

Abbreviated Title: Immunotherapy for vulvar HSIL
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Title: A Phase II Study of Immunotherapy with E7 T Cell Receptor T Cells for Vulvar High-Grade Squamous Intraepithelial Lesions

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Investigational Agents:

Drug Name:	E7 TCR
IND Number:	16959
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Manufacturer:	CC DTM

Commerical Agents: None

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 a HPV-16 E7-specific T cell receptor (E7 TCR T cells).

Objective:

- Determine the complete response rate for E7 TCR T cells in 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.
- Eastern Oncology Cooperative Group Performance Score of 0 or 1.

Design:

- This is a phase II clinical trial
- Simon minimax two-stage design with initial enrollment of 12 patients and expansion to 16 patients if one or more complete response(s) is/are observed in the initial patients.
- Subjects will receive 1×10^{11} E7 TCR T cells
- No conditioning regimen or aldesleukin will be given
- Re-enrollment will be allowed for a small number of subjects.

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1. INTRODUCTION

1.1 STUDY OBJECTIVES

Objectives:

1.1.1 Primary Objective

- To determine the complete response rate for E7 TCR T cells in the treatment of patients with vulvar HSIL.

1.1.2 Secondary Objectives

- Characterize the safety profile of E7 TCR T cells administered in a low intensity setting without conditioning or systemic aldesleukin.

1.1.3 Exploratory Objectives

- To evaluate a novel assay to genotype HPV using a ddPCR-based blood test.
- Test for clearance of HPV infection from vulvar tissues.
- Evaluate the expansion, survival, trafficking, phenotype, and function of E7 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 E7 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). HPV-18 causes only 6% of cases of high-grade vulvar HSIL (3, 4). Spontaneous

regression of vulvar HSIL is rare, reportedly occurring in fewer than 1.5% of patients per year (5).

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 (6). 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 (7). Thus, surgery may be an unsatisfactory treatment option as it often fails to prevent disease recurrence, results in morbidity, and negatively impacts psychological and sexual health (8).

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 (6). 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 (9).

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 limits its use and it is not currently FDA-approved (10). 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(11, 12). Recently, a DNA-based vaccine against HPV-16 E6, E7 and HPV-18 E6,E7 showed an improvement in histopathological regression in cervical intraepithelial neoplasia compared to placebo (49% vs 30%)(13, 14). These results led to a phase II clinical trial comparing the DNA-based vaccine alone or in combination with imiquimod for patients with vulvar HSIL. This trial is actively accruing patients (NCT03180684). 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(15).

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

In a clinical trial of HPV-16 E7 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 E7 TCR T cells and aldesleukin (protocol 16-C-0154). A total of 12 patients were treated. Four patients experienced confirmed partial responses. Two patients had unconfirmed partial responses. One dose-limiting toxicity occurred in the 12 patients on this protocol. The patient had impaired lung function from rapidly progressing cancer in the lungs and experienced severe lung, cardiovascular, and kidney toxicity that required temporary mechanical ventilation, pressors, and hemodialysis, that resulted in soft tissue injury to the distal lower extremities. No other patients experienced dose-limiting toxicities. No off-target toxicity or healthy tissue targeting was observed. The maximum administered dose was deemed the maximum tolerated dose (100 billion E7 TCR T cells). 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 ([12](#), [16](#)). Adoptive T cell therapy has not been reported for the treatment of premalignant HPV-related diseases.

Tumors appear to acquire mutations that confer resistance to T cell immunity as they progress ([13](#), [17](#), [18](#)). This may make targeting of pre-malignant conditions more successful than in the setting of advanced disease.

The amount of E7 TCR T cell engraftment required to treat this condition is not known. Furthermore, the high cell dose (100 billion cells) will increase the chance of engraftment in the absence of conditioning chemotherapy compared to lower cell doses used in our previous protocol with E6 TCR T cells (7 to 70 billion cells).

1.2.4 E7 TCR discovery and characterization

E7₁₁₋₁₉ is a naturally processed epitope of HPV-16 E7 that binds to HLA-A*02:01 and that has been isolated from the surface of HPV-16+ HLA-A*02:01+ tumor cells ([19](#)). We identified the nucleotide sequence of a TCR targeting E7₁₁₋₁₉ from the cervix-infiltrating T cells of a patient with cervical intraepithelial neoplasia who received a therapeutic cancer vaccine targeting HPV-16 E7. The nucleotide sequence was codon optimized for expression in human tissues and the TCR constant regions were swapped for their mouse counterparts, which in other receptors has improved TCR alpha/beta chain pairing. TCR expression was improved by reversing the order of the alpha and beta genes, and by making cysteine substitutions in the TCR constant regions and hydrophobic substitutions in the transmembrane region of the alpha chain constant region. ([20](#), [21](#)) The TCR sequence insert was cloned into the MSGV1 retroviral vector (**Figure 1**), which was chosen for this clinical trial based on its excellent safety record in treating greater than 200 patients.

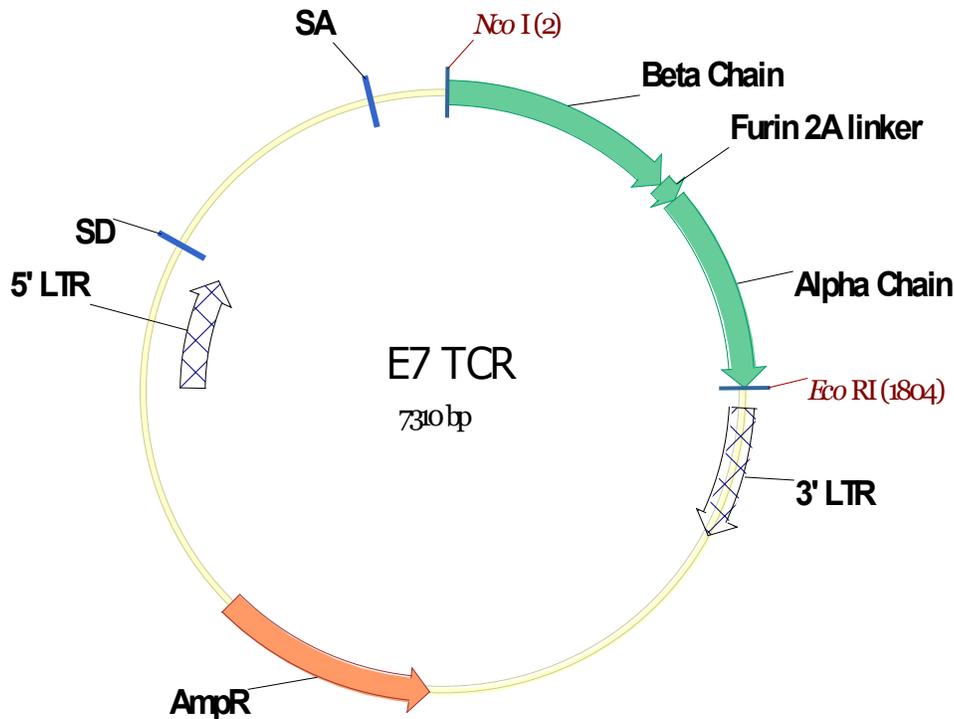


Figure 1: E7 TCR vector map. A TCR targeting E7₁₁₋₁₉ was isolated from the cervix-infiltrating lymphocytes of a patient with cervical intraepithelial neoplasia. The nucleotide sequence of the alpha and beta chains was determined, codon optimized for expression in human tissues, and the constant regions swapped for their mouse counterparts. A MSGV1 retroviral vector encoding this TCR was constructed. This retroviral vector consists of 7,310 base pairs and includes a 5'LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites (SA). Alpha and beta chains of the E7 TCR are linked by a furin 2A peptide.

