCR cells will occur in the DTM according to standard operating procedures and the E6 TCR investigational new drug application. E6 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 ETIB the following day. Contacts in the ETIB preclinical core are Fran Hakim and Jeremy Rose (301-594-5339).

Before infusion, the percentage of T cells expressing the E6 TCR will be determined by flow cytometry. Successful TCR gene transfer will be defined as greater than 20% TCR positive cells. In addition to flow cytometry, further testing of the cells will take place prior to infusion to evaluate for microbial contamination, replication-competent retroviruses, and viability. Details of this testing can be found in the appropriate DTM SOPs. Any remaining pretreatment PBMC collected on this protocol and cryopreserved as described in Section **3.1.2** will be transferred from the Department of Transfusion Medicine to the Principal Investigator of this protocol for storage in the ETIB preclinical core and possible use in research and banked according to protocol 16-C-0061 (ETIB Tissue Procurement Protocol).

3.1.4 Treatment Phase

E6 TCR T cells will be infused while the patient is admitted to the hospital on treatment day 0. A central line catheter may be placed for cell infusion. The day after cell infusion, patients will receive aldesleukin 720,000 IU/kg IV q 12 hours x up to 2 doses. The second dose of aldesleukin will not be given if a patient develops any of the following: 1) hypotension or oliguria that require fluid boluses, 2) mental status changes, 3) hypoxia that requires supplemental oxygen, 4) diarrhea or nausea that are not controlled with symptomatic therapy. The second dose of aldesleukin may also be held for any safety concern at the discretion of the treating physician, and will not be given if a grade 3 or higher AE occurs following the first dose.

The patient will remain an inpatient for aldesleukin administration. Patients also will be observed overnight as inpatients following aldesleukin administration, and will not be discharged until cleared by the treating physician. Patients will receive one course of treatment.

3.1.5 Dose Limiting Toxicities (DLTs)

All treatment related Grade 3 and greater AEs occurring within 30 days of the cell infusion will be considered DLTs with the exception of the following expected transient effects of aldesleukin.

- Grade 3 fever or chills responsive to symptomatic treatment that resolve to ≤ grade 2 in 48 hours.
- Grade 3 hypotension or oliguria responsive to ≤ 1.5L of IV fluid boluses in 24 hours that resolves to ≤ grade 2 in 48 hours.
- Grade 3 dyspnea/hypoxia that improves to ≤ grade 2 or less with supplemental oxygen and resolves to ≤ grade 2 without supplemental oxygen in 48 hours.
- Grade 3 creatinine or electrolyte abnormalities that resolve to ≤ grade 2 in 48 hours.
- Grade 3 or higher white blood cell count decreased and/or lymphocyte count decreased that resolves to ≤ grade 2 in 72 hours.

Further outline of expected AEs with aldesleukin and their management is included in **Appendix B** and **Appendix C**.

3.1.6 Dose Escalation

The protocol will follow a 3+3 phase 1 dose escalation design. Three patients will be treated initially on dose level -1. If 2 of these 3 patients experience DLTs, the study will be stopped. If none of the 3 patients experiences a DLT, enrollment can start on dose level 1. If 1 of the first 3 patients experiences a DLT on dose level -1, 3 more patients will be treated at that dose level. If a second patient of the 6 has a DLT, the study will be stopped; otherwise, accrual can proceed to dose level 1. On dose level 1, if 2 patients have DLTs at any time during the treatment of 6 patients, then treatment on dose level 1 will be stopped, and 3 additional patients will be accrued at dose level -1 to reach a total of 6, if only 3 were previously treated. The maximum tolerated dose is the highest dose at which a maximum of 1 of 6 patients has a DLT.

There will be a minimum of a 7-day delay in treatment between the patients and a minimum of a 14-day delay in treatment between cohorts.

The total number of E6 TCR T cells transferred for each cohort will be:

- **Dose level -1** 0.7×10^{10} transduced T cells
- **Dose level 1** 0.7×10^{11} transduced T cells

The cell dose administered will be in a range of +/- 30% of the target dose above. The frequency of transduced T cells will be determined by flow cytometry with anti-mouse TCR beta-chain antibody. The number of transduced cells to be administered will be determined by multiplying the frequency of transduced T cells by the total number of viable T cells. If fewer than the target number of cells are generated the patient will be treated but toxicity data from the patient will not be used in determining the MTD, and that patient will be replaced in the protocol enrollment.

3.1.7 Safety Protocol Stopping Rules

The study may be halted if any of the following safety conditions are met:

1. If one or more deaths occur, we will immediately stop accrual and promptly discuss this with the NCI IRB and the FDA.
2. If two or more patients develop a Grade 3 or greater toxicity not attributable to the aldesleukin (or circumstances unrelated to the study) that does not resolve to Grade 2 within 24 hours.
3. If two patients develop Grade 3 or greater Immune Related Adverse Event (IRAE) related to aldesleukin that cannot be resolved to less than or equal to a Grade 2 within 10 days.
4. If 2 DLTs occur on dose level 1.

3.2 DRUG ADMINISTRATION

Inpatient treatment will be according to the following schedule. Cell infusions may be slowed or delayed as medically indicated. Administration of diuretics, hydration, and electrolyte monitoring and replacement will be performed as clinically indicated.

Day 0

E6 TCR cells

The E6 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) and an identification of the product and documentation of administration are entered in the patient's chart (as is done for blood banking protocols). E6 TCR T cells will be administered intravenously over 20 to 30 minutes via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping. A peripheral IV may be used if access is adequate (in general, two 18 gauge or larger IVs should be established). A PICC line or other central line may be used if peripheral access is not adequate.

Prophylactic antibiotics

Prophylactic antibiotics will be administered starting the morning of cell infusion and continue until 24 hours after the last dose of aldesleukin per PI discretion. Oxacillin, clindamycin, or other antibiotic with equivalent gram positive coverage may be chosen based on patient's history of allergies and physician discretion.