Peripheral blood T cells transduced to express the E7 TCR display high avidity for the E7₁₁₋₁₉ peptide (Figure 2) and CD8-independent HLA-A*02:01/E7₁₁₋₁₉ tetramer binding (Figure 3). They specifically recognize a panel of HPV-16+ HLA-A*02:01+ cervical and oropharyngeal cancer cell lines but not cell lines that lack HLA-A*02:01 or HPV-16 (Figure 4). Thus, gene engineered T cells expressing the E7 TCR can specifically target HPV-16+ HLA-A*02:01+ cancers. In contrast to TCRs that have had unexpected cross-reactivity against normal human proteins, this TCR was isolated directly from a human T cell (2). Hence, it was subjected to thymic selection and is unlikely to possess avid reactivity against self-antigens. The complementarity determining regions of the TCR have not been modified; therefore, there is no chance that cross-reactivity has been artificially introduced. The target epitope is derived from a viral protein, and no more than 6 of its 9 amino acids are shared with any human protein (Table 1). There is no cross reactivity of this TCR with epitopes of human proteins that share six amino acids or five amino acids plus a conservative amino acid substitution (Figure 5). In addition, alanine scanning of E7₁₁₋₁₉ identified four important residues for recognition (

Figure 6). Cross reactivity was not detected against epitopes of human proteins that shared these residues (**Table 1**).

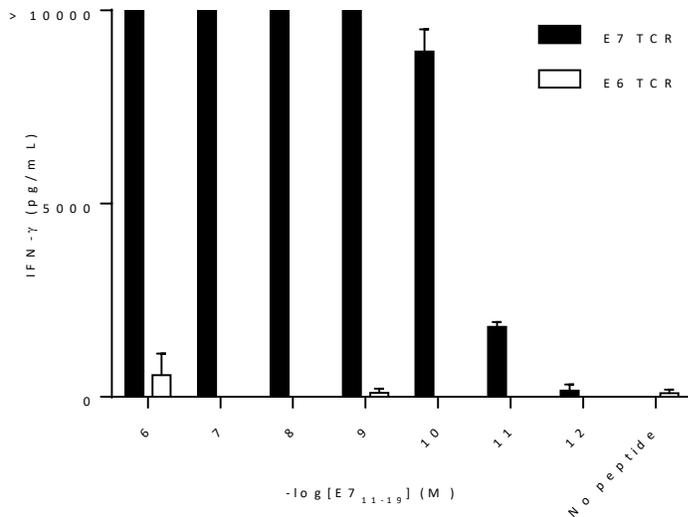


Figure 2: T cells transduced to express the E7 TCR demonstrated high avidity for the E7₁₁₋₁₉ peptide. T cells from PBMC were transduced to express the E7 TCR. Functional avidity was tested by coculture with T2 cells pulsed with titrated concentrations of E7₁₁₋₁₉ peptide.

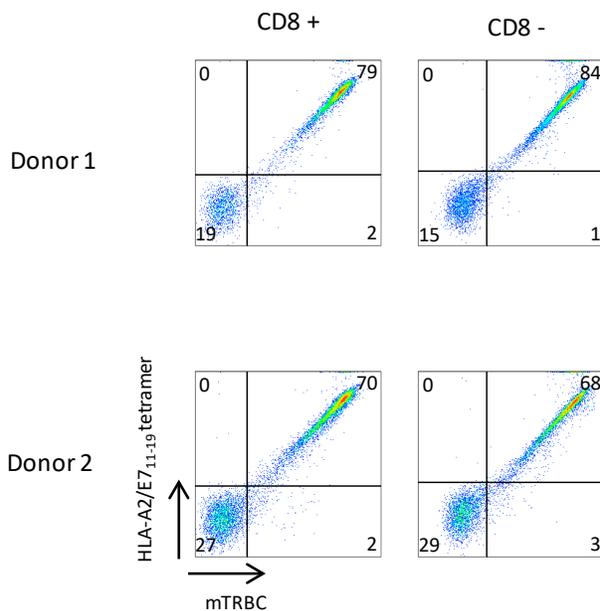


Figure 3: Peripheral blood T cells transduced to express the E7 TCR display CD8-independent HLA-A*02:01/E7₁₁₋₁₉ tetramer binding. T cells from PBMC were transduced to express the E7 TCR. Dot plots shown are gated on PI- lymphocytes and either CD8+ or CD8- cells as indicated above the dot plots. The x-axis is mouse T cell receptor beta chain expression. The y-axis is tetramer binding.

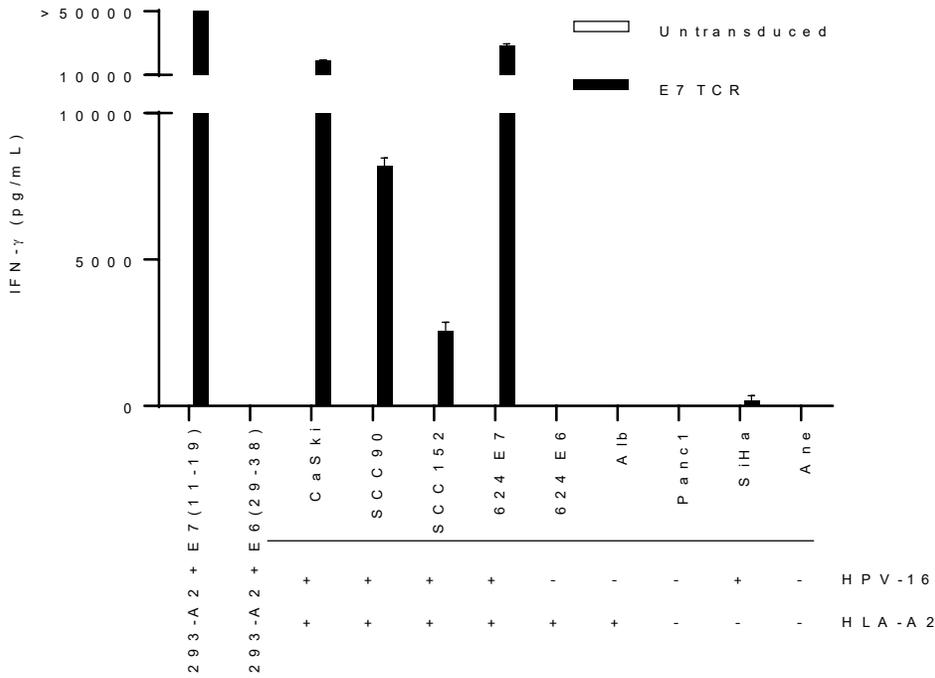
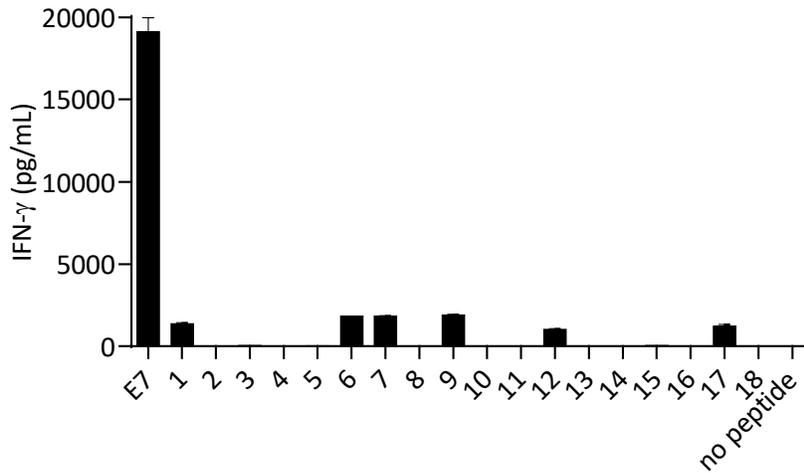


Figure 4: T cells transduced to express the E7 TCR specifically recognized HPV-16+ HLA-A*02:01+ tumor lines T cells transduced with E7 TCR were cocultured with targets expressing HPV-16 and HLA-A2 or with negative controls. Target cell line expression of HPV-16 and HLA-A2 is indicated below each label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



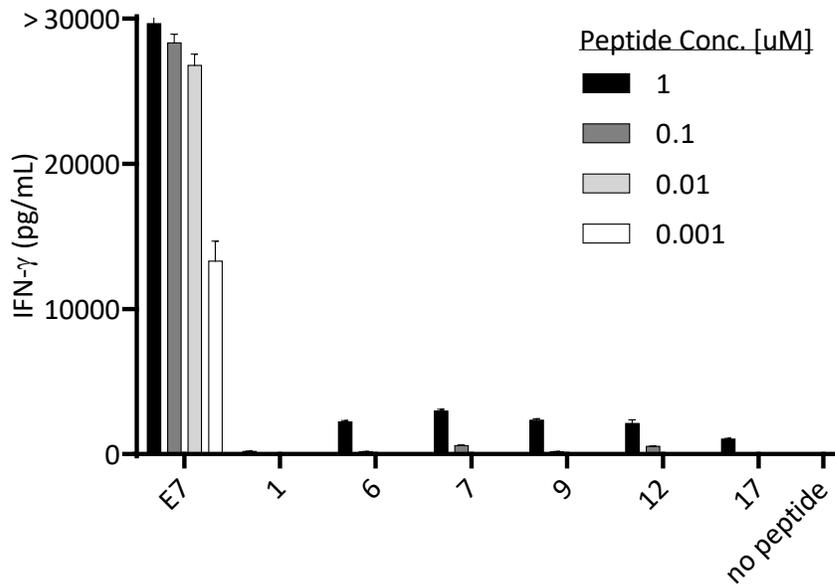


Figure 5: E7 TCR transduced T cells did not show cross reactivity against human peptides. E7 TCR transduced human T cells were tested for recognition of peptides identified by the BLAST search shown in [Table 1](#). Target cells were T2 cells loaded with either the E7 peptide (E7) as a positive control,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

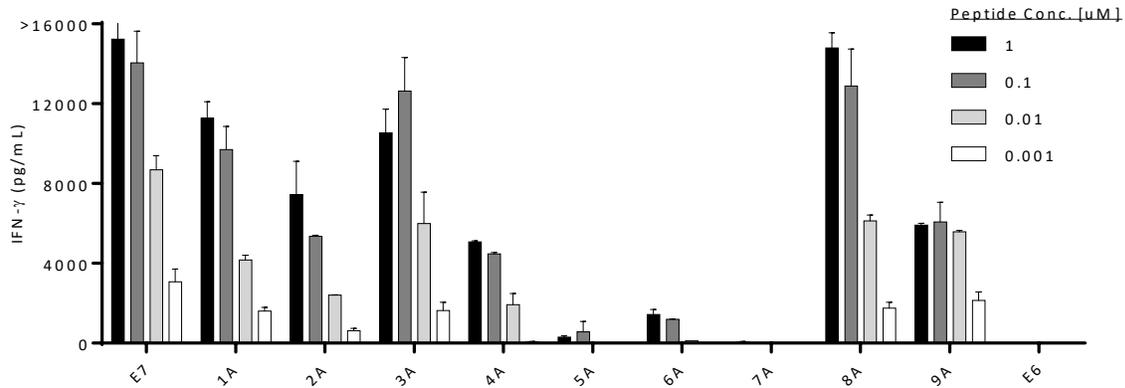


Figure 6: Serial alanine substitutions to the E7₁₁₋₁₉ target peptide revealed positions 4-7 to be the most crucial for recognition by E7 TCR transduced T cells. Human T cells were transduced to express the E7 TCR. The transduced cells were cocultured with T2 cells loaded with varying concentrations of E7₁₁₋₁₉ peptide (E7) or E7₁₁₋₁₉ with an alanine substitution at the position indicated by the x-axis labels. E7 peptide (E7) is an HLA-A2 restricted negative control peptide.

Peptide No.	Protein	Sequence
	E7 (11-19)	YMLDLQPET
1*	endophilin-B1 isoform 4 [Homo sapiens]	YMLDLQkq1
2	uncharacterized serine/threonine-protein kinase SBK3 [Homo sapiens]	gLLDLdPET
3	zinc finger protein 236 [Homo sapiens]	aMLDLEPQh
4	zinc finger protein GLIS1 [Homo sapiens]	sgLgLQPET
5	tensin-1 [Homo sapiens]	lMLDLEPas
6*	clathrin coat assembly protein AP180 isoform c [Homo sapiens]	dLLDLQPDF
7*	translational activator GCN1 [Homo sapiens]	mgLDLQPDl
8	phosphatidate phosphatase LPIN3 isoform X2 [Homo sapiens]	agaDLQPDT
9*	GH3 domain-containing protein isoform 3 precursor [Homo sapiens]	lgLNLQPEq
10	GH3 domain-containing protein isoform 1 precursor [Homo sapiens]	elLNLQPEq
11	protocadherin alpha-9 isoform 2 precursor [Homo sapiens]	lsyELQPET
12*	integrin alpha-IIb preproprotein [Homo sapiens]	YiLDIQPQg
13	tripartite motif-containing protein 66 [Homo sapiens]	pvsDMQPET
14	neural cell adhesion molecule L1 isoform 3 precursor [Homo sapiens]	tqwDLQPDT
15	receptor-type tyrosine-protein phosphatase S isoform X8 [Homo sapiens]	vitNLQPET
16	collagen alpha-1(XII) chain long isoform precursor [Homo sapiens]	meiNLQPET
17*	sacsin isoform 2 [Homo sapiens]	nrLDLQPDl
18	protein AHNAK2 [Homo sapiens]	isgDLQPDT