Day 1

Beginning in the morning of day 1, Aldesleukin will be given 720,000 IU/kg IV q12 hours for up to 2 doses. Infusion will take place over 15 minutes. The second dose of aldesleukin will be given at the treating physician's discretion and will not be given if a grade 3 or higher AE occurs following the first dose.

3.3 STUDY CALENDAR

Procedure	***Screening/ Baseline*** [2]	***Day 0***	***Day 1***	***Day 2***	***Day 3***	***Daily until d/c***	***Follow-up Period***			
							1 wk *(Days 5-8)*	***2 wk*** *(Days 10-14)*	***Monthly f/u*** [8, 9] (Month *1 is also End of Treatment Visit)*	***Annual Followup***
History, physical exam, and review of systems[1]	X	X[1]	X[1]	X[1]	X[1]	X[1]	X	X	X	
Vital signs	X	X	X	X	X	X	X	X	X	
Concomitant medications	X	X	X	X	X	X	X	X	X	
ECOG performance status	X						X	X	X	
Chest x-ray	X									
EKG/Cardiac stress test (as per guidelines in Section 2.2)	X									
Screening labs: HIV, HBV serology, HCV serology, thyroid panel, PT/PTT, Beta-HCG pregnancy test, HLA-A typing for HLA-A*02:01 allele positivity	X									
HPV-16 verification and pathology review for confirmation of diagnosis[3]	X									
Acute care panel, hepatic panel, mineral panel, LD, total protein, CK, uric acid, CBC with differential and TBNK	X						X	X	X	

Procedure	***Screening/ Baseline*** [2]	***Day 0***	***Day 1***	***Day 2***	***Day 3***	***Daily until d/c***	***Follow-up Period***			
							1 wk *(Days 5-8)*	***2 wk*** *(Days 10-14)*	***Monthly f/u*** [8, 9] (Month *1 is also End of Treatment Visit)*	***Annual Followup***
HSIL measurements, vulvar biopsy, and photographs[4]	X							X	X	
Leukapheresis	X							X		
Replication Competent Retrovirus (RCR) testing[5]	X								X[5]	
Research samples: peripheral blood lymphocytes, plasma, and serum[6]	X		X		X	X[6]	X	X	X	
E6 TCR T cells		X								
Aldesleukin			X							
Prophylactic antibiotics[7]		X[7]	X[7]	X[7]						
Response Evaluation									X	
HSIL and patient status update[11]										X
Adverse Events		X	X	X	X	X	X	X	X	

[1] Patients will have a full history and physical at screening, on admission to the hospital, and on follow-up visits. While inpatient, patients will have a daily review of systems and physical exam.

[2] Exact timeline is indicated in Section **2.2**

[3] Vulvar HSIL must be HPV-16+ by PCR, RNA, or in situ hybridization test from a CLIA certified laboratory prior to treatment.

[4] Vulvar biopsies will be performed as a punch biopsy under local anesthetic in the outpatient clinic within 8 weeks prior to starting therapy, at 2-week follow-up (day 10-14), and at 1-month follow-up (day 27-34) for all patients. An additional biopsy will be obtained at the time a patient comes off-treatment (e.g. at time of progression, at time of complete response, or at 6-month visit for patients with continuing partial response). Biopsies will be performed only if technically feasible, if they do not interfere with response assessment, and if the patient provided consent. The investigators may elect not to perform biopsies at their discretion (i.e. safety concerns, patient concerns, etc.). HSIL photographs will occur at each follow up visit.

[5] Patients' blood samples will be obtained and undergo analysis for detection of replication competent retroviruses (RCR) by PCR prior to cell infusion and at approximately 3, 6, and 12 months post cell administration. Blood samples will be archived annually thereafter if all previous testing has been negative with a brief clinical history. The ETIB preclinical core staff will cryopreserve 2 vials of cells if possible. If less than 10 million cells are available, only 1 vial of cells will be cryopreserved.

[6] Research samples will be collected at screening, pre-treatment (within four weeks prior to cell infusion); posttreatment on post-therapy day 1, post-therapy day 3, thereafter any MWF while still an inpatient for one week, and thereafter weekly while still inpatient; and post-treatment at each research visit as described in section **3.5**. For each time point, six 8mL CPT tubes will be drawn (5 for PBMC and 1 for plasma) and one 8mL SST tube (for sera). Processing and storage are as described in section **5**.

[7] Prophylactic antibiotics will be administered starting the morning of cell infusion and continue until 24 hours after the last dose of aldesleukin per PI discretion. If patients are discharged prior to completing antibiotics they will be given a prescription to complete the course at home. Oxacillin, clindamycin, or other antibiotic with equivalent gram positive coverage may be chosen based on patient's history of allergies and physician discretion.

[8] All patients follow- up 1, 2, and 3 months after cell infusion. Patients with no response at the 3-month evaluation will come off-treatment and receive standard of care therapy. Patients with a partial or complete response at the 3-month evaluation will continue follow up at 4, 5, and 6 months for safety, response, and research evaluations. After the 6-month response assessment patients with partial response will receive standard of care therapy and patients with a complete response will continue to have follow-up every 3 months (+/- 30 days) for 2 visits, then every 6 months (+/- 30 days) for 2 visits (2 years of follow up total). Patients with disease progression at any time will come off treatment and receive standard of care therapy either at the NIH, with their primary physician, or another institution. For follow up visits, if unwilling or unable to travel to the NIH Clinical Center, patients will be contacted by telephone regarding their status, and may be asked to send labs and physical exam reports to fulfill follow up visit requirements. Adverse events associated with standard of care therapy will not be recorded or reported on this study.

[9]As indicated in **3.5** (+/- 14 days)

[10]As indicated in section **10.4**, all subjects ≥ age 18 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.