Peptide No.	Protein	Sequence
	E7 (11-19)	<u>YMLDLQPET</u>
19	Hermansky-Pudlak syndrome 1 protein isoform X8 [Homo sapiens]	pAv <u>DLQP</u> pA
20	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase epsilon-1 isoform 2 [Homo sapiens]	eLi <u>DLQP</u> lI
21	dystrophin isoform X9 [Homo sapiens]	rLs <u>DLQP</u> QI
22	junctophilin-4 [Homo sapiens]	iAq <u>DLQP</u> mL
23	Werner syndrome ATP-dependent helicase [Homo sapiens]	iLq <u>DLQP</u> fL
24	fibronectin isoform 6 preproprotein [Homo sapiens]	tLs <u>DLQP</u> gV

Table 1 BLAST search for peptides with at least 6 (or least 5 identical + 1 conservative change) amino acids shared with E7₁₁₋₁₉. Capital/Underlined = Amino Acid Identical to E7 epitope, Capital/Not Underlined = Conservative change. *=Peptides that demonstrates weak cross-reactivity only at supraphysiological concentrations.

1.2.5 Safety Considerations

HPV-16 E7 TCR

The safety of infusion of large numbers of retrovirally modified tumor reactive T-cells has been demonstrated in clinical studies conducted at the NIH Clinical Center. (22, 23) Patients at the NIH Clinical Center have been treated with up to 1.77×10^{11} gene engineered cells. The clinical safety of E7 TCR T cells has been studied in a protocol for the treatment of metastatic HPV-16 cancer (16-C-0154). 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 one dose-limiting toxicity for E7 TCR T cell infusion was encountered at the highest dose level of cells. The one patient who experienced a dose-limiting toxicity developed rapid progression of metastatic disease in her lungs during conditioning chemotherapy. The patient went on to develop grade 4 hypoxia following infusion of E7 T cells. No other patients at that dose level experienced a dose-limiting toxicity. The E7 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 (1×10^{11} transduced cells).

In the present protocol, the safety margin of treatment has been increased by the following measures: 1) No conditioning chemotherapy will be given. 2) No aldesleukin will be given.

Other protocols at the NIH Clinical Center have administered over 1×10^{11} tumor infiltrating lymphocytes (TIL) with widely heterogeneous reactivity including CD4, CD8, and NK cells without infusional toxicities. Experience at the NIH Clinical Center treating more than 200 patients with advanced cancers with genetically engineered T cells indicates that these cells do not have a significant risk of malignant transformation in this setting. While the risk of insertional mutagenesis is a known possibility using retroviral vectors, this has only been observed in the setting of infants treated for XSCID, WAS and X-CGD using retroviral vector-mediated gene transfer into CD34+ bone marrow cells. It has also been reported with lentiviral transduction in a patient who received CD19 CAR-T cells (it did not cause malignant transformation and may have enhanced the efficacy of the T cells(24). In the case of retroviral vector-mediated gene transfer into mature T cells there has been no evidence of long-term toxicities including leukemia since the first NCI sponsored gene transfer study in 1989. Although continued follow-up of all gene therapy patients will be required, data suggest that administration of retrovirally transduced mature T cells is a safe procedure. While the risk of insertional mutagenesis is extremely low, the proposed

protocol follows all current FDA guidelines regarding testing and follow up of patients receiving gene transduced cells.

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 **6.3.1** and one or more of the following criteria:
 - a. Failure of surgery to control disease (i.e. positive margins after surgery or recurrence of HSIL after surgery).
 - b. Multifocal or extensive disease for which surgery would result in disfigurement 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. Patients with recurrent disease must have histologically documented recurrence on new biopsy and a measurable lesion that meets the above criteria.
- 2.1.1.5 Patients must have the HLA-A*02:01 allele
- 2.1.1.6 Age \geq 18 years.
- 2.1.1.7 ECOG performance status of 0-1 as defined by **Appendix A**.
- 2.1.1.8 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 E7 TCR 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 Protocol20C0051), 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 $\geq 3,000/\text{mcL}$
 - absolute neutrophil count $\geq 1,000/\text{mcL}$
 - platelets $\geq 100,000/\text{mcL}$
 - hemoglobin $\geq 9.0 \text{ g/dL}$
 - total bilirubin within 1.5X normal institutional limits except in patients with Gilbert's Syndrome who must have a total bilirubin $< 3.0 \text{ mg/dL}$
 - AST(SGOT)/ALT(SGPT) Serum ALT/AST $< 3X \text{ ULN}$
 - creatinine $< 1.5X \text{ baseline, } < 1.5X \text{ ULN}$
- OR
- creatinine clearance $\geq 60 \text{ mL/min/1.73 m}^2$ 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 E7 TCR, breastfeeding should be discontinued if the mother is treated with E7 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 Cardiac stress test and pulmonary function tests maybe required for patients with a clinical history of significant cardiopulmonary disease. Patients with active cardiac ischemia or severe chronic obstructive pulmonary disease are not eligible.
- 2.1.2.7 Patients with any active invasive cancer are not eligible.
- 2.1.2.8 Patients with vulvar HSIL that is not HPV-16+ or is associated with multiple types of high-risk HPV at the time of eligibility assessment are not eligible.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

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

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

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

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

2.2.2.1 Any time prior to starting the therapeutic regimen:

- a. HLA-Testing
- 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 in the NCI Laboratory of Pathology.
- d. Cervical high-risk HPV testing
- e. Venous assessment (as per apheresis clinic policy)

2.2.2.2 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 and/or pulmonary function tests may be needed for patients with significant cardiopulmonary disease
- e. HIV antibody titer, HbsAG determination, and anti-HCV antibody. (Note: may be performed within 3 months prior to therapy start date).
- f. Past medical history including family history, social history, allergies, and current medications.

2.2.2.3 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, LDH, Total Protein, Total CK, Uric Acid).
- b. TSH
- c. CBC with differential and TBNK
- d. PT/PTT
- e. Beta-HCG pregnancy test (serum or urine) on all women of child-bearing potential
- f. ECOG performance status assessment

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

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

2.3.1 Treatment assignment and randomization procedures:

Cohorts

Number	Name	Description
1	Patients	Patients with vulvar HSIL

Arms

Number	Name	Description
1	Arm 1	1×10^{11} E7 TCR T cells will be administered intravenously over 20 to 30 minutes on day 0.