[11]As indicated in section **3.5.2**, subjects will be contacted to collect information annually for five years after the date of cell infusion. This follow up can be conducted via telephone.

3.4 On-Study Evaluations

3.4.1 Prior to starting cell infusion:

Within 14 days prior to therapy, patients will have a complete blood count, serum chemistries performed including electrolytes, BUN, creatinine, pregnancy test and liver function

tests. If any results are beyond the criteria established for eligibility, the patient will not proceed until the abnormalities can be resolved.

- Pre-treatment biopsy within 8 weeks prior to therapy
- Blood samples for analysis for detection of Replication Competent Retrovirus (RCR) by PCR as described in Section **5.3**.

3.4.2 After Cell Infusion:

- Vital signs will be monitored hourly (+/- 15 minutes) for 4 hours and then routinely (every 4-6 hours) unless otherwise clinically indicated for the duration of the hospital stay

3.4.3 During Hospitalization:

Every 1-2 days.

- Daily review of systems and physical exam
- Other tests will be performed as clinically indicated.

3.5 Post Treatment Evaluation (Follow-Up)

Follow-up visits will be done according to the following schedule.

- 1 week safety and research visit (days 5-8)
- 2 week safety and research visit (days 10-14)
- 1 month safety, research, and response visit (+/- 7 days)
- 2 month safety, research, and response visit (+/- 7 days)
- 3 month safety, research, and response visit (+/- 7 days)
- 4 month safety, research, and response visit (+/- 7 days)
- 5 month safety, research, and response visit (+/- 7 days)
- 6 month safety, research, and response visit (+/- 7 days)
- As clinically indicated subsequently.

Note: Patients may be seen more frequently as clinically indicated

3.5.1 Evaluation:

- Patients will follow up 1 and 2 weeks after cell infusion for safety assessment and research studies as indicated on the **Study Calendar**. Response will not be measured at these visits.
- Patients will follow up 1, 2, and 3 months after cell infusion for safety assessment, research studies, and response evaluations.
- Patients with no response at the 3 month evaluation will come off-treatment and receive standard of care therapy.
- Patients with a partial or complete response at the 3-month evaluation will follow up at 4, 5, and 6 months for safety, response, and research evaluations.
- After the 6-month response assessment patients with partial response will receive standard of care therapy. Patients with a complete response will thereafter have follow-up every 3 months (+/- 30 days) for 2 visits, then every 6 months (+/- 30 days) for 2 visits (2 years of follow up total).
- Patients with disease progression at any time will come off treatment and receive standard of care therapy.

- Patients who come off treatment will follow up as required for gene transfer studies or as clinically indicated.

3.5.2 At each Response Evaluation, patients will undergo the following:

- Physical examination
- Chem 20 equivalent: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (Bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LD, Total Protein, Total CK, Uric Acid).
- Complete Blood Count with differential and TBNK
- Toxicity assessment, including a review of systems.
- Serum, plasma, and peripheral blood lymphocytes.
- HSIL measurements and photographs will be taken for documentation of response.
- Optional biopsies for research purposes will be obtained at baseline (within 8 weeks prior to starting treatment); at 2 week (day 10-14) safety and research visit; at 1 month (day 27 to 34) safety, response, and research visit; and at the time the patient comes off-treatment (i.e. attains a complete response, experiences progression, or reaches the 6-month time point without a complete response). Biopsies will be performed only if technically feasible, if they do not interfere with response assessment, and if the patient provides consent. The investigators may elect not to perform biopsies at their discretion (i.e. safety concerns, patient concerns, etc.). Biopsies for clinical concern will be performed as clinically indicated. Biopsies will be processed as per Section **5.1.3**.
- An approximately 5 liter leukapheresis will be performed at the 2 week (day 10-14) 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 each response visit (see **Study Calendar** for sampling details). Peripheral blood mononuclear cells will be cryopreserved for immunologic testing and will be banked under protocol 16-C-0061 (ETIB Tissue Procurement Protocol). PBMC from apheresis will be stored in 10 vials at $1x10^8$ cells per vial with remaining cells stored at $3x10^8$ cells per vial.
- Cervical testing for high-risk HPV if the screening assessment was positive for HPV-16.
- The following information will be collected annually for a total of five years after the date of cell infusion: 1) procedures or treatments for vulvar HSIL, 2) procedures or treatments for cervical, vaginal, or anal intraepithelial neoplasia, 3) occurrence of vulvar, cervical, or anal cancer.

3.5.3 Patients who are unable or unwilling to return for follow up evaluations

They will be followed via phone or e-mail contact. Patients may be asked to send laboratory, imaging and physician exam reports performed by their treating physician.

3.5.4 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, 15-C-0141. 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.

3.6 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 30 days following the last dose of study therapy. Since patients will be completing a one-month follow up visit, the end of treatment visit will be the 30 day followup visit as indicated on the Study Calendar in Section **3.3**

3.6.1 Criteria for removal from protocol therapy

- Completion of protocol therapy.
- Grade 3 or greater autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs).
- Positive pregnancy test
- If a patient experiences grade 3 or 4 toxicity due to cell infusion (reaction to cellular product or infusion reaction), the infusion will be stopped and the patient will receive no further treatment (i.e. aldesleukin).
- Progression of disease
- Participant requests to be withdrawn from active therapy
- Investigator discretion

3.6.2 Off-Study Criteria

- Screen failure
- Completed study follow-up period
- Inability to generate a cell product. A second attempt may be made to generate a cell product from the patient. If the second attempt fails, that patient will be removed from the study and replaced with another patient.
- Participant requests to be withdrawn from study
- There is significant noncompliance
- General or specific changes in the patient's condition render continued participation of the patient unacceptable for further follow up in the judgment of the investigator.
- Death

3.6.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off study. A Participant Status Update Form from the web site **(**http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office ncicentralregistration-l@mail.nih.gov .

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 STEROIDS

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

4.1.1 Blood Product Support

Patients are unlikely to require blood product support as a side effect of therapy. However, any patient on study may receive platelets and packed red blood cells (PRBC's) in clinically needed. As a general guideline, patients may be transfused for:

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

All blood products will be irradiated and leukocyte filters will be utilized to decrease sensitization to transfused WBC's.

4.2 OTHER CONCOMITANT MEDICATIONS/MEASURES

Concomitant medications to control side effects of therapy may be given. Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg q4h), indomethacin (50-75 mg q8h) and ranitidine (150 mg g12h). If patients require steroid therapy they will be taken off treatment. Patients who require transfusions will receive irradiated blood products. Ondansetron will be administered per standard dosing instructions as needed for nausea and vomiting. Additional antiemetics will be administered as needed for nausea and vomiting uncontrolled by ondansetron. Antibiotic coverage will be provided at the discretion of the investigator.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH

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

5.1.1 Pre cell infusion evaluations

- The following samples will be drawn for research and sent to the ETIB preclinical core within 4 weeks prior to cell infusion. Send to Dr. Fran Hakim, Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.
    - 6 CPT tubes (8mL each). One CPT tube per time point will be used to collect 4mL of plasma, which will be frozen in 4mL vials. PBMC from the remainder of the CPT tubes will be frozen in aliquots of 10 x 106 cells/vial
    - 1 SST tube (8mL) for sera. Serum will be processed in the ETIB Pre-Clinical Core and aliquoted into vials of 0.5-1 mL each.
- During screening, one 10 mL tube of blood will be collected from each patient in a Cell-Free DNA BCT tube. Specimens may be drawn at the patient's local doctor's office or laboratory and FedExed to the assigned research nurse. Specimens will be transported by the assigned research nurse to Dr. Christian Hinrichs' lab for sample labeling. Contact Stacey Doran, Bldg 10, room 4B-04. HPV-16 in peripheral blood via ddpCR assay samples will be coded with a patient identification number, and linked to samples by that identification number in LabMatrix. Access to personally identifiable information (PII) is

limited to the PI and study personnel who interact directly with the patient and their samples. Once labeled, samples will be sent to the laboratory of Liang Cao, Genetics Branch, CCR for HPV-16 ddpCR assay testing. See **Appendix E** for collection and processing instructions, which will be sent to the patient.

5.1.2 Post cell infusion evaluations

- The following samples will be drawn for research and sent to the ETIB preclinical core While the patient is inpatient, samples will be drawn on post-treatment day 1, post-treatment day 3, thereafter every Monday/Wednesday/Friday x5 days, and then weekly until discharge. Samples will also be collected at each outpatient follow-up visit. Send to Dr. Fran Hakim, Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.
    - 6 CPT tubes (8mL each). One CPT tube per time point will be used to collect 4mL of plasma, which will be frozen in 4mL vials. PBMC from the remainder of the CPT tubes will be frozen in aliquots of 10 x 10^6 cells/vial
    - 1 SST tube (8mL) for sera. Serum will be processed in the ETIB Pre-Clinical Core and aliquoted into vials of 0.5-1 mL each.

  Aliquots of serum samples will be provided to the laboratory of Liang Cao, Genetics Branch, CCR for testing of plasma or serum for HPV DNA.

5.1.3 Optional vulvar Biopsies

- Biopsies of vulvar lesions for clinical purposes will be obtained within 8 weeks prior to starting treatment if clinically indicated to rule out invasive cancer. Biopsies will be performed in the outpatient clinic under local anesthetic.
- Biopsies for research purposes will be obtained at baseline (within 8 weeks of starting treatment), 10 to 14 days after treatment, 27 to 34 days after treatment, and at the time the patient comes off-treatment (i.e. attains a complete response, experiences progression, or reaches the 6-month time point without a complete response). Clinically indicated biopsies may be obtained at any time by PI discretion if there is concern for invasive cancer.
- Biopsies for research will be performed with a standard punch biopsy instrument of up to 4 mm diameter. At each time point, up to 3 biopsies of HSIL lesions and up to 2 biopsies of adjacent normal tissue will be performed. A formalin-fixed paraffin embedded specimen will be transported to the NCI Laboratory of Pathology for review and archiving. The contact will be Stacey Doran, Bldg 10, Room 4B04, contact 301-491-6957. Specimens will then be transported to the ETIB preclinical core for further processing and storage. They will be delivered to Dr. Fran Hakim, Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C216 contact phone: 301-594-5339.
- Processing of biopsies will depend upon the amount of tissue obtained. Examples of processing may include OTC blocks, formalin-fixed paraffin embedding, snap freezing, placing in RNAlater or other required laboratory processing technique.
- Biopsies will not be performed if they are not technically feasible, if they will interfere with response assessment, or if a patient declines consent.

- Tissue samples will be coded and deidentified prior to transfer to the laboratory of Liang Cao, Genetics Branch, CCR for quantification of HPV DNA.
- Some of these samples will be archived and analyzed under another protocol 16-C-0061 (ETIB Tissue Procurement Protocol) if the subject is also enrolled on that study.