Arm Assignment

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

2.4 BASELINE PROCEDURES

2.4.1 Within 14 days prior to starting therapy (unless otherwise indicated):

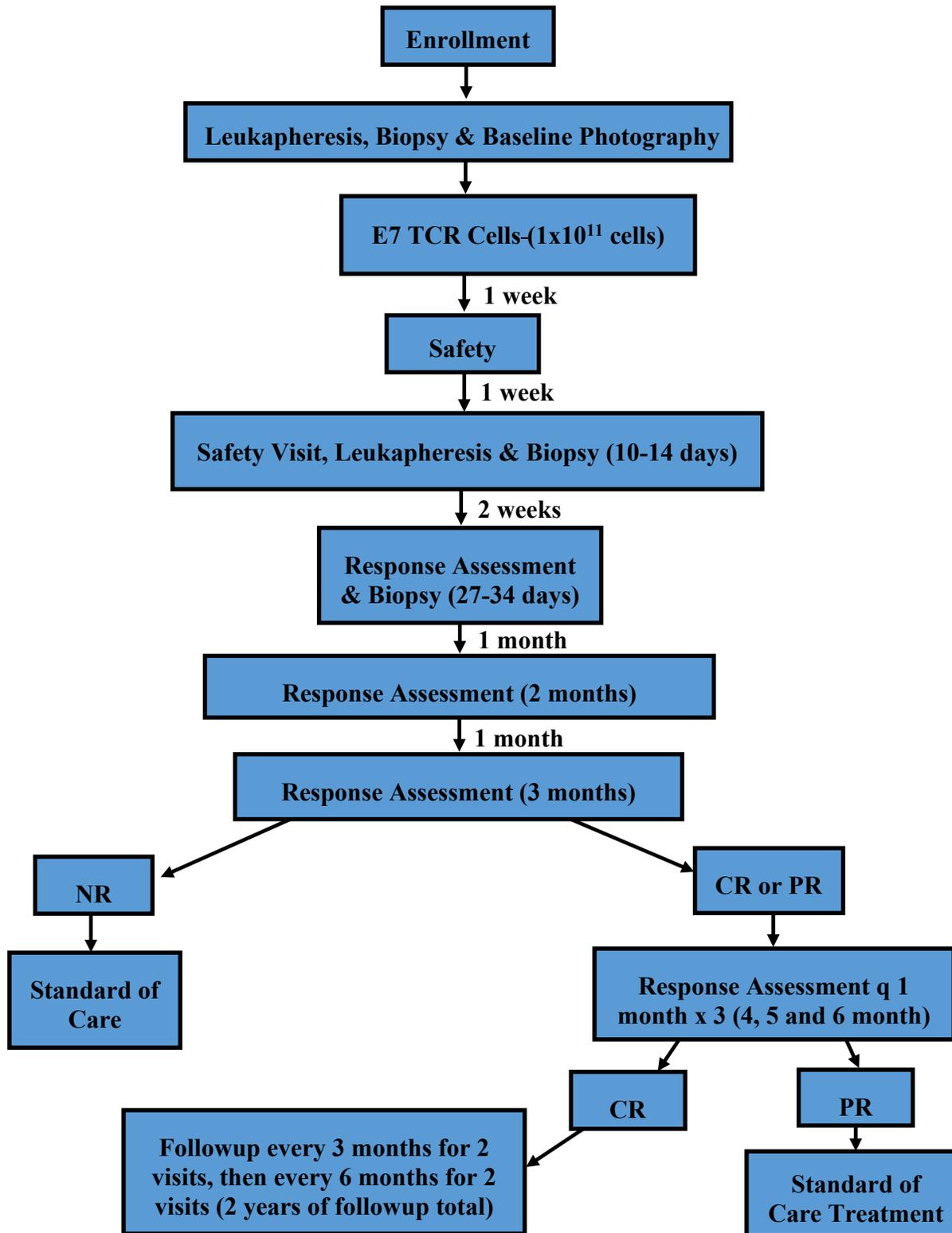
- a. Complete history and physical examination
- a. Chem 20 equivalent: (Sodium (Na), Potassium (K), Chloride (Cl), Total CO₂ (Bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Inorganic Phosphorus, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid).
- b. TSH
- c. CBC with differential and TBNK
- d. PT/PTT
- e. Beta-HCG pregnancy test (serum or urine) on all women of child-bearing potential
- f. ECOG performance status assessment Baseline photographs and 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. 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. Replication Competent Retrovirus (RCR) testing. Leukapheresis as described in section 3.1.2.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a single arm phase II clinical trial using a Simon minimax two-stage design. Twelve patients will be treated initially, which will be expanded to sixteen patients if one or more patient(s) have a complete response in the initial 12 patients.

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 $6-10 \times 10^9$ 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 100×10^6 cells per vial with remaining cells stored at 300×10^6 cells per vial for research and banked on this study and protocol 16-C-0061 (ETIB Tissue Procurement Protocol) if the subject coenrolled on that study. Patients previously treated on protocol 17-C-0116 will not undergo leukapheresis to generate a cell product.

3.1.3 E7 TCR T cell preparation

After cells are obtained by apheresis, further cell processing to generate E7 TCR cells will occur in the DTM according to standard operating procedures and the E7 TCR investigational new drug application. E7 TCR cells can be produced in approximately 21 to 27 days. Cell products may be cryopreserved during production to accommodate patient treatment schedules. Either freshly-collected cells or cryopreserved cells can be used to initiate the cell-preparation process. Peripheral blood mononuclear cells (PBMC) will be isolated. Sufficient cells for three complete cell productions (2-3 vials at $3-4.5 \times 10^9$ cells/vial) will be retained in the DTM; the remaining cells will first be frozen in 10 vials at 100×10^6 cells per vial with excess frozen at 300×10^6 cells/vial. Cells will be frozen in the DTM and then transferred to ETIB the following day. The ETIB preclinical core contact is Jeremy Rose (301-594-5339).

Before infusion, the percentage of T cells expressing the E7 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. When a patient is no longer eligible for retreatment (see section 3.4) on this protocol due to meeting any of the off-study criteria listed in Section 3.6.2, 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 PDCMF and possible use in research and banked according to protocol 16-C-0061 (ETIB Tissue Procurement Protocol) if the subject coenrolled on that study. Cell products for patients treated on protocol 17-C-0116 will be generated from previously collected PBMC products retained in the DTM.

3.1.4 Treatment Phase

E7 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. Patients will be observed overnight as inpatients following administration of cells, and will not be discharged until cleared by the treating physician. Patients will receive one course of treatment unless eligible for retreatment as discussed in section 3.4.

3.1.5 Safety Protocol Stopping Rules

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

1. If one or more deaths (other than death related to progressive disease) occurs within 30 days of E7 TCR T cells.
2. If 2 of the first 6 patients or the fraction of patients $> 33\%$ after the initial 6 patients develop grade 4 or greater adverse events (except lymphocyte count increased) possibly related to E7 TCR T cells.