5.1.4 Immunological Testing

- Leukapheresis will be performed prior to treatment and 10-14 days after treatment.
- Processing of leukapheresis will be in the Department of Transfusion Medicine (DTM). Cell product will be frozen in 10 vials at concentration 100 x 10^6 cells/mL and additional vials at 300 x 10^6 cells/mL. Cells will be stored in a -80 degrees Celsius freezer in DTM overnight and transferred to the ETIB Preclinical Core the following day. Samples will be sent to Dr. Fran Hakim, Pre-Clinical Core lab; Attention Jeremy Rose, Bldg 10, room 12C216. Contact phone: 301-594-5339.
- At other time points, peripheral blood lymphocytes (PBL) and plasma will be obtained from whole blood by purification using centrifugation. These samples will be transferred directly to the ETIB Pre-Clinical Core lab for processing. Plasma will be frozen in 4mL vials. PBL will be frozen in aliquots of 10 x 10^6 cells/vial.
- A variety of tests including but not limited to the following will be used to evaluate immunologic correlates: specific lysis and cytokine release, intracellular FACS of cytokine production, ELISPOT assays, and lymphocyte subset analysis.
- Samples of all infused cell products will be cryopreserved, and extensive retrospective analysis of infused cell phenotype and function will be performed to attempt to *find in vitro* characteristics of the infused cells which correlate with in vivo activity.
- Peripheral blood lymphocytes (PBL) may be tested by cytolysis assays, cytokine release, limiting dilution analysis, and other experimental studies. Immunological monitoring will consist of quantifying T cells reactive with tumor cells using established techniques such as intracellular FACS, cytokine release assays, and ELISPOT assays. Immunological assays will be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the T cells cryopreserved at the time of infusion.
- Blood and tissue 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 (ETIB Tissue Procurement Protocol).

5.2 SUMMARY OF SAMPLE COLLECTION

Test/assay	Volume blood (approx)	Type of tube	Collection point (+/- 48hrs)	Location of specimen analysis
Whole Blood	10 mL	Cell-free DNA BCT	Screening	Processed in Christian Hinrichs Lab, then transported to Dr. Liang Cao's lab for analysis
Plasma/PBMC	48 mL	CPT	<4 weeks prior to cell infusion	Pre-Clinical core lab (Dr. Fran Hakim's lab)

Test/assay	Volume blood (approx)	Type of tube	Collection point (+/- 48hrs)	Location of specimen analysis
Serum	8 mL	SST	<4 weeks prior to cell infusion	Pre-Clinical core lab (Dr. Fran Hakim's lab)
Plasma/PBMC	48 mL	CPT	Post cell infusion day 1, 3, every Mon/Wed/Fri x 5 days, weekly until discharge	Pre-Clinical core lab (Dr. Fran Hakim's lab)
Serum	8mL	SST	Post treatment day 1, 3, every Mon/Wed/Fri x 5 days, weekly until discharge	Pre-Clinical core lab (Dr. Fran Hakim's lab)
Optional Vulvar Biopsy	N/A	N/A	< 8 weeks prior to treatment, 10-14 days after treatment, 27-34 days after treatment, and when patient comes off treatment.	NCI Laboratory of Pathology (for review and archiving) Processed in Christian Hinrichs Lab, then transported to Pre-Clinical core lab (Dr. Fran Hakim's lab) Dr. Liang Cao's lab

5.3 GENE-THERAPY-SPECIFIC FOLLOW-UP

- Patients will be enrolled on the Long Term Followup Gene Therapy Study (Protocol 15-C-0141) in order to collect information to meet current FDA requirements. All followup as required by the FDA will occur via the 15-C-0141 protocol.

5.4 SAMPLE STORAGE, TRACKING AND DISPOSITION

5.4.1 Storage/Tracking in the Preclinical Development and Clinical Monitoring Facility (PDCMF)

- Samples will be ordered in CRIS and tracked through the 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 the NIH without IRB notification and an executed MTA.
- Patient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the Experimental Transplantation and Immunology Branch (ETIB), may be archived by the ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF). All data associated with archived clinical research samples is entered into the ETIB PDCMF's Microsoft Excel databases on frozen cells and serum. These databases are stored on the NCI group drive in the ETIB 'PRECLINSERVICE' folder. Access to this folder is limited to PDCMF staff and ETIB clinical staff, requiring individual login and password. All staff in the PDCMF laboratory receive annually updated NIH/CIT training and maintain standards of computer security.

- The data recorded for each sample includes the patient ID, trial name/protocol number, date drawn, treatment cycle/post-transplant time point, cell source (e.g. peripheral blood, marrow,) as well as box and freezer location. Patient demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI/ETIB clinical records. As of January 2007, all newly received samples receive a unique bar code number, which is included in the sample record in the PDCMF database. Only this bar code is recorded on the sample vial and the vials will not be traceable back to patients without authorized access to the PDCMF database. All non-coded samples previously archived will be stripped of identifiers prior to distribution for any use other than as a primary objective of the protocol under which they were collected.

- Samples are stored in freezers. All samples will be labeled solely with a bar code (which includes the date, and serially determined individual sample identifier). The key will be available to a restricted number of ETIB investigators and associate investigators on the protocol. Coded samples will be stored frozen at -20°, -80° or liquid nitrogen vapor phase to -180 C according to the stability requirements in a single location under the restricted control of the PDCM Facility of ETIB.

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

5.4.3 Storage/Track in the Christian Hinrichs lab

Blood, plasma, serum, and tissue samples will be processed, labeled, and stored in the ETIB Pre-Clinical Core. Samples will be requested and transferred to Dr. Hinrichs' lab as needed for analysis. The contact will be Stacey Doran, Bldg 10, Room 4B04, phone 301-491-6957.

Once received, the samples will be coded utilizing LabMatrix. The bar-coded samples will be stored at -80ºC or in liquid nitrogen until time of use. Any unused samples will be returned to the PDCMF laboratory at the completion of all studies associated with the protocol.

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

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

5.4.4 Storage/Tracking in the Liang Cao lab

Serum samples will be processed in the ETIB Pre-Clinical Core and 1 ml aliquots will be transferred to Dr. Cao's lab in batches for circulating tumor DNA analysis. Samples will be sent to Dr. Liang Cao, Molecular Targets Core Lab of Genetics Branch; Attention Dr. Zhigang Kang, Bldg 37, room 6134. Contact phone: 301-443-2817.

Once received, the bar-coded samples will be stored at -80ºC until the time of testing. Serum DNA will be isolated with an automated purification system by Promega Corp using a system-attached bar-code reader to track samples and DNA products. The circulating tumor/HPV DNA will be quantified with a digital droplet PCR system from Bio-Rad to obtain precise quantification.

5.4.4 Protocol Completion/Sample Destruction

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

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

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

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

The date on which screening consent is signed is the same date informed consent is signed.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for at least 30 days after removal from study treatment or until off-study, whichever comes first.

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

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.1.1 Exclusions to Routine Adverse Event Reporting:

Adverse events in patients who go on to standard of care therapy will not be collected or reported for this study.

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__ De-identified data in an NIH-funded or approved public repository.

_x__ De-identified data in BTRIS (automatic for activities in the Clinical Center)

_x__ De-identified 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. Insert name or names: 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

6.3.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of E6 TCR T cell infusion. Toxicity data will be collected for all patients.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received an E6 TCR T cell infusion, and have had their disease re-evaluated for response will be considered evaluable for response. These patients will have their response classified according to the definitions stated below.

6.3.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension as $\geq$ 5 mm. All measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <5 mm), are considered non-measurable disease.

Target lesions. All measurable lesions will be identified as target lesions and recorded and measured at baseline.

Non-target lesions. All other lesions (or sites of disease) will 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 will be noted throughout follow-up.

6.3.3 Methods for Evaluation of Measurable Disease

Lesions will be photographed, described in detail, and measured at each response evaluation. Measurements will be recorded in metric notation using a ruler or calipers. Baseline evaluations will be performed no more than 8 weeks before starting treatment.

The same method of assessment will be used to characterize each identified and reported lesion at baseline and during follow-up. Lesions will be documented by digital color photography at each response evaluation.

6.3.4 Response Criteria

6.3.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. No appearance of new lesions.

Partial Response (PR): A $\geq$ 50% decrease in the sum of the product of the longest perpendicular diameters of target lesions, taking as reference the baseline measurements. No appearance of new lesions. No increase of greater than 25% of any index lesion.

Progressive Disease (PD): A $\geq$ 25% increase in sum of the product of the longest perpendicular diameters of target lesions, taking as reference the smallest product on study (this includes the baseline product if that is the smallest on study). In addition to the relative increase of 25%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Non-CR/Non-PD: Neither sufficient decrease to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest product of diameters while on study.

Note: In the event of lesion regression with a residual abnormality of unclear significance, a biopsy may be obtained. If HSIL is present in the pathology specimen the response will be considered less than complete. If HSIL is not present the response will be considered complete.

6.3.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of non-target lesions.

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions. It must be representative of the overall non-target disease rather than an increase in a single lesion.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s).

6.3.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-CR/Non-PD/not evaluated	No	PR
Non-CR/Non-PD	Non-CR/Non-PD/not evaluated	No	Non-CR/Non-PD

PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD

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

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

6.3.5 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).

Duration of overall CR: 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 overall non-CR/non-PD: Non-CR/non-PD disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.4 TOXICITY CRITERIA

Careful evaluation to ascertain the toxicity, immunologic effects and anti-HSIL 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 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1.1 Adverse Event

An adverse event is defined as any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

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 drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- 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 event 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.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

7.2.1 NCI-IRB and NCI Clinical Director Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

To ensure safety using this treatment, the NCI ETIB will review safety data on all protocols semi-annually at the time of continuing review. Data will be presented for both the recent 6 month period and for the entire length of time the protocol has been open. The toxicity data for review will include all toxicities captured on the protocol and will include a table that describes all incidences of intubation including the duration of and reason for intubation.

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:

- All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
- All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
- All Grade 5 events regardless of attribution;
- All Serious Events regardless of attribution.

NOTE: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.3 IND SPONSOR REPORTING CRITERIA

During the first 30 days after the subject receives the investigational agent, an investigator must immediately report to the sponsor, using the mandatory MedWatch form 3500a or equivalent, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur more than 30 days after the last administration of the investigational agent, only report those that have an attribution of at least possibly related to the agent/intervention.

Required timing for reporting per the above guideline:

- Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

7.3.1 Reporting Pregnancy/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. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents (s) should be documented in box B5 of the MedWatch form "Describe Event or Problem".

Pregnancy itself is not regarded as an AE unless there is a suspicion that the study treatment under study may have interfered with the effectiveness of a contraceptive medication. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as **Grade 3** "***Pregnancy, puerperium and perinatal conditions - Other (pregnancy)***" under the ***Pregnancy, puerperium and perinatal conditions*** SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The

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

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within 1 day, i.e., immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of E6TCR 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 E6 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.

7.4.2 Annual Reports to IBC

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

7.4.2.1 Clinical Trial Information

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

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

- if the trial has been completed, a brief description of any study results.

7.4.2.2 Progress Report and Data Analysis

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

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

7.5 DATA AND SAFETY MONITORING PLAN

7.5.1 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. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS.

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

7.5.2 Sponsor Monitoring Plan

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

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

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

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

7.5.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-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 STATISTICAL CONSIDERATIONS

The primary objective of this phase I trial is to determine the safe dose level of E6 TCR T cells for the treatment of vulvar HSIL. Secondary objectives include an assessment of the clinical responses in subjects receiving this treatment and exploratory analyses of translational research endpoints.

This is a phase I trial with a standard 3+3 design and two dose levels. The maximum number of patients required to determine the MTD on this trial is 12. Six additional patients will be evaluated at the MTD, for a maximum intended enrollment of 18 patients. To allow for patients who enroll but are not evaluable for the primary endpoint and screen failures, up to 200 patients total may be enrolled.

The expected accrual rate is 10-15 patients per year. It should therefore take 1.25-2 years to complete accrual of patients.

The clinical responses in 12 patients tested at the MTD may be used in the design of a subsequent phase II trial.