3.2 DRUG ADMINISTRATION

3.2.1 Cell infusion and other 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

E7 TCR cells

The E7 TCR cells (1×10^{11} 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). E7 TCR T cells (1×10^{11} 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.

3.3 STUDY CALENDAR

<i>Procedure</i>	<i>Screening/ Baseline</i> ²	<i>Day 0</i>	<i>Day 1</i>	<i>Day 2</i>	<i>Day 3</i>	<i>Daily until d/c</i>	<i>Follow-up Period</i>			
							<i>1 wk (Days 5-8)</i>	<i>2 wk (Days 10-14)</i>	<i>Monthly f/u</i> ⁷ <i>(Month 1 is also End of Treatment Visit)</i>	<i>Annual Followup for 5 years after infusion</i>
History, physical exam, and review of systems ¹	X	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	X									
Screening labs: HIV, HBV serology, HCV serology, thyroid panel, PT/PTT, Beta-HCG pregnancy test in women of childbearing potential, HLA-A typing for HLA-A*02:01 allele positivity	X									
HPV-16 verification and pathology review for confirmation of diagnosis ³	X									

<i>Procedure</i>	<i>Screening/ Baseline</i> ²	<i>Day 0</i>	<i>Day 1</i>	<i>Day 2</i>	<i>Day 3</i>	<i>Daily until d/c</i>	<i>Follow-up Period</i>			
							<i>1 wk (Days 5-8)</i>	<i>2 wk (Days 10-14)</i>	<i>Monthly f/u</i> ⁷ <i>(Month 1 is also End of Treatment Visit)</i>	<i>Annual Followup for 5 years after infusion</i>
Cervical high-risk HPV testing	X									
Cardiac stress test and/or pulmonary function tests ¹¹	X									
Sodium (Na), Potassium (K), Chloride (Cl), Total CO ₂ (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, CK, uric acid, CBC with differential and TBNK	X						X	X	X	
HSIL measurements, vulvar biopsy, and photographs ⁴	X							X	X	
Leukapheresis ¹²	X							X		
Replication Competent Retrovirus (RCR) testing ⁵	X								X ⁵	

<i>Procedure</i>	<i>Screening/ Baseline</i> ²	<i>Day 0</i>	<i>Day 1</i>	<i>Day 2</i>	<i>Day 3</i>	<i>Daily until d/c</i>	<i>Follow-up Period</i>			
							<i>1 wk (Days 5-8)</i>	<i>2 wk (Days 10-14)</i>	<i>Monthly f/u</i> ⁷ <i>(Month 1 is also End of Treatment Visit)</i>	<i>Annual Followup for 5 years after infusion</i>
Research samples: peripheral blood lymphocytes, plasma, and serum ⁶	X		X		X	X ⁶	X	X	X	
E7 TCR T cells		X								
E7 TCR assay ¹⁰	X		X				X	X	X	
Response Evaluation									X	
NIH Advance Directives Form ⁸	X									
HSIL and patient status update ⁹										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 Sections 2.2 and 2.4

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

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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. HSIL measurements will occur at baseline and at each monthly 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 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. 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 All patients will follow-up at 1, 2, and 3 months after cell infusion (+/- 7 days). Patients with no response at the 3-month evaluation will be referred for standard of care therapy. Patients with a partial or complete response at the 3-month evaluation will continue to follow up at 4, 5, and 6 months for safety, response, and research evaluations (+/- 7 days). After the 6-month response assessment patients with partial response will be referred for 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).

⁸ As indicated in section 10.4, all subjects \geq 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.

⁹ Subjects or local treating physician will be contacted to collect information annually for 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. This followup can be conducted via telephone.

¹⁰ Clinical assay may be performed at these time points by the NCI Flow Cytometry Laboratory in the Laboratory of Pathology.

¹¹ If applicable, see section 2.1.2.6.

¹² Except for patients previously treated on protocol 17-C-0116.

3.4 POTENTIAL REPEAT TREATMENT

Patients may be retreated if an E7 T cell product can be generated from cells collected prior to any T cell gene therapy treatment. Patients eligible for retreatment include those who had partial or complete response to E7 TCR T cell therapy that subsequently progress. Patients must meet the original eligibility criteria to be considered for retreatment. Research assessments will be performed at the same time intervals used for initial treatment. Patients who develop grade 3 or 4 toxicity due to cell infusion will not be retreated. A maximum of 1 retreatment course may occur. The second treatment will not begin until at least 12 weeks after initial T cell therapy but must be within 5 years from the date of progressive disease.

Note: Response data for all treatments will be captured in the database however only the response data from the first treatment with E7 TCR T cells will be used in determination of response.

3.5 ON-STUDY EVALUATIONS

Please see the study calendar in Section **3.3** for details regarding on-study evaluations.

3.5.1 Long-term Follow-Up:

Long-term follow-up of patients receiving gene transfer is required by the FDA and must continue even after the patient comes off the study. Long-term follow-up will be done under a different long-term gene therapy follow-up protocol (20C0051).

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.

3.6.1 Criteria for removal from protocol therapy

Note: Subjects removed from protocol therapy may still be eligible for up to one course of retreatment per section **3.4**. The same off treatment criteria apply after the second potential course as for the first. Data collection and assessments required while off therapy are addressed in sections **6.1** and **3.3** respectively.

- Completion of protocol therapy
- Positive pregnancy test
- Progression of disease
- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Unacceptable toxicity

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
- The investigators decide to end the study
- The investigators decide it is in the patient's best interest
- Death

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 PROHIBITED MEDICATIONS

Patients needing systemic steroid therapy greater than the equivalent of 5 mg of prednisone may not participate in this study.

4.2 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/mm³

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

4.3 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 q12h). 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.

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 may be drawn for research and sent to the ETIB preclinical core within 4 weeks prior to cell infusion. Send to 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 may be used to collect 4mL of plasma, which may be frozen in 4mL vials. PBMC from the remainder of the CPT tubes may be frozen in aliquots of 10×10^6 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.

5.1.2 Post cell infusion evaluations

- The following samples may be drawn for research and sent to the ETIB preclinical core. While the patient is inpatient, samples may be drawn on post-treatment day 1, post-treatment day 3, thereafter every Monday/Wednesday/Friday x5 days, and then weekly until discharge. Samples may also be collected at each outpatient follow-up visit. Send to 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 may be used to collect 4mL of plasma, which will be frozen in 4mL vials. PBMC from the remainder of the CPT tubes may be frozen in aliquots of 10×10^6 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.

5.1.3 Optional vulvar Biopsies

- 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). This biopsy schedule may be repeated for patients receiving retreatment. 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 Scott Norberg, Bldg 10, Room 4B04, contact 301-275-9668. Specimens will then be transported to the ETIB preclinical core for further processing and storage. They will be delivered to 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.
- Samples will be stored for this protocol. Some of these samples may be archived and analyzed under another protocol 16-C-0061 (ETIB Tissue Procurement Protocol) if the subject also coenrolled on that study.