9 COLLABORATIVE AGREEMENTS

9.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

A CRADA (# 03022) between NCI and Kite Pharma is in place. Kite Pharma will be providing funding for this study.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

The patients to be entered in this protocol have vulvar HSIL that is unable to be completely resected to negative margins without causing deformity. As only women develop vulvar HSIL, men will be excluded from the study.

Subjects from all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. If differences in outcome that correlate to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

10.2 Strategies/Procedures for Recruitment

Patients for this protocol will be recruited via standard CCR mechanisms 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.

10.3 Participation of Children

The efficacy of this treatment is unknown, and no safety data in children exist; children are therefore excluded from participation. In addition, vulvar HSIL is exceedingly rare in children so a small number of children, if any, would be eligible to participate.

10.4 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 **10.6**), 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) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 and NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.5 Evaluation of Benefits and Risks/Discomforts

The experimental treatment has well-defined risks based on experience with the administration of E6 TCR T cells and aldesleukin. The benefit of the treatment is unknown. The alternative to treatment on the protocol is surgical resection, which carries risks of disfiguration, pain, anesthesia complications, wound infections, functional impairment, and disease recurrence.

The additional risks associated with potential study procedures have been outlined and described in detail in the consent form.

10.6 Risks/Benefits Analysis (including who are or may become unable to consent)

All patients in this protocol have vulvar HSIL, and therefore an increased risk of vulvar cancer. In addition, their standard treatment option is surgery with unacceptable cosmetic or functional results and a high risk of disease recurrence. The protocol has defined risks based on prior studies with E6 TCR T cells and extensive clinical testing of aldesleukin. In this study, aldesleukin is administered after cell infusion to promote growth and survival of the infused cells. Although aldesleukin can cause serious adverse events, it's use in this protocol is essential. The success of this effort cannot be predicted now. Because all patients in this protocol have vulvar HSIL and therefore an increased risk of vulvar cancer the potential benefit is thought to

outweigh the potential risks. Although, this protocol involves greater than minimal risk, it presents the potential for direct benefit to individual subjects.

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10.7 CONSENT PROCESS AND DOCUMENTATION

The patient, along with family members or friends, will be presented with a detailed description of the protocol treatment. The specific requirements, objectives, and potential advantages and disadvantages will be presented. The Informed Consent document is given to the patient who is requested to review it and to ask questions prior to agreeing to participate in the treatment portion of this protocol. The patient will be reassured that participation on trial is entirely voluntary and that she can withdraw or decide against treatment at any time without adverse consequences. The research nurse, Principal Investigator or his designee is responsible for obtaining written informed consent from the patient.

If new safety information results in significant changes in the risk/ benefit assessment, the consent form will be reviewed and updated as necessary. All subjects (including those already being treated) will be informed of the new information, be given a copy of the revised form, and asked to give their consent to continue in the study.

For optional biopsies, patients will be required to sign a separate consent for all biopsies conducted during the study. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and the research record.

10.7.1 Telephone consent and re-consent

Consent for screening and re-consent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the screening requirements or change (s) in the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent.

The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone.

A fully executed copy will be returned via mail for the subject's records. The informed consent process will be documented on a progress note by the consenting investigator.

10.7.2 Enrollment of non-English Speaking Subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation. Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

11 PHARMACEUTICAL INFORMATION

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

11.1.1 How Supplied:

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

11.1.2 Formulation/Reconstitution:

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

11.1.3 Storage:

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

11.1.4 Dilution/Stability:

Reconstituted aldesleukin should be further diluted with 50 mL of 5% Human Serum Albumin (HSA). The human serum albumin should be in the final container (bottle or bag) prior to the addition of aldesleukin. 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.

11.1.5 Administration:

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

11.1.6 Toxicities:

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

11.2 CELL PREPARATION (E6 TCR TRANSDUCED PBL) (IND # 16078)

The procedure for the expanding the human PBL and the Certificate of Analysis (CoA) are similar to those approved by the Food and Drug Administration, and used at the NCI in ongoing protocols evaluating cell therapy in the Surgery Branch. The CoA is included **Appendix D**. The PBL will be transduced with retroviral supernatant containing the E6 TCR.

11.2.1 Retroviral Vector Containing the E6 TCR gene

The retroviral vector supernatant (PG13-MSGV1-E6-TCR) encoding a T cell receptor directed against HPV16 $E6_{29-36}$) was prepared and preserved following a cGMP conditions in the Surgery Branch Vector Production Facility (SBVPF).

The retroviral vector E6 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 E6 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 the NIH Clinical Center Department of Transfusion Medicine. These storage facilities are equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in *ex vivo* transduction of patient PBL. There will be no re-use of the same unit of supernatant for different patients. Retroviral titer has been shown to be stable after immediate thawing and immediate administration (coating the tissue culture wells previously coated with Retronectin). Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2). The specific guidelines for Biosafety Level-2 (BSL-2) can be viewed at http://bmbl.od.nih.gov/sect3bsl2.htm

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13 APPENDICES

13.1 APPENDIX A -PERFORMANCE STATUS CRITERIA

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

* 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.