5.1.4 Mandatory 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.

5.1.4 Immunological Testing

- Leukapheresis will be performed prior to treatment (expect for patients previously treated on protocol 17-C-0116) and 10-14 days after treatment.
- Processing of leukapheresis will be in the Department of Transfusion Medicine (DTM). Cell product may be frozen in 10 vials at concentration 100×10^6 cells/mL and additional vials at 300×10^6 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 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 may 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 may be frozen in 4mL vials. PBL may be frozen in aliquots of 10×10^6 cells/vial.
- The following tests may be used to evaluate immunologic correlates: specific lysis and cytokine release, intracellular FACS of cytokine production, ELISPOT assays, engraftment of E7 TCR T cells and lymphocyte subset analysis.
- Samples of all infused cell products will be cryopreserved, and retrospective analysis of infused cell phenotype and function may be performed including functionality measured by cytokine release in co-culture assays (ELISPOT, ELISA), cytotoxicity measured by cell impedance assay and characterization of T cell subsets by surface marker expression detected by flow cytometry and/or RNA sequencing.
- Peripheral blood lymphocytes (PBL) may be tested by cytotoxicity assays, cytokine release and limiting dilution analysis. Immunological monitoring may consist of quantifying T cells reactive with tumor cells using established techniques such as intracellular FACS, cytokine release assays, and ELISPOT assays. Immunological assays may be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the T cells cryopreserved at the time of infusion.
- Blood and tissue specimens collected in the course of this research project will be stored for this protocol and may be used in the future to investigate new scientific questions related to this study. If the patient is enrolled on protocol 16-C-0061 (ETIB Tissue Procurement Protocol) their blood and tissue specimens may be used on that protocol as well.

5.2 SUMMARY OF SAMPLE COLLECTION

Test/assay	Volume blood (approx)	Type of tube	Collection point (+/- 48hrs)	Location of specimen analysis
Plasma/PBMC	48 mL	CPT	<4 weeks prior to cell infusion	Pre-Clinical core lab (PDCMF) (Jeremy Rose)

Serum	8 mL	SST	<4 weeks prior to cell infusion	Pre-Clinical core lab (PDCMF) (Jeremy Rose)
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 (PDCMF) (Jeremy Rose)
Serum	8mL	SST	Post treatment days 1 & 3, then every Mon/Wed/Fri x 5 days, then weekly until discharge	Pre-Clinical core lab (PDCMF) (Jeremy Rose)
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.	Processed in Christian Hinrichs Lab, then transported to Pre-Clinical core lab (PDCMF) (Jeremy Rose)

5.3 GENE-THERAPY-SPECIFIC FOLLOW-UP

- Patients will be enrolled on the Long Term Followup Gene Therapy Study in order to collect information to meet current FDA requirements. All followup as required by the FDA will occur via the 20C0051 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 appropriate approvals and/or agreements, if required.
- Patient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the Experimental Transplantation and Immunotherapy Branch (ETIB), may be archived by the ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF). All data associated with archived clinical research samples is entered into LabMatrix. Access is limited to PDCMF staff and 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.

5.4.2 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 Scott Norberg, Bldg 10, Room 4B04, phone 240-858-3303

Once received, the samples will be coded utilizing LabMatrix. The bar-coded samples will be stored at -80°C 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

5.4.3 Protocol Completion/Sample Destruction

- Once research objectives for the protocol are achieved, researchers can request access to remaining samples, providing they have both approval of the Principal Investigator of the original protocol under which the samples or data were collected and either an IRB

approved protocol and patient consent or an OHSRP exemption indicating that the activity is exempt from IRB review.

- The 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 record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section 7.2.

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.

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

Document AEs from the first study intervention, Study Day 0 through 28 days after removal from the study treatment, or until off-study, or until they initiate a new treatment, whichever comes first. Beyond 28 days after the last intervention, or initiation of new treatment as applicable, only adverse events and serious adverse events that are at least possibly related to E7 TCR T cells will be recorded. Grade 1 adverse events will not be recorded.

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

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

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

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section [7.2.1](#).

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

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

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

How and where will the data be shared?

Data will be shared through:

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

When will the data be shared

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

6.3 RESPONSE CRITERIA

6.3.1 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension as ≥ 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.

6.3.2 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.

6.3.3 Response Criteria

6.3.3.1 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.

6.3.3.2 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).

6.3.3.3 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
<p>* In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.</p> <p><u>Note:</u> 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 “<i>symptomatic deterioration</i>.” Every effort should be made to document the objective progression even after discontinuation of treatment.</p>			

6.3.4 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 5.0 for toxicity and adverse event reporting. A copy of the CTCAE version 5.0 can be downloaded from the website <http://ctep.cancer.gov>. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found [here](#).

7.2 OHSRP OFFICE OF COMPLAINE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

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

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found [here](#).

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

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

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

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of E7TCR transduced PBL cells as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the E7 TCR transduced PBL, but are not fatal or life-threatening, much be reported to the NIH IBC as soon as possible, but not

later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.4.2 Annual Reports to IBC

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

7.4.2.1 Clinical Trial Information

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

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

7.4.2.2 Progress Report and Data Analysis

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

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

7.5 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient.

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

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

7.5.2 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NIH Intramural IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 SPONSOR SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

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

- Death,
- A life-threatening adverse event (see section [8.1.3](#))
- Inpatient hospitalization or prolongation of existing hospitalization
 - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.

- A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
- Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

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

8.1.4 Severity

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

8.1.5 Relationship to Study Product

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

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

8.2 ASSESSMENT OF SAFETY EVENTS

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

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as

the site principal investigator or sub-investigator.

- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section [6.1](#). All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

8.3 REPORTING OF SERIOUS ADVERSE EVENTS

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

All SAE reporting must include the elements described in section [8.2](#).

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at:

<https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842>

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

8.4 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

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

8.5 REPORTING PREGNANCY

8.5.1 Maternal exposure

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

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

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

8.6 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected in expedited manner to the FDA in accordance to 21 CFR 312.32. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety

report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

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

9 CLINICAL MONITORING

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

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

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

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

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESES

10.1.1 Primary Efficacy endpoints

The primary efficacy endpoint of this trial is to determine the complete response rate for E7 TCR T cells in the treatment of patients with vulvar HSIL.

10.1.2 Secondary Efficacy endpoint

The secondary objective of this trial is to characterize the safety profile of E7 TCR T cells administered in a low intensity setting without conditioning or systemic aldesleukin.