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

Body System	% Patients
Body as a Whole	
Chills	52
Fever	29
Malaise	27
Asthenia	23
Infection	13
Pain	12
Abdominal pain	11
Abdomen enlarged	10
Cardiovascular	
Hypotension	71
Tachycardia	23
Vasodilation	13
Supraventricular tachycardia	12
Cardiovascular disorder[a]	11
Arrhythmia	10
Digestive	
Diarrhea	67
Vomiting	50
Nausea	35
Stomatitis	22
Anorexia	20
Nausea and vomiting	19
Hemic and Lymphatic	
Thrombocytopenia	37
Anemia	29
Leukopenia	16
Metabolic and Nutritional Disorders	
Bilirubinemia	40
Creatinine increase	33
Peripheral edema	28
SGOT increase	23
Weight gain	16
Edema	15
Acidosis	12
Hypomagnesemia	12
Hypocalcemia	11
Alkaline phosphatase incr	10
Nervous	
Confusion	34
Somnolence	22
Anxiety	12
Dizziness	11
Respiratory	
Dyspnea	43
Lung disorder[b]	24
Respiratory disorder[c]	11
Cough increase	11
Rhinitis	10
Skin and Appendages	
Rash	42
Pruritus	24
Exfoliative dermatitis	18
Urogenital	
Oliguria	63

a Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.
b Lung disorder: physical findings associated with pulmonary congestion, rales, rhonchi.
c Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.
[1]Source: Proleukin® Prescribing Information – June 2007

13.3 APPENDIX C: EXPECTED ALDESLEUKIN TOXICITIES AND THEIR MANAGEMENT

Toxicity	Grade	Supportive Medications	Considered DLT*
Chills	3	IV Meperidine 25-50mg IV q1hr, prn	No
Fever	3	Acetaminophen 650mg po q4hr; Indomethicin 50-75mg po q8h	No
Pruritus	3	Hydroxyzine HCl 10-20mg po q6h, prn; Diphenhydramine HCl 25-50mg po q4h prn	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
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 48h despite all supportive measures
Malaise	3 or 4	Bedrest	If other toxicities occur simultaneously
Hyperbilirubinemia	3 or 4	Observation	Yes
Anemia	3 or 4	Transfusion with PRBCs	Yes
Thrombocytopenia	3 or 4	Transfusion with platelets	Yes
Edema/Weight gain	3	Diuretics prn	No
Hypotension	3	Fluid resuscitation, Vasopressor support	No, unless requires more than 1.5L/24 hours of bolus IV fluids or vasopressor support
Dyspnea	3 or 4	Oxygen or ventilator support	If requires more than nasal cannula oxygen support or requires oxygen for greater than 48 hours after last dose

Toxicity	Grade	Supportive Medications	Considered DLT*
Oliguria	3 or 4	Fluid boluses	If requires more than 1.5L/24 hours of bolus IV fluids
Increased Creatinine	3 or 4	Observation	If grade 3 lasts more than 48 hours or for any grade 4.
Renal Failure	3 or 4	Dialysis/CVVH	Yes
Pleural Effusion	3	Thoracentesis	Yes
Bowel Perforation	3	Surgical intervention	Yes
Confusion	3	Observation	Yes
Somnolence	3 or 4	Intubation for airway protection	Yes
Arrhythmia	3	Correction of fluid and electrolyte imbalances; chemical conversion or electrical conversion therapy	Yes
Elevated Troponin Levels	3 or 4	Observation	Yes
Myocardial Infarction	4	Supportive care	Yes
Elevated Transaminases	3 or 4	Observation	Yes
Hyperbilirubinemia	3 or 4	Observation	Yes
Electrolyte Imbalances	3 or 4	Electrolyte replacement	If uncontrolled after 48 hours despite all supportive measures
Neutropenia	4	Observation	Yes

*Unless the toxicity is not reversed within 24 hours of last aldesleukin, in which case all AEs are considered DLTs.

13.4 APPENDIX D: CERTIFICATE OF ANALYSIS HPV-16 E6 TCR

Date of preparation of final product:

Patient:

Tests performed on final product:

Test	*Method*	*Limits*	*Results*	*Initials/Date*
Cell viability[1]	Trypan blue exclusion	>70%		
Total viable cell number[1]	Visual microscopic count	>1x10^9		
Tumor reactivity[3]	Gamma-IFN release vs peptide pulsed T2 cells	>200 pg/mL and > 2 times background		
TCR expression[2]	FACS analysis of the transduced cells	PBL, >10%		
Microbiological studies	Gram stain[1,3]	No micro-organisms seen		
	Aerobic culture[3,4]	No growth		
	Fungal culture[3,4]	No growth		
	Anaerobic culture[3,4]	No growth		
	Mycoplasma test[5]	No growth		
Endotoxin	Limulus assay[1]	<5 E.U./kg		
RCR	S+L- Assay[4] RCR-PCR[6]	Negative		

[1]Performed on sample of the final product immediately prior to infusion. Results are available at the time of infusion.

[2]Performed 2 to 10 days post-transduction. Results are available at the time of infusion.

[3]Performed 2 to 4 days prior to infusion. Results are available at the time of infusion but may not be definitive.

[4]Sample collected from the final product prior to infusion. Results will not be available before cells are infused into the patient.

[5]Performed 2 to 10 days prior to infusion. Results are available at the time of infusion.

[6]Performed on sample approximately 1 to 4 days prior to infusion. Results are available at the time of infusion.

Prepared by: _____ Date:_____

QC sign-off: _____ Date: _____

Qualified Clinical or Laboratory Supervisor

13.5 APPENDIX E: HPV-16 DDPCR PERIPHERAL BLOOD PROCESSING INSTRUCTIONS TO PATIENT AND PHYSICIAN

PATIENT INSTRUCTIONS:

Please take this instruction sheet, along with the sample tube that was sent to you, to your physician or the laboratory that will be drawing these labs.

PHYSICIAN/LABORATORY INSTRUCTIONS:

1. Draw peripheral blood into one 10 mL Cell-Free DNA BCT tube (provided).
2. After blood draw, immediately mix the tube by gentle inversion 8 to 10 times.
3. Do not vortex tubes, as cellular lysis could occur.
4. Keep the tubes at 6° C to 37°C. Do NOT refrigerate or freeze.
5. Ship the tube via FedEx at 6° C to 37°C to ensure the arrival of tubes within 5 days of date of blood draw.
6. Once shipped, notify Erin Ferraro at erin.ferraro@nih.gov

Address for Shipping: Erin Ferraro, RN
National Institutes of Health
10 Center Drive
Building 10-CRC Room 3-3330
Bethesda, MD 20814