10.1.3 Sample Size Determination

This trial will be conducted using a Simon minimax two-stage phase II trial design(25) to rule out an unacceptably low CR rate of 5% ($p_0=0.05$) in favor of an improved complete response rate of 25% ($p_1=0.25$). With $\alpha=0.10$ (probability of accepting a poor treatment=0.10) and $\beta = 0.20$ (probability of rejecting a good treatment=0.20), the first stage will enroll 12 evaluable patients, and if 0 of the 12 have a complete response, then no further patients will be accrued. If 1 or more of the first 12 patients have a complete response, then accrual would continue until a total of 16 evaluable patients have been treated. As it may take up to several months to determine if a patient has experienced a complete response, a temporary pause in the accrual may be necessary to ensure that enrollment to the second stage is warranted. If there are 1-2 patients with a complete response out of 16 patients, this would be an uninterestingly low

complete response rate. If there were 3 or more of 16 (18.75%) who experienced a complete response, this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (5% complete response rate), the probability of early termination is 54.0%. In patients that undergo retreatment, only the first response to E7 TCR T cell treatment will be used in the calculation of complete response rate.

Since patients will be screened on this protocol, to allow for patients who enroll but are not evaluable for the primary endpoint, as well as screen failures, up to 200 patients total may be enrolled. It is expected that up to 10-12 treated patients can be enrolled within one year; thus, up to 18 months may be required to accrue 16 evaluable patients. Up to 16 patients may be treated in order to allow for a small number of inevaluable, treated patients.

10.1.4 Populations for Analyses

Toxicity Analysis: All patients will be evaluable for toxicity from the time of E7 TCR T cell infusion. Toxicity data will be collected for all patients.

Response Analysis - Intention to treat: any subjects who enroll onto the trial and provide consent and who receive at least one cycle of treatment will be included in analyses.

10.2 STATISTICAL ANALYSES

10.2.1 General Approach

The fraction of patients who experience a complete response will be reported along with confidence intervals. Only the first treatment course with E7 TCR T cells will be used for this analysis.

10.2.2 Analysis of the Primary Endpoints

The fraction of patients who experience a complete response will be reported along with 80% and 95% two-sided confidence intervals.

10.2.3 Analysis of the Secondary Endpoint

The safety of the E7 TCR T cells will be monitored and any toxicities identified will be reported by type and grade. All treatment courses with E7 TCR T cells will be considered in this analysis.

10.2.4 Safety Analyses

If two of the first six patients develop any grade 4 event related to E7 TCR T cells or grade 3 events (except lymphocyte count increased) that does not resolve to a grade 2 or less in 48 hours or the fraction of participants with these events is greater than or equal to 33% after the initial 6 patients, then no further patients will be accrued as soon as this has been determined. Accrual may resume after appropriate treatment revision is described in approved amendment. All treatment courses with E7 TCR T cells will be considered in this analysis.

10.2.5 Baseline Descriptive Statistics

Baseline demographic characteristics will be reported.

10.2.6 Planned Interim Analyses

As indicated in the two-stage design, the number of complete responses noted after 12 evaluable patients have been treated will be noted and will be used to determine if enrollment to the second stage of accrual may proceed.

10.2.7 Sub-Group Analyses

None.

10.2.8 Tabulation of individual Participant Data

None.

10.2.9 Exploratory Analyses

Optionally, the following analyses may be done if appropriate and adequate data exist to perform them:

- To evaluate a novel assay to genotype HPV using a ddPCR-based blood test.
- Test for clearance of HPV infection from vulvar tissues.
- Evaluate the expansion, survival, trafficking, phenotype, and function of E7 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.

Any exploratory evaluations which generate quantitative measures will be done using descriptive statistics including confidence intervals when appropriate. Any statistical tests performed for evaluation of exploratory objectives will be done without formal adjustment for multiple comparisons, but in the context of the number of tests performed.

11 COLLABORATIVE AGREEMENTS

11.1 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.

12 HUMAN SUBJECTS PROTECTIONS

12.1 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.

12.2 STRATEGIES/PROCEDURES FOR RECRUITMENT

Patients for this protocol will be recruited via standard CCR mechanisms. This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms. All advertisements, letters and other recruitment efforts will be submitted to the IRB for approval prior to their implementation.

12.3 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. The protocol has defined risks based on prior studies with E7 TCR T cells.

12.4 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 12.6), all subjects \geq age 18 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 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.

12.5 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

The experimental treatment has well-defined risks based on experience with the administration of E7 TCR T cells. The benefit of the treatment is unknown. The alternative to treatment on the protocol is surgical resection, which carries risks of disfigurement, 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.

12.6 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 E7 TCR T cells. 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.

12.7 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.

12.7.1 Telephone consent (for screening consent only) and re-consent

13 CONSENT FOR SCREENING AND RE-CONSENT ON THIS STUDY MAY BE OBTAINED VIA TELEPHONE. TELEPHONE CONSENT WILL BE OBTAINED AND DOCUMENTED PER OHSRP/IRBO AND CCR POLICIES AND PROCEDURES. PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

13.1 DESCRIPTION OF THE INVESTIGATIONAL PRODUCT (IND #16959)

13.1.1 Cell Preparation (E7 TCR Transduced PBL)

The procedure for the expanding the human PBL is similar to those approved by the Food and Drug Administration, and used at the NCI in ongoing protocols. The PBL will be transduced with retroviral supernatant containing the E7 TCR.

13.1.2 Retroviral Vector Containing the E7 TCR gene

The retroviral vector supernatant (PG13-MSGV1-E7-TCR) encoding a T cell receptor directed against HPV16 E7₁₁₋₁₉ was prepared and preserved following cGMP conditions in the Surgery Branch Vector Production Facility (SBVVPF). The E7 TCR vector was produced by the Surgery Branch Vector Production Facility. The backbone is the MSGV1 retrovirus that has been used in prior gene therapy clinical trials. It was produced using a PG13-based packaging line.

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

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Version Date: 4/27/2020

The physical titer will be determined by transduction of PBL with serial dilutions of the vector. TCR expression on the cell surface will be measured using FACS following staining with an anti-mouse constant region antibody. The titer will be measured as transducing units per milliliter. Portions of the supernatant will be stored at -80C at Surgery Branch, NCI, American Type Culture Collection (ATCC), Rockville, MD, and the NIH Clinical Center Department of Transfusion Medicine. These storage facilities are equipped with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in *ex vivo* transduction of patient PBL. There will be no re-use of the same unit of supernatant for different patients. Retroviral titer has been shown to be stable after immediate thawing and immediate administration (coating the tissue culture wells previously coated with Retronectin). Handling of the vector should follow the guidelines of Biosafety Level-2 (BSL-2). The specific guidelines for Biosafety Level-2 (BSL-2) can be viewed at http://www.cdc.gov/biosafety/publications/bmbl5/BMML5_sect_IV.pdf.

13.1.3 Source

After cells are obtained by apheresis, further cell processing to generate E7 TCR cells will occur in the DTM according to standard operating procedures and the E7 TCR investigational new drug application Toxicities

13.1.4 Storage and Stability

There is a 4-hour expiration time from the point of harvest to the end of infusion. This is based on stability studies showing no changes in cell viability or cell count at 4 hours from cell harvest.

13.1.5 Administration procedures

Please see section [3.2](#).

13.1.6 Potential Drug Interactions

There are no known drug interactions.

14 REFERENCES

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15 APPENDICES

15.1 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.

